NN 8201

N° 571

substrate utilization and respiration in relation to growth and maintenance in higher plants

F. W. T. PENNING DE VRIES

N08201.571

BIBLIOTHEEK
LANDBOUWHOGESCHOOL
WAGENINGEN

substrate utilization and respiration in relation to growth and maintenance in higher plants

F. W. T. PENNING DE VRIES

SUBSTRATE UTILIZATION AND RESPIRATION IN RELATION TO GROWTH AND MAINTENANCE IN HIGHER PLANTS

F.W.T. Penning de Vries

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD

VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,

OP GEZAG VAN DE RECTOR MAGNIFICUS,

PROF.DR.IR. H.A. LENIGER,

IN HET OPENBAAR TE VERDEDIGEN OP

VRIJDAG 21 DECEMBER 1973 DES NAMIDDAGS OM VIER UUR

IN DE AULA VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

STELLINGEN

\- 1 -

Leerboeken der plantenfysiologie beschrijven gewoonlijk het begrip "fotosynthese" te eng als de vorming van koolhydraten uit koolzuur en water onder invloed van licht. Het is beter "fotosynthese" te definiëren als de som van alle syntheseprocessen die onder onvloed van licht in de groene plant gebeuren, en dan de verschillende onderdelen, te weten de reductieve koolzuurassimilatie, de nitraatreductie en bijbehorende processen, en de synthese van diverse polymeren, afzonderlijk aan te duiden.

(dit proefschrift)

\- 2 -

Bij het bestuderen van ademhaling van planten behoort in veel grotere mate dan tot nu toe gewoon is, nadruk te worden gelegd op haar functionele karakter.

(dit proefschrift)

\- 3 -

De snelheid van heterotrofe groei van een plant of orgaan kan eenvoudig en non destructief worden afgeleid uit metingen van de ademhalingssnelheid.

(dit proefschrift)

\- 4 -

Het is niet mogelijk rassen van de belangrijkste landbouwgewassen te kweken die hun assimilaten efficiënter benutten voor biosynthese dan de huidige rassen. Het is wel zinvol te zoeken naar planten die lagere onderhoudskosten hebben dan de bestaande planten, of te proberen een verlaging van deze kosten te bewerkstelligen.

(dit proefschrift)

- 5 -

Voor kandidaatspractica fysiologie biedt het experimenteren met simulatiemodellen vaak grotere mogelijkheden dan het doen van proeven met reële objecten.

- 6 -

Simuleren is de beste, en toch goedkope leidraad bij het bestuderen van het verloop van processen binnen een betrekkelijk afgesloten geheel, dat in grote trekken bekend is.

- 7 -

Veel rekencentra zijn nog niet ingesteld op klanten die geen belang stellen in de werkwijze van de computer.

- 8 -

Planten die zijn opgegroeid in klimaatkamers bij een lichtintensiteit van minder dan 300 $W\ m^{-2}$ zijn ongeschikt voor het bepalen van de intensiteit van processen onder veldomstandigheden.

- 9 -

Om de gemotiveerdheid voor het verlenen en ontvangen van steun aan arme landen te bevorderen, is het noodzakelijk de geestelijke afstand tussen hulpverlenende en hulpontvangende groepen te verkleinen. Hiertoe kunnen het uitwisselen van personen en het mogelijk maken van informatieve, goedkope reizen belangrijk bijdragen.

- 10 -

Leidinggevend personeel is als enzym-eiwit in een levende cel: beide zijn duur om te vormen en te onderhouden, het aanpassingsvermogen van de eenheid waartoe ze behoren is mede afhankelijk van hun "turnover", en wanneer de groei stilstaat leidt opeenhoping van inactieve elementen tot verstarring en steeds slechter functioneren.

F.W.T. Penning de Vries
Wageningen, 21 december 1973

BEKNOPTE SAMENVATTING VAN DIT PROEFSCHRIFT

De betekenis en regulering van ademhalingsprocessen in planten zijn bestudeerd om vast te stellen hoeveel van de koolstof die door fotosynthese is vastgelegd door ademhaling weer verloren gaat. Het grootste deel van de ademhaling van veldgewassen is het gevolg van groeiprocessen. Op theoretische gronden zijn verbanden tussen groei, substraatgebruik en ademhaling nauwkeurig berekend. Experimenteel zijn deze ook bevestigd. Een ander deel van de ademhaling wordt veroorzaakt door onderhoudsprocessen die cellen intact houden. Deze zijn nog weinig onderzocht. Daardoor is ook de grootte van de onderhoudsademhaling nog niet precies bekend. Wel is een inzicht verkregen in de processen die hierbij een rol spelen.

CURRICULUM VITAE

De auteur is geboren op 26 maart 1946 in Nijmegen. In 1963 behaalde hij daar het H.B.S.-B diploma aan het Dominicus College, en begon daarna met de Biologie-studie aan de Katholieke Universiteit. Het hoofdvak plantenfysiologie is in Wageningen bewerkt onder leiding van dr.ir. J.F. Bierhuizen. In maart 1969 legde hij het doctoraal examen af en in april van dat jaar stelde de Landbouwhogeschool hem aan als wetenschappelijk medewerker bij de Afdeling Theoretische Teeltkunde. Hier werkt hij mee aan het verbeteren en uitbouwen van simulatiemodellen over gewasgroei. Het proefschrift over de betekenis van de ademhaling van planten is bewerkt onder leiding van prof.dr.ir. C.T. de Wit, hoogleraar in de Theoretische Teeltkunde aan de Landbouwhogeschool en prof.dr. A.H. Stouthamer, hoogleraar in de Microbiologie aan de Vrije Universiteit te Amsterdam. De proeven bij deze onderzoekingen genomen, zijn uitgevoerd in nauwe samenwerking met het Instituut voor Biologisch en Scheikundig Onderzoek van Landbouwgewassen te Wageningen.

This thesis comprises:

1. A general INTRODUCTION and SUMMARY in ENGLISH (7 pages)

2. Een algemene INLEIDING en SAMENVATTING in het NEDERLANDS (7 bladzijden)

and three papers:

3. PRODUCTS, REQUIREMENTS AND EFFICIENCY OF BIOSYNTHESIS, A QUANTITATIVE APPROACH. Journal of Theoretical Biology, 1974 (in press) (51 pages)

4. USE OF ASSIMILATES IN HIGHER PLANTS. In: Photosynthesis and productivity in different environments. Ed. J.P. Cooper, Cambridge University Press, 1974 (in press) (29 pages)

5. THE COST OF MAINTENANCE PROCESSES IN PLANT CELLS Annals of Botany, 1974 (in press) (38 pages)

F.W.T. Penning de Vries

October 1973

SUBSTRATE UTILIZATION AND RESPIRATION IN RELATION TO GROWTH AND MAINTENANCE IN HIGHER PLANTS

F.W.T. Penning de Vries

Department of Theoretical Production Ecology

Agricultural University, Wageningen

The Netherlands

This paper contains a summary of the references 3, 4 and 5.

Introduction

The recent development of simulation techniques to study growth of field crops actualized the question how much of the carbon fixed by photosynthesis gets lost by respiratory processes (1). A coherent picture can hardly be constructed from literature data on carbon dioxide production and oxygen consumption of different organs in various conditions, and certainly none that allows extrapolation to other situations. This study tries to answer this question by the investigation of the significance and regulation of respiratory processes in plants. Although this problem is focussed to higher plants it is of a much broader nature.

Five groups of processes were recognized in which carbon dioxide evolution and oxygen uptake are involved:

1. Processes related to the biochemical conversion of substrate into the compounds found in organisms, or shortly: growth processes. The central question is how many grams of carbon dioxide are produced and how many grams of oxygen are consumed if 1.0 gram of glucose is converted into biomass. The question how many grams of biomass are formed simultaneously is closely related, but the answer to the second question does not follow from the answer to the first because also water is involved.
2. Processes related to maintenance of already existing cells and their structures. Energy for these processes is provided by respiration. From respiration measurements it appears that the intensity of maintenance processes in plants is low compared to animals and micro-organisms. In many situations, however, these processes do consume an important fraction of the assimilates.
3. Processes related to active transport of organic compounds across cell membranes and in phloem vessels. Nearly all substrate for growth is transported because substrate production usually does not occur in growing cells. Watertransport is a passive process, which active regulation requires very little respiratory energy.
4. Processes without usefull outcome. A considerable fraction of the carbon dioxide evolution in plants has often been ascribed to "uncoupled respiration": a process without any (known) use. It is pressupposed for this study that these processes are unimportant, and this is demonstrated in a few cases. To decide that these processes are also absent in other conditions, simultaneous measurements are lacking of rates of respiration, protein turnover and ion fluxes across membranes.

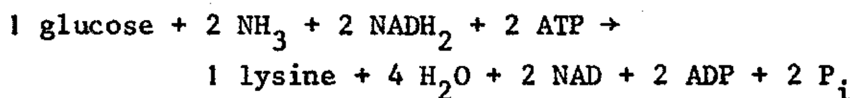
5. Photorespiration. The calculation of carbon dioxide assimilation of a canopy is usually based on the carbon dioxide assimilation light respons curve of a single leaf in similar conditions. A possible decrease of the rate of assimilation due to photorespiration is already included in this curve, and photorespiration is not important any longer for such calculations. Because in general photosynthesizing cells do not grow and none or little photosynthesis occurs in growing cells, photorespiration does not contribute to formation of any other product than sugars and amino acids, which amount is known already.

The energy consumption in other active processes in plants is negligible.

In this way the initial problem is restricted and clarified considerably It is now very similar to that of production-microbiologists, but is still new for many plant physiologists.

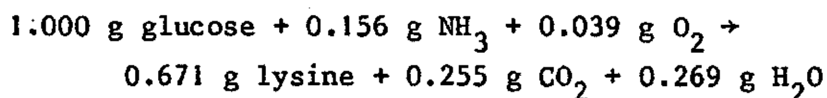
Methods

To determine the amount of biomass formed from a certain amount of substrate and the concomitant respiration, the "reaction equation" of the biochemical conversions may be calculated. For example, if lysine is the end product and glucose and ammonia substrates, handbooks of biochemistry indicate that in plants this conversion can be represented by the reaction equation:



$$1 \text{ glucose} + 2\, NH_3 + 2\, NADH_2 + 2\, ATP \rightarrow 1 \text{ lysine} + 4\, H_2O + 2\, NAD + 2\, ADP + 2\, P_i$$

Heterotrophic cells obtain the hydrogen ($NADH_2$) and energy (ATP) required by oxidation of glucose. The maximum efficiency of substrate and energy utilization is always used, so that the total conversion process, expressed in grams, is represented by the equation:



$$1.000 \text{ g glucose} + 0.156 \text{ g } NH_3 + 0.039 \text{ g } O_2 \rightarrow 0.671 \text{ g lysine} + 0.255 \text{ g } CO_2 + 0.269 \text{ g } H_2O$$

Such equations can be made for all important conversions. To do so only a basic knowledge is required of the well known processes that are the direct cause of carbon dioxide formation and oxygen consumption. When synthesizing complex products the reaction equations of the constituting monomers are added according to their relative importance, and cost of polymerization are accounted for. In this way a conversion reaction can

also be constructed for synthesis of a complex end product, such as biomass. The equation contains only weights of glucose, oxygen, minerals, the end product, carbon dioxide and water. In addition the active uptake of glucose and minerals requires respiratory energy. Finally, the computations were extended to include also some processes that require little energy.

Much of the basic information for such calculations has been obtained from micro-organisms, but it seems correct to conclude that differences in this respect between various kinds of organisms are unimportant.

The above calculations can be applied in principle to higher plants, because heterotrophic, growing cells are generally separated from autotrophic non growing cells. The substrate for growth mostly consists of sucrose and amino acids.

Costs of maintenance processes are calculated from the intensity of these processes and their specific costs. The latter concern mainly costs of (re)synthesis and are fairly well known, but their intensity has been determined only in a few cases. There are also still remarkable few measurements of maintenance respiration in plants.

Conclusions

A first question is whether the yield of growth processes calculated in this way agrees with what is found in plants, or that it is of purely theoretical value. A simple experiment by Kandler (2) answers this. He grew maize embryo's in darkness at 27°C in a petri disk with a nutrient solution containing glucose and nitrate, and determined the weight increase of the embryo's and the amount of glucose consumed over a five days period. On basis of an estimate of the composition of the biomass synthesized a simplified calculation of how much glucose is required theoretically for synthesis is made (table 1). In addition to 66 mg glucose needed for the synthesis of 47.5 mg dry matter, uptake of glucose and minerals consumes the energy of 3.7 mg glucose. Maintenance of the material already formed consumes 2 to 5 mg glucose, so that the total glucose consumption is calculated to be 71.5 to 74.5 mg glucose. This is almost identical to the experimental result (75.4 $\pm$ 2.4 mg). The ratios of the volumes of carbon dioxide and oxygen involved in the calculation (1.35-1.29) and the experiment (1.2 $\pm$ 0.1) also agree fairly well. It is therefore concluded that the reaction equation derived from basic biochemical data reflects reality at least under good growing conditions, and that enzymatic conversions

and transport processes in plants occur at almost maximum efficiency. Because the cost of synthesis and transport are independent of temperature, the result is almost independent of temperature.

Table 1. A calculation of the amounts of glucose, CO_2 and O_2 involved in synthesis of 47.5 mg of maize plant in darkness. Meaning of the columns: (1) weight increase in mg of the fraction considered (only "total" and "nitrogenous compounds" have been observed), (2) amount of the fraction considered, in g, formed from 1.0 g of glucose, (3) mg glucose required for biosynthesis, (4) amount of CO_2, in g, released during conversion of 1.0 g of glucose into the fraction considered, (5) mg CO_2 released, (6) amount of O_2, in g, required for conversion of 1.0 g glucose into the fraction considered, (7) mg O_2 required. The figures in the columns (2), (4) and (6) are standard values.

fractions of the biomass formed	(1)	(2)	(3)	(4)	(5)	(6)	(7)
carbohydrates	32.6	0.826	39.50	0.102	4.02	0.082	3.23
nitrogenous compounds	5.6	0.404	13.85	0.673	9.35	0.174	2.42
organic acids	3.0	1.104	2.71	-0.050	-0.14	0.298	0.81
lignin	2.4	0.465	5.16	0.292	1.51	0.116	0.60
minerals	2.4	--	--	--	--	--	--
lipids	1.5	0.330	4.54	0.530	2.41	0.116	0.53
total	47.5	--	65.76	--	17.15	--	7.59

A computer program has been formulated to execute these detailed calculations. A sensitivity analysis indicated that the rough chemical composition of the end product (as indicated in table 1), and the form in which nitrogen was supplied (as nitrate or ammonia), have the most effect on the amount of biomass formed and the respiration. Much less important are more precise data on the composition of the end product (such as the amino acid composition of protein), cellular compartmentation of processes, maintenance cost of used enzymes, and even the efficiency of oxidative phosphorylation between 100 % and 50 % of its maximal value is of minor importance. Consequently a simplified scheme was derived to calculate the reaction equation of conversions (3).

The above calculations need not much change if applied to autotrophic plants: only the synthesis of many nitrogenous compounds consumes amino

acids and sucrose instead of glucose and minerals, and cost of active transport has to be included. The transport cost for translocation over short distances is probably mainly that of passing membranes of the cell and phloem vessels, but transport over many meters may be much more expensive. There is also an important difference: in most agricultural crops the majority of nitrate reduction occurs in the leaves in the light. If the light intensity is in the light saturated part of the carbon dioxide assimilation light respons curve, the rate of carbon dioxide assimilation is limited by the rate of carbon dioxide diffusion into the leaf, and because then more energy is available in the green cell due to the high light intensity the other energy requiring processes do not decrease the carbon dioxide assimilation. This is important especially for an expensive process such as nitrate reduction. For this reason nitrate reduction in field crops consumes much less energy from assimilates than is expected from its high reduction cost in darkness. Also other processes can use energy that has not been fixed in assimilates, but these are usually less important. Mainly due to nitrate reduction energy absorption by leaves is often 5 to 15 % higher than is expected according to the reaction: carbon dioxide plus water plus light energy gives glucose plus oxygen (4).

Since formation of biomass from assimilates causes a predictable carbon dioxide production, it is possible to determine non destructively the rate of growth by measuring the rate of respiration. This is confirmed by experiments in which substrate utilization, respiration and growth of whole plants is known, as for example in constant conditions in the light, where the rates of carbon dioxide assimilation and of growth (and therefore that of dissimilation) are related. A comparison of measured and calculated ratios of assimilation to dissimilation of young plants of various species at some temperatures demonstrates that also these plants utilize their substrates at the biochemically maximal efficiency (4). It seems thus impossible to increase the efficiency of plants in this sence by plant breeding.

Maintenance processes require an unimportant amount of energy in rapidly growing tissues, i.e. at a relative growth rate of 0.3 $g\ g^{-1} day^{-1}$ or more, but a considerable fraction in other cases (3,5). Measurements of maintenance respiration indicate that these processes consume about 1 to 4 % of the weight of the dry matter in the form of carbohydrates per day. Knowledge of the individual maintenance processes indicates that the main part of it is used for continuous breakdown and resynthesis of

proteins, while another important fraction is required to maintain ion concentrations in cells. To determine the amount of substrate required from the calculated energy consumption in these processes it is essential to know the efficiency of oxidative phosphorylation. However, due to technical difficulties this information is still very limited in plants.

The intensity of protein turnover, and therefore its cost, probably depends on the metabolic activity of the cells, which may be expressed as the daily carbon dioxide assimilation. The cost of maintaining ion concentrations depends mainly upon the environment. The first conclusion arises mainly from measurements of the maintenance respiration rate in leaves, and the few basic data do not support or oppose this. The second conclusion is derived mainly from basic data and was only indirectly confirmed. It seems worthwhile to investigate how to reduce protein turnover, a process that may have lost much of its importance in present agricultural conditions. Unlike increasing the efficiency of synthetic processes in plants, which is considered to be impossible, it seems feasible to control the rate of maintenance processes, and thus to influence the crop yield considerably (5). Cost of maintenance processes probably depend on external conditions, such as temperature, salinity and water stress, but this cannot be quantified as yet.

Many minor questions have not been answered because of lack of basic data. Probably because the rate of synthetic processes in the investigated organ is unknown, quite some unexplained observations remain also. It is likely that many of such observations will not be explained before a better insight is obtained into the processes and factors that start, regulate and influence biochemical conversions and transport.

Literature

1. De Wit, C.T., R. Brouwer and F.W.T. Penning de Vries, 1970: The simulation of photosynthetic systems. In: Prediction and measurement of photosynthetic productivity. Pudoc, Wageningen, The Netherlands.
2. Kandler, O., 1953: Ueber den "Synthetischen Wirkungsgrad" in vitro cultivierter Embryonen, Wurzeln and Sprosse. Z. Naturforschg. 8b, 109-117.
3. Penning de Vries, F.W.T., A.H.M. Brunsting and H.H. van Laar, 1974: Products, requirements and efficiency of biosynthesis. A quantitative approach. J. Theoret. Biol., in press.

4. Penning de Vries, F.W.T., 1974a: Use of assimilates in higher plants. In: Photosynthesis and productivity in different environments. Ed. J.P. Cooper, Cambridge University Press, in press.
5. Penning de Vries, F.W.T., 1974b: The cost of maintenance processes in plant cells. Ann. Bot., in press.

SUBSTRAATBENUTTING EN ADEMHALING IN VERBAND MET GROEI EN ONDERHOUD IN HOGERE PLANTEN

F.W.T. Penning de Vries

Vakgroep Theoretische Teeltkunde

Landbouwhogeschool, Wageningen

Dit geschrift is een samenvatting van de literatuurverwijzingen 3, 4 en 5.

Inleiding

De recente ontwikkeling van simulatiemethodieken voor het bestuderen van de groei van te velde staande gewassen actualiseerde de vraag hoeveel van door fotosynthese vastgelegde koolstof verloren gaat door ademhalingsprocessen (1). Uit literatuurgegevens over koolzuurafgifte en zuurstofopname van verschillende organen in diverse omstandigheden kan nauwelijks een samenhangend beeld worden verkregen, en zeker geen beeld dat extrapolaties naar andere situaties toelaat. In deze studie is gepoogd de bovenstaande vraag te beantwoorden door onderzoek naar de betekenis en regulering van ademhalingsprocessen in planten. Hoewel de vraagstelling is toegespitst op planten in deze in wezen breder en van toepassing op alle organismen.

De processen waarbij koolzuurgasvorming en zuurstofopname zijn betrokken zijn in vijf groepen te onderscheiden:

1. Processen die samenhangen met de biochemische omzettingen van substraat in de verbindingen die in organismen worden aangetroffen, of kortweg: groeiprocessen. De kernvraag is hier hoeveel gram koolzuur er vrijkomt en hoeveel gram zuurstof er wordt opgenomen wanneer 1.0 gram glucose wordt omgezet in biomassa. De vraag hoeveel gram biomassa hierbij wordt gevormd houdt hiermee nauw verband, maar het antwoord op de tweede vraag volgt niet uit het antwoord op de eerste omdat ook water bij de reacties is betrokken.
2. Processen die samenhangen met het in stand houden van reeds bestaande cellen en hun structuren. De energie voor deze actieve processen wordt door ademhaling geleverd. Uit ademhalingsmetingen blijkt dat de intensiteit van onderhoudsprocessen in planten laag is vergeleken bij dieren en micro-organismen, maar in veel situaties consumeren deze processen toch een belangrijk deel van de assimilaten.
3. Processen die verbonden zijn met actief vervoer van organische verbindingen door celmembranen en in de vaatbundels. Vrijwel alle substraat voor groei wordt getransporteerd, omdat substraatproduktie meestal niet plaatsvindt in groeiende cellen. Watertransport is een passief gebeuren, waarvan de actieve regulering uiterst weinig ademhalingsenergie vergt.
4. Processen die geen nuttig resultaat afwerpen. Herhaaldelijk is gesuggereerd dat een aanzienlijke fractie van de koolzuurontwikkeling in planten moet worden toegeschreven aan "ontkoppelde ademhaling": een proces zonder enig (herkenbaar) nut. Een uitgangspunt voor deze studie was dat

dergelijke processen niet van belang zijn, hetgeen in enkele gevallen ook werd aangetoond. Het is nog onzeker of deze processen nooit optreden, maar om dit vast te stellen ontbreken vooralsnog gelijktijdige waarnemingen van ademhalingssnelheden, van eiwitturnover en van ionfluxen door celmembranen.

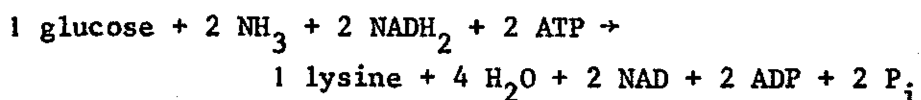
5. Fotorespiratie. Het is gebruikelijk om de koolzuurassimilatie van een gewas te berekenen met de koolzuurassimilatie-licht-respons-curve van een blad in overeenkomstige omstandigheden. Een eventuele verlaging van de assimilatie-snelheid door fotorespiratie is dan al in deze curve verrekend, en fotorespiratie is dus niet meer van belang voor zulke berekeningen. Omdat in het algemeen fotosynthetiserende cellen niet meer groeien, en in groeiende cellen geen of weinig fotosynthese plaatsvindt, levert fotorespiratie geen bijdrage tot de vorming van andere produkten dan de reeds bekende hoeveelheid suikers en aminozuren.

In planten vragen de andere actieve processen een te verwaarlozen hoeveelheid ademhalingsenergie.

Het aanvankelijke probleem is daarmee aanzienlijk ingeperkt en verduidelijkt. Het komt nu sterk overeen met dat van produktie-microbiologen, maar is nog nieuw voor veel plantenfysiologen.

Werkwijze

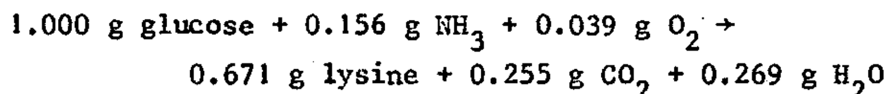
Het vaststellen van de hoeveelheid biomassa die in een groeiproces kan worden gevormd van een zekere hoeveelheid substraat, en de grootte van de bijbehorende gaswisseling, kan gebeuren door het berekenen van de "reactievergelijking" van de biochemische omzettingen. Wanneer, bijvoorbeeld, het aminozuur lysine het eindprodukt is en glucose en ammoniak het substraat, blijkt uit biochemie-handboeken dat deze omzetting in plantencellen kan worden weergegeven met de reactievergelijking:



$$1 \text{ glucose} + 2\, NH_3 + 2\, NADH_2 + 2\, ATP \rightarrow 1 \text{ lysine} + 4\, H_2O + 2\, NAD + 2\, ADP + 2\, P_i$$

Heterotrofe cellen verkrijgen de benodigde waterstof ($NADH_2$) en energie (ATP) door glucose te verbranden. Bij alle omzettingen is steeds de grootst bekende graad van benutting van substraat en energie aangehouden, zodat het gehele omzettingsproces, uitgedrukt in grammen, wordt weergegeven

door de vergelijking:



$$1.000 \text{ g glucose} + 0.156 \text{ g } NH_3 + 0.039 \text{ g } O_2 \rightarrow$$
$$0.671 \text{ g lysine} + 0.255 \text{ g } CO_2 + 0.269 \text{ g } H_2O$$

Dergelijke vergelijkingen kunnen worden opgesteld voor alle belangrijke omzettingen. Van de processen die de onmiddellijke oorzaak zijn van koolzuurafgifte en zuurstofopname, en die door diepgaand onderzoek goed bekend zijn, is daarbij slechts elementaire kennis vereist. Bij de synthese van samengestelde eindprodukten worden de reactievergelijkingen van de constituerende monomeren gewogen opgeteld, en polymerisatiekosten in rekening gebracht. Ook voor synthese van een gecompliceerd eindprodukt, zoals biomassa, wordt op deze wijze een reactievergelijking vastgesteld, met als enige termen de gewichten van glucose, zuurstof, mineralen, het eindprodukt, koolzuurgas en water. Daarenboven vraagt het actieve opnemen van glucose en mineralen uit het medium ademhalingsenergie. De berekeningen zijn tenslotte verfijnd doordat ook met weinig energie vragende processen rekening is gehouden.

Veel van de benodigde basisinformatie is verkregen met behulp van micro-organismen, maar de conclusie lijkt gewettigd dat deze niet belangrijk afwijkt in verschillende soorten organismen.

Bovenstaande berekeningen kunnen in beginsel ongewijzigd worden toegepast in hogere planten, omdat als regel heterotrofe, groeiende cellen gescheiden zijn van autotrofe, niet groeiende cellen. Het substraat voor groei bestaat dan meestal uit sucrose en aminozuren.

De berekening van de kosten van onderhoudsprocessen geschiedt op basis van intensiteit van deze processen en hun specifieke kosten. Deze laatste betreffen voornamelijk kosten van (her)synthese en zijn redelijk goed bekend, maar hun intensiteit is nog in weinig gevallen vastgesteld. Ook zijn er nog opvallend weinig goede metingen van de onderhoudsademhaling in planten.

Conclusies

Een eerste vraag is of de aldus berekende opbrengst van groeiprocessen overeenkomt met wat gevonden wordt in planten, of dat het een puur theoretisch maximum is. Een eenvoudige proef van Kandler (2) geeft hierover uitsluitsel. Hij liet maisembryo's op een petrischaal met een steriele voedingsoplossing met glucose en nitraat groeien in het donker bij 27°C, en bepaalde de gewichtstoename van de embryo's en de opgenomen hoeveelheid

glucose over een periode van vijf dagen. Op basis van een zo goed mogelijke schatting van de samenstelling van de gevormde droge stof is een vereenvoudigde berekening gemaakt van de glucose die theoretisch nodig is voor synthese (tabel 1).

Tabel 1. Een berekening van de hoeveelheden glucose, O_2 en CO_2 die betrokken zijn bij synthese van 47.5 mg maisplant in het donker. Betekenis van de kolommen: (1) gewichtstoename in mg in de betreffende fractie (alleen "totaal" en "stikstofhoudende verbindingen" zijn waargenomen), (2) aantal g van de betreffende fractie dat wordt gevormd uit 1.0 g glucose, (3) aantal mg glucose nodig voor biosynthese, (4) aantal g CO_2 dat vrijkomt bij de omzetting van 1.0 g glucose in de betreffende fractie, (5) aantal mg CO_2 dat vrijkomt, (6) aantal g O_2 dat nodig is bij de omzetting van 1.0 g glucose in de betreffende fractie, (7) aantal mg O_2 dat nodig is. De getallen in de kolommen (2), (4) en (6) zijn standaard waarden.

fracties van de gevormde biomassa	(1)	(2)	(3)	(4)	(5)	(6)	(7)
koolhydraten	32.6	0.826	39.50	0.102	4.02	0.082	3.23
stikstofhoudende verbindingen	5.6	0.404	13.85	0.673	9.35	0.174	2.42
organische zuren	3.0	1.104	2.71	-0.050	-0.14	0.298	0.81
lignine	2.4	0.465	5.16	0.292	1.51	0.116	0.60
mineralen	2.4	--	--	--	--	--	--
lipiden	1.5	0.330	4.54	0.530	2.41	0.116	0.53
totaal	47.5	--	65.76	--	17.15	--	7.59

Behalve ongeveer 66 mg glucose die nodig zijn voor de synthese van 47.5 mg droge stof, vereisen de opname van glucose en mineralen de energie van 3.7 mg glucose. Voor onderhoud van het reeds gevormde materiaal is 2 tot 5 mg glucose nodig, zodat detotale glucose-consumptie volgens berekening 71.5 tot 74.5 mg bedraagt. Dit is nagenoeg identiek met hetgeen experimenteel was vastgesteld (75.4 $\pm$ 2.4 mg). De verhouding tussen de betrokken volumina koolzuurgas en zuurstof in de berekening (1.29 - 1.35) en de meting (1.2 $\pm$ 0.1) stemmen ook vrij goed overeen. Blijkbaar weerspiegelt de van basisgegevens afgeleide omzettingsvergelijking tenminste onder goede groeiomstandigheden de werkelijkheid, en verlopen enzymatische omzettingen en transportprocessen in planten vrijwel maximaal efficiënt.

Omdat de kosten voor synthese en transport niet van de temperatuur afhangen is dit resultaat ook bijna onafhankelijk van de temperatuur.

De gedetailleerde berekeningen worden uitgevoerd door een computerprogramma. Uit een gevoeligheidsanalyse bleek dat de grove chemische samenstelling van het eindprodukt (zoals in tabel 1 aangegeven) en de vorm waarin stikstof wordt aangeboden (als nitraat of als ammoniak) de meeste invloed hebben op de gevormde hoeveelheid biomassa en de gaswisseling. Van veel minder belang zijn een verdere precisering van de samenstelling van het eindprodukt (zoals de aminozuursamenstelling van eiwit), de cellulaire compartimentatie van processen, de onderhoudskosten van gebruikte enzymen, en zelfs de efficiëntie van oxidatieve fosforylering tussen 100 % en 50 % van zijn maximale waarde. Een sterk vereenvoudigd schema om de reactie-vergelijkingen van omzettingen te berekenen werd daarom afgeleid (3).

Bovenstaande berekeningen zijn vrijwel ongewijzigd geldig voor autotrofe planten: alleen synthese van veel stikstofhoudende verbindingen gebeurt van aminozuren en sucrose in plaats van glucose en mineralen, en de kosten van actief transport moeten worden verrekend (3,4). Bij vervoer over korte afstanden zijn transportkosten waarschijnlijk vooral die van het passeren van de membranen van cel- en vaatbundel, maar het is nog niet uitgemaakt of transport over vele meters niet veel duurder is. Er is echter ook een belangrijk verschil: in de meeste landbouwgewassen vindt het overgrote deel van de nitraatreductie plaats in de bladeren in het licht. Wanneer de lichtintensiteit in het lichtverzadigde deel van de koolzuurassimilatie-licht-respons-curve is, wordt de koolzuurassimilatiesnelheid bepaald door de snelheid waarmee koolzuurgas het blad in diffundeert, en omdat door het vele licht dan nog meer energie de groene cel ter beschikking staat, verlopen de andere energie-vragende processen niet ten koste van de koolzuurassimilatie. Met name voor een duur proces als nitraatreductie is dit een belangrijk gegeven. Om deze reden vraagt nitraatreductie in landbouwgewassen beduidend minder energie van assimilaten dan op grond van de hoge reductiekosten in het donker zou worden verwacht. Ook andere processen kunnen niet in assimilaten vastgelegde energie benutten, maar zijn vaak minder belangrijk. Vooral door nitraatreductie is de energieabsorptie van bladeren dikwijls 5 tot 15 % hoger dan wordt verwacht volgens de reactie: koolzuurgas plus water plus stralingsenergie geeft glucose plus zuurstof (4).

Omdat vorming van biomassa van assimilaten een voorspelbare koolzuurontwikkeling veroorzaakt is het mogelijk de groeisnelheid non-destructief vast te stellen door de ademhalingssnelheid te meten. Dit wordt bevestigd door proeven waarin het substraatverbruik, de ademhaling en de groei van gehele planten bekend is. Dit is het geval in constante omstandigheden in het licht, waar de snelheden van koolzuurassimilatie en van groei (en dus die van dissimilatie) op elkaar zijn afgesteld. Een vergelijking van gemeten en berekende verhouding tussen assimilatie en dissimilatie van jonge planten van verschillende soorten bij enige temperaturen toont dat ook deze hun substraat met de biochemisch maximale efficiëntie benutten (4). Het lijkt dan ook niet mogelijk om door veredelingswerk de efficiëntie van planten in deze zin te verhogen.

Onderhoudsprocessen vragen een relatief onaanzienlijke hoeveelheid energie in snel groeiende weefsels, dit is: bij een relatieve groeisnelheid van 0.3 g $g^{-1} dag^{-1}$ of meer, maar een geenszins te verwaarlozen hoeveelheid in andere gevallen (3,5). Admehalingsmetingen wijzen uit dat deze processen dagelijks ongeveer 1 tot 4 % van het droge stof gewicht aan koolhydraten consumeren. De nog geringe kennis van de individuale onderhoudsprocessen duidt erop dat het grootste deel hiervan gebruikt wordt voor voortdurende afbraak en opbouw van eiwitten, en dat een ander belangrijk deel nodig is om ionenconcentraties in de cellen te handhaven. Om uit berekende energiekosten van deze processen de substraatkosten vast te stellen is kennis van de efficiëntie van oxidatieve fosforylering essentieel, maar voor planten is deze door technische moeilijkheden nog beperkt.

De intensiteit van eiwitturnover, en dus de kosten van dit proces, hangt vermoedelijk voor een groot deel samen met de metabolische activiteit van de cellen, bijvoorbeeld uitgedrukt als de dagelijkse koolzuurassimilatie. De kosten van het handhaven van ionenconcentraties hangen vooral af van het milieu. De eerste conclusie volgt voornamelijk uit metingen van de intensiteit van onderhoudsademhaling in bladeren, en wordt door de weinige basisgegevens niet ondersteund of tegengesproken; de tweede conclusie volgt vooral uit de basisgegevens en wordt slechts indirect bevestigd. Het lijkt zinvol onderzoek te verrichten naar het reduceren van eiwitturnover, welk proces onder de huidige landbouwomstandigheden mogelijk veel in betekenis heeft ingeboet. In tegenstelling tot het onmogelijk geachte verhogen van de efficiëntie van syntheseprocessen lijken er namelijk wel mogelijkheden aanwezig om de snelheid van onderhoudsprocessen te beheersen, en zo de

opbrengst van het gewas belangrijk te beïnvloeden (5). Het is waarschijnlijk dat de kosten van onderhoudsprocessen afhankelijk zijn van uitwendige omstandigheden zoals temperatuur, zoutgehalte van de bodem en waterspanning, hoewel hierover nog niet voldoende gegevens beschikbaar zijn om deze mening te kwantificeren.

Vanzelfsprekend zijn nog veel detailvragen onbeantwoord bij gebrek aan basisgegevens. Ook zijn er nog heel wat onverklaarde meetresultaten, vermoedelijk vooral omdat de intensiteit van syntheseprocessen in het onderzochte orgaan vaak onopgemerkt blijft. Het lijkt daarom waarschijnlijk dat veel waarnemingen van ademhalingssnelheden niet volledig kunnen worden verklaard vóór een beter inzicht is verkregen in de processen en faktoren die biochemische omzettingen en transport in gang zetten, reguleren en beïnvloeden.

Literatuur

1. De Wit, C.T., R. Brouwer and F.W.T. Penning de Vries, 1970: The simulation of photosynthetic systems. In: Prediction and measurement of photosynthetic productivity. Pudoc, Wageningen, The Netherlands.
2. Kandler, O., 1953: Ueber den "Synthetischen Wirkungsgrad" in vitro cultivierter Embryonen, Wurzeln und Sprosse. Z. Naturforschg. 8b, 109-117.
3. Penning de Vries, F.W.T., A.H.M. Brunsting and H.H. van Laar, 1974: Products, requirements and efficiency of biosynthetis. A quantitative approach. J. Theoret. Biol., in press.
4. Penning de Vries, F.W.T., 1974a: Use of assimilates in higher plants. In: Photosynthesis and productivity in different environments. Ed. J.P. Cooper, Cambridge University Press, in press.
5. Penning de Vries, F.W.T., 1974b: The cost of maintenance processes in plant cells. Ann. Bot., in press.

PRODUCTS, REQUIREMENTS AND EFFICIENCY OF BIOSYNTHESIS

A quantitative approach

F.W.T. Penning de Vries, A.H.M. Brunsting and H.H. van Laar

Department of Theoretical Production Ecology

Agricultural University, Wageningen

The Netherlands

Received March 9, 1973; accepted
for publication in Journal of
Theoretical Biology

CONTENTS

Summary 1

1. Introduction 2

2. Short review of related studies 3

3. Some general considerations about biosynthesis 6

   3.1. The reaction balance for synthesis of monomers 9

   3.2. Inorganic molecules 10

   3.3. Synthesis of polymers and formation of cellular structures 11

      3.3.1. Polymerization 11

      3.3.2. Tool maintenance 11

      3.3.3. Biomass synthesis from polymers 13

   3.4. Non synthetic activities during biosynthesis 14

4. Variables characterizing biosynthesis 15

5. Modifications of variables characterizing a biosynthetic process induced by changes in conditions 19

   5.1. Compartmentation 20

   5.2. Alternative pathways 21

   5.3. Effect of the P/O ratio on pv, cpf and orf 22

   5.4. Temperature and other environmental factors 23

6. Maintenance of cellular structures and other respiration processes 24

7. Conclusions 24

8. References 27

PRODUCTS, REQUIREMENTS AND EFFICIENCY OF BIOSYNTHESIS

A quantitative approach

F.W.T. Penning de Vries[1], A.H.M. Brunsting[2] and H.H. van Laar[3]

Department of Theoretical Production Ecology, Agricultural University
Wageningen, The Netherlands

SUMMARY

The question of how many grams of an organism can grow heterotrophically from only 1.0 gram of glucose and adequate minerals has been put forward many times. Only a few attempts have been made to answer this question theoretically and these attempts were rather rough. In this paper, it is demonstrated that the yield of a growth process may be accurately computed by considering the relevant biochemistry of conversion reactions and the cytological implications of biosynthesis and growth. Oxygen consumption and carbon dioxide production by these processes are also computed. The weight of the biomass synthesized from 1.0 gram of substrate and the quantities of gases exchanged are independent of temperature.

These results are obtained by adding the individual equations describing the formation of each compound synthesized by the organism from the substrate supplied. The sum represents an equation which accounts for all substrate molecules required for biosynthesis of the carbon skeletons of an end-product, whose chemical composition is given. It is then calculated how much energy is required for the non-synthetic processes which form a part of biosynthesis, such as intra- and intercellular transport of molecules and maintenance of RNA and enzymes. The additional amount of substrate required to provide this energy by combustion is easily calculated. Adding this substrate to the amount used for skeleton synthesis gives an overall equation which quantifies the substrate and oxygen demand as well as carbon dioxide evolution during biosynthesis of 1.0 gram biomass. For example, it requires 1.34 gram of glucose with adequate ammonia and minerals to synthesize 1.0 gram maize plant biomass in darkness; during this process 0.14 gram oxygen

1) Partial fulfillment of a Ph.D. thesis of the senior author
2) Doctorate student in 1969
3) Technical assistant

are consumed and 0.24 gram carbon dioxide are produced. It has been described elsewhere that similar results were obtained experimentally with growing plants.

Such results depend considerably upon the chemical composition of the biomass being synthesized and upon the state (oxidized or reduced) of the nitrogen source. Other parameters, such as the number of ATP molecules required for protein synthesis, the possibility for utilization of alternative pathways for synthesis or energy production, the presence or absence of compartmentation of synthetic processes and variations in the P/O ratio between 2 and 3, under many conditions affect results of the computation less than 10 %.

Since maintenance of cellular structures is not considered, the approach concerns the gross yield of biosynthesis. It predicts therefore the dry matter yield of heterotrophic cells from a given quantity of substrate at high relative growth rates.

1. INTRODUCTION

What determines the actual efficiency of conversion of substrate into biomass by living organisms and the maximal efficiency under optimal growth conditions is a problem which has attracted much attention. It is a challenging question for microbiologists and zoologists since they are often confronted with substrate-yield relationships. Many simultaneous measurements of growth and substrate consumption have been made, with results expressed in weight and energy units. The highest efficiencies reported are about 30 % to 70 % on a weight basis, and slightly higher on an energy basis.

One might also try to calculate the efficiency of an anabolic process. The pathways by which substrate molecules are converted into the variety of end-product molecules found in cells are described in many textbooks, and the amounts of energy required for these conversions and for polymerization are known. Information is becoming available on the energetics of cytological aspects of biosynthesis, such as active transport across membranes and maintenance of the tools for biosynthesis (nucleic acids and enzymes). The substrate requirement to provide the respiratory energy for these activities can be calculated. The sum of the amounts of substrate for material and for energy represents the total substrate requirement for biosynthesis of an end-product. The total of these biochemical and cellular processes will be called biosynthesis in this paper.

To simplify the problem of how to calculate the yield of biosynthetic processes, only the quantitative relation between substrate and end product is of interest, rather than the exact way in which the end product is obtained. Thus, order and rate of individual reactions are not important. It is a legitimate simplification if the rate of a biochemical reaction does not change the stoichiometry of its chemical reaction equation or that of others, which seems to be generally agreed in biochemistry. Stimulation of synthesis of product A by synthesis of product B does not disturb the calculations: then the end product, whose chemical composition must be established experimentally in all cases, will contain more of product A.

Energy consumption for the maintenance of cell structures interferes with dry matter production. An estimate of the substrate requirement for maintenance processes in plant cells is given in another paper (Penning de Vries, 1974b). The term "growth" will be used to indicate biosynthesis accompanied by maintenance of cell structures, and corresponds to dry weight increase.

The present paper deals with the theoretical derivation from biochemical data of the reaction balance for conversion of substrate into a particular product, and with the determination of values characterizing this conversion process. Such values are the weight of the dry matter synthesized from 1.0 gram of substrate and the weight of the corresponding oxygen uptake and carbon dioxide production. This study is focussed on higher plants, of which cells grow heterotrophically under aerobic conditions. This applies to many situations, since even in leaf cells often much growth occurs before the cells obtain the capacity to photosynthesize. Synthesis of organic material from glucose is considered in detail, but the same approach can be used with a variety of substrates. The effect of unfavorable conditions for biosynthesis, such as extreme temperatures, water stress or mineral shortage, are not considered.

2. SHORT REVIEW OF RELATED STUDIES

The first studies about the quantitative relation between dry matter production and substrate consumption were performed by Pasteur around 1875, by Pfeffer around 1890 and by Rubner (1904). They emphasized the energy efficiency of growth. In the nineteen twenties and thirties the relationship between dry matter yield and the amounts of substrate consumed was investigated in fungi. Tamiya (1932) demonstrated that the heat of combustion of the substrate is not a major determinant of yield: the yield expressed

in g organisms per unit of chemical energy present in the substrate proved to be variable.

From the "molecular formula" of fungi, $C_{86} H_{160} O_{45} N_7$, Tamiya (1932) derived that for synthesis of 1.0 gram yeast 1.467 gram of glucose is required if all substrate carbon is incorporated in the organism. He established experimentally that 2.2 gram of glucose was required per gram yeast formed, and concluded that 0.73 gram of glucose was burned to supply energy for the progress in the conversions and the activation of compounds, for replacement of "lost heat" and for achievement of cellular organization. A theoretical basis why 0.73 gram of glucose per gram yeast was respired, and not twice or half this amount, could not be given. A distinction was made between biosynthetic and maintenance processes, in both of which substrate is consumed (Tamiya and Yamagutchi, 1933, see also Terroine and Wurmser, 1922). The observed fixed ratio of the number of grammulecules of substrate consumed and the maximum dry weight of the microbes synthesized from or with it (Monod, 1942; Siegel and Clifton, 1950; Rippel-Baldes, 1952) caused DeMoss et al. (1951) to express their results as dry weight of the organic matter formed per mole of the substrate utilized. This "molar growth yield" is fairly constant for a large number of bacterial species, but depends considerably on the chemical nature of the substrate.

Bauchop and Elsden (1960) expressed the yield of a growth process as dry weight synthesized per mole ATP available from the substrate and called this the ATP yield (Y_{atp}) of the growth process. This was an improvement of the gram organism per unit of energy ratio, since the ATP-yield accounts for the availability of the chemical energy in the substrate to the organism. They demonstrated that this ratio is remarkably constant for a large number of bacterial species and substrates under anaerobic conditions, and is approximately 10.5 g organism per mole of ATP. The substrates they use consisted of a mixture of amino acids and other essentials for cell synthesis and some carbohydrate, which provided the energy for all processes but was not assimilated. Only the ATP derived from carbohydrate was counted in the calculation of Y_{atp}. In later experiments both lower and higher values of Y_{atp} have been observed in aerobic and anaerobic media (Stouthamer, 1969; De Vries et al., 1970; Stouthamer and Bettenhaussen, 1973). Calculating Y_{atp} under aerobic conditions the amount of ATP formed per mole of substrate must be known. The P/O ratio, however, which is of major importance, may deviate considerably from its maximum value of 3 in these micro-organisms (Stouthamer, 1969). Growth yields expressed in g organism per Joule or per

gram-electron available from the substrate are less constant than Y_{atp} (Payne, 1970), which suggests that the fraction of chemical energy in a substrate molecule that can be transferred to ATP is more meaningful for biosynthetic processes than its total chemical energy.

In his book "Plant Respiration", James (1953) described correlations of plant respiration with activities and growth processes, but could not decide whether respiration is essentially an unavoidable and useless loss, or whether it results from useful processes. About synthesis of alkaloids and polyphenols he remarked: "it seems little better than an even chance that a metabolic reaction occurring in a plant tissue will be of any real importance to it". In a review Goddard and Meeuse (1950) state that in rapidly growing cells only a small fraction of the energy released in respiration is related to growth. Beevers (1961), like Needham (1964) and Kleiber (1961) for animals, treated correlations of plant processes and activities with respiration and gave a detailed account of the glycolysis and the Krebs cycle. Also Thornley (1970), McCree (1970) and Thornley and Hesketh (1972) have correlated growth and maintenance processes with respiration by mathematical analysis of experimental data.

Animal husbandry has supplied information about the return of food with varying chemical composition in higher animals in terms of increase in live weight per weight of the substrate. The maximal yields reported exceed 0.5 g animal (live weight) per g food, and approach sometimes 0.7, depending on the growth rate and upon the type of food and product (Brody, 1945; Blaxter, 1962; Needham, 1964). The complexity of the processes accompanying biosynthesis, such as digestion, transport and movements, maintenance, and the practical problem of measuring the dry matter accumulation in large animals has made it very difficult for this discipline to rise essentially above an empirical level, except in cases of lactate and fat production (Baldwin, 1968; Van Es, 1971).

A more fundamental approach was used by Gunsalus and Shuster (1961). They demonstrated how the ATP requirements may be calculated for bacteria growing from a substrate containing all "building blocks", the monomers, used in cell synthesis. Forrest and Walker (1971) calculated the amount of ATP needed for synthesis of the monomers from glucose and their polymerization, but neglected energy required for active uptake of substrate molecules. Both sets of calculations predicted maximum Y_{atp} values of about 30, which is approached by the highest values reported in literature (about 24, by De Vries et al., 1970). Penning de Vries (1972, 1974a) calculated the dry

matter yield of growth in maize, bean and sunflower plants from the substrates glucose and photosynthate, probably accounting for all the important processes. A good correspondence between the theoretically established relative yield and experimental data was shown, indicating that the metabolism in higher plants may operate at nearly the maximum efficiency allowed by the biochemical scheme.

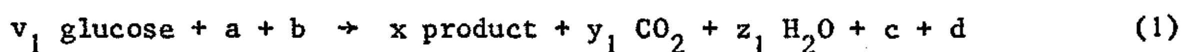
3. SOME GENERAL CONSIDERATIONS ABOUT BIOSYNTHESIS

On basis of the assumption that all substrate carbon will be found in the end product a reaction balance for a conversion can be written with a knowledge of only the molecular compositions of substrate and end-product. This procedure has been followed by Tamiya (1932), Chen (1964) and Morowitz (1968). The assumption, however, is not correct: not all substrate carbon is converted into biomass carbon, and not all biomass carbon comes from the substrate, since some carbon is split off as carbon dioxide molecules during carbon skeleton reformation, and carboxylation reactions may occur. It also gives incorrect answers because it does not consider the energy required for conversion, polymerization and other active processes inherent to biosynthesis, and thus neglects the substrate required for the production of energy and its combustion products. Depending upon the type of substrate, product, and conversion reaction, these two aspects may be responsible for considerable deviations from the balance calculated using the molecular formula (Green cells growing while photosynthesizing are an exception. Net carbon uptake and dry matter production are then related, and the exact ratio between "substrate", consumption and biomass increase can be calculated from the biomass "molecular formula". The situation, however, in which the substrate provides carbon and energy is the usual one, and will be considered in this paper).

The procedure followed in calculating a conversion reaction balance is summarized below and schematized in figure 1. The summation of steps of reaction chains yields an overall balance for the synthesis of a monomer, such as an amino acid. Adding the balances of a number of amino acids and accounting for polymerization cost gives the balance for a protein. Balances for carbohydrates and other substances can be made similarly. Ultimately, the substrate demand for the synthesis of aggregates of compounds, like living organisms, can be established.

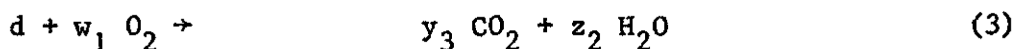
Glucose is the direct substrate for synthesis of many monomers, but during formation of nitrogenous compounds and fatty acids acetyl co-enzyme A,

Figure 1. A schematic presentation of synthesis of a complex product from glucose, including simultaneous non-synthetic processes. Oxygen is the only co-substrate and carbon dioxide and water the only by-products. v, w, x, y and z represent the number of moles involved and a, b, c and d the types.



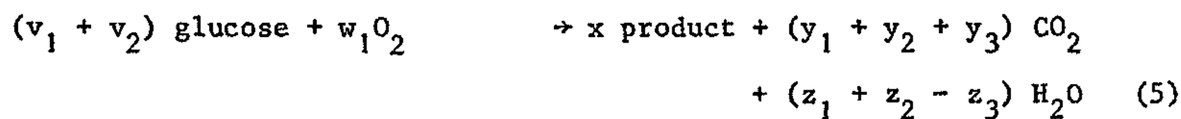
$$v_1 \text{ glucose} + a + b \rightarrow x \text{ product} + y_1\, CO_2 + z_1\, H_2O + c + d \quad (1)$$

$$c \rightarrow b + y_2\, CO_2 \quad (2)$$

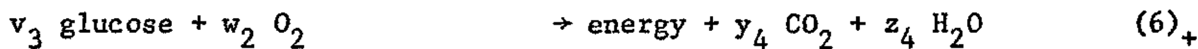


$$d + w_1\, O_2 \rightarrow y_3\, CO_2 + z_2\, H_2O \quad (3)$$

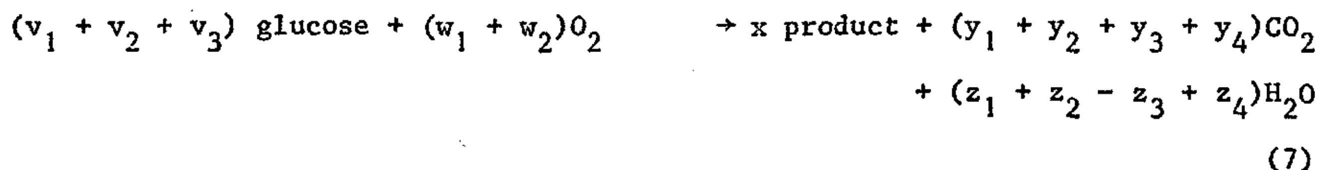
$$v_2 \text{ glucose} + z_3\, H_2O \rightarrow a \quad (4)_+$$



$$(v_1 + v_2) \text{ glucose} + w_1 O_2 \rightarrow x \text{ product} + (y_1 + y_2 + y_3)\, CO_2 + (z_1 + z_2 - z_3)\, H_2O \quad (5)$$



$$v_3 \text{ glucose} + w_2\, O_2 \rightarrow \text{energy} + y_4\, CO_2 + z_4\, H_2O \quad (6)_+$$



$$(v_1 + v_2 + v_3) \text{ glucose} + (w_1 + w_2) O_2 \rightarrow x \text{ product} + (y_1 + y_2 + y_3 + y_4) CO_2 + (z_1 + z_2 - z_3 + z_4) H_2O \quad (7)$$

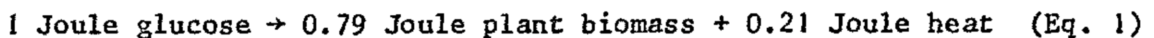
pyruvic acid, oxalo-acetate, aspartate and glutamate (represented by a and b in fig. 1, eq. 1) may also be used. By-products of synthesis in an aerobic environment are carbon dioxide and water, while other compounds may also remain (eq. 1, c and d). In some micro-organisms and in anaerobic conditions reaction by-products consist of partly oxidized molecules, like acetate, ethanol, lactate etc. Under aerobic conditions, not fully oxidized by-products of single reactions may be used in other reactions (eq. 2, c) and those not used are oxidized (eq. 3, d). Synthesis of the intermediates which are not a by-product of other reactions (eq. 4, a) completes the sequence. Summation of the equations (1) - (4) yields the reaction balance for synthesis of the required end-product from glucose with oxygen and completely oxidized by-products (eq. 5). Equation (6) represents the production of energy consumed in non-synthetic processes related to growth, so that equation (7) gives the total of substrate required for biosynthesis of this end-product. The final equation represents the most efficient conversion possible, given the biochemical machinery. With this equation, conversion processes can easily be expressed in terms of grams of glucose, oxygen, end-product, carbon dioxide and water.

An actual conversion balance generally includes ATP and $NADH_2$; these are recycling intermediates and are not oxidized to carbon dioxide and water. ATP, CTP, GTP, TTP and UTP are taken to be similar with respect to their ability to transfer energy. It is supposed that an ATP $\rightarrow$ AMP conversion can be replaced by 2 ATP $\rightarrow$ ADP reactions. The dehydrogenase co-enzymes NAD, NADP and FAD all transfer protons, but oxidation of $FADH_2$ in the "respiratory chain" yields only 2 ATP molecules. Whereas $NADH_2$ yields 3; $NADPH_2$ is not oxidized in this multi-enzyme-complex. The hydrogen of $NADH_2$ can be transferred to NADP only with energy supplied from ATP, but the reverse does not yield ATP. For simplification of the calculations and presentation only the reactions ATP $\rightarrow$ ADP and $NADH_2$ $\rightarrow$ NAD will be used in this paper. If necessary, calculations will be adjusted for this difference. In the pentose phosphate pathway $NADPH_2$ results directly from substrate degradation, but this pathway is not intensively used in plants (Beevers, 1961). It is assumed that all the energy and hydrogen production occurs via the Krebs cycle and none in the pentose phosphate pathway. Because of the small difference between these two pathways in these respects, the error resulting from this simplification is nearly always negligible.

When the overall reaction balance of a biosynthetic process is simplified to its final version in which only glucose and oxygen are

substrate (with nitrogen and sulphur if necessary) and the required products, carbon dioxide and water are formed (fig. 1, eq. 7), variables characterizing the conversion can be calculated, being the "production value" (pv), the "oxygen requirement factor" (orf) and the "carbon dioxide production factor" (cpf). These are defined in table 1. Water and heat will not be considered separately because mostly their formation is difficult to measure. Pv (table 1) resembles Pfeffers' "Oekonomischen Koeffizient" (see Tamiya, 1932),which represents the number of grams of organism formed per 100 g of substrate used, but pv differs from the latter as pv characterizes bio-synthesis and the processes inherent to it, while the Ö.K. accounts for structure maintenance processes as well. Pv, orf and cpf are characteristics strictly for gross dry matter formation.

The energy conversion equation



1 Joule glucose → 0.79 Joule plant biomass + 0.21 Joule heat (Eq. 1)

can easily be derived from equation 1, which represents the same biosynthetic process expressed in grams. A similar computation as the one made to obtain equation 1 can, in principle, be made to obtain equation 2 using the specific heats of combustion of compounds. However, this is much more difficult, if at all possible. Both sets of calculations start with the molecular reaction equations for simple conversions (fig. 1, eq. 1-4). These equations can easier and more accurate be "translated" into a weight balance than into an energy balance. The amounts of oxygen and carbon dioxide involved cannot be expressed in Joules, so that the energy equation does not provide information on the gas exchange of the conversions. The fraction of the energy from ATP molecules retained in reactions is difficult to establish, and it depends, among others, on the concentration of compounds in the cell. But the number of moles ATP used in biosynthesis can be counted easily, because many reactions operate with the energy supply of exactly one ATP molecule, independent of the efficiency of its utilization. The remainder of ATP energy is lost as heat. In phosphorylation, for instance, one ATP molecule is always involved and not an amount of energy that can be calculated from the change in free energy of formation of the reactants divided by the average energy efficiency of processes. It could be argued that instead of one ATP molecule per reaction two or more could be involved, for instance, in case of a high end-product concentration. This is unlikely, because the energy supplied by ATP is generally in excess of the needs (Krebs and Kornberg, 1957; Lehninger, 1965). It is therefore concluded that the "energy efficiency" of biosynthesis of an organism from

Table 1. The variables characterizing biosynthetic processes.

Units: $g.g^{-1}$ and gmole. g^{-1}.

Name	Symbol	Definition
production value	pv	$\frac{\text{weight of the end product}}{\text{weight of substrate required for C-skeletons and energy production}}$
oxygen requirement factor	orf	$\frac{\text{weight of oxygen consumed}}{\text{weight of substrate required for C-skeletons and energy production}}$
carbon dioxide production factor	cpf	$\frac{\text{weight of carbon dioxide produced}}{\text{weight of substrate required for C-skeletons and energy production}}$
hydrogen requirement factor	hrf	$\frac{\text{gmoles of } NADH_2 \text{ required}}{\text{weight of end product}}$
energy requirement factor	erf	$\frac{\text{gmoles of ATP required}}{\text{weight of end product}}$

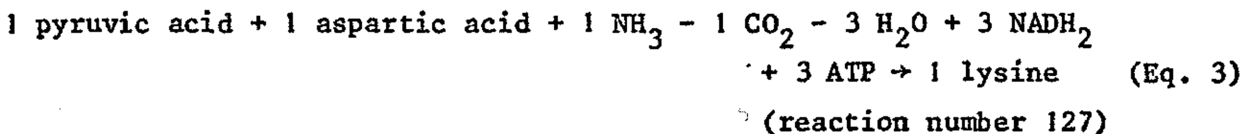
a substrate can be determined experimentally, like the "weight efficiency" by measuring heats of combustion instead of weights. Both values can be useful and converted into one another. But the "weight efficiency" is also predictable from more basic data, while in practice the "energy efficiency" is not.

3.1. THE REACTION BALANCE FOR SYNTHESIS OF MONOMERS

The reaction equations used in the calculations described in this paper were constructed from pathway data as presented in the excellent book by Dagley and Nicholson (1970), showing many steps of nearly all relevant conversions in detail. The pathways for synthesis of lignin, deoxyribose, fructosan and chitin could not be found and therefore estimated. The characterization of compounds like hemicellulose has been greatly simplified. Table 2 presents the reaction balances used in calculating the values characterizing a conversion process from glucose into organic dry matter in a standard format; ADP and NAD are not quoted. All reactions are in the form:

1 glucose + 1 ATP → 1 fructose (Eq. 2)
(reaction number 205)

and



1 pyruvic acid + 1 aspartic acid + 1 NH_3 - 1 CO_2 - 3 H_2O + 3 $NADH_2$ + 3 ATP → 1 lysine (Eq. 3)
(reaction number 127)

Note that whenever $NADPH_2$ is required, for instance for fatty acid synthesis, this is presented as $NADH_2$ plus 1 ATP per $NADH_2$ molecule. Alternative synthetic pathways are listed in an order of decreasing probability in higher plants, in so far as indications could be found. The figures in the table represent the number of molecules involved in the synthesis of 1 molecule of the product. Glucose, pyruvic acid, acetyl coenzym A, serine, aspartic acid and glutamic acid are taken to be the (co)-substrates. As there are "families" of derivates from one common intermediate, the procedure followed during computation was first to determine the required amounts of intermediates and, in a second phase, to calculate the amount of glucose required for synthesis of these compounds.

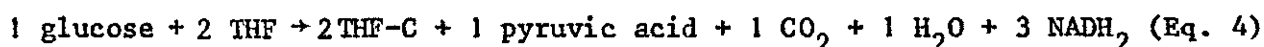
Table 2. Basic data for conversion reactions, as derived from Dagley and Nicholson (1970). Each balance gives the number of moles involved in synthesis of one mole end-product (prod), the name of which is given in the last column. Substrate for conversion may be glucose (gluc), pyruvic acid (pyr), acetyl coenzym-A (AcoA), serine (ser), aspartic acid (aspa) or glutamic acid (glua). Furthermore, the NH_3, H_2S, CO_2, O_2, H_2O, $NADH_2$ and ATP involved in the reactions are indicated. The first column gives the number of the reaction. MW represents the molecular weight of the end-product. Meaning of notes (column 16):

1.1 production of one THF-C group during conversion
-2.1 consumption of one THF-CH_2 group during conversion
-3.1 " " one THF-CO " " "
-3.2 " " two THF-CO groups " "
-3.3 " " three THF-CO groups during conversion
10 does not occur in plants
11 authors estimate
12 Embden-Meyerhof-Parnas pathway or Etner-Douderoff pathway
13 cellulose, glycogen, starch and amylopectine
14 polymer of mannose and xylose
15 polychitobiose
16 polyconiferylalcohol, pathway estimated

TABLE 2, CONTINUED

NO.	GLUC	PYR.	ACoA	SER.	ASPA	GLUA	NH_3	H_2S	CO_2	O_2	H_2O	$NADH_2$	ATP	PROD	NOTE	M.W	NAME
101		1.0					1.0				-1.0	1.0		1.0		89	alanine 1
102					1.0				-1.0					1.0		89	" 2
103						1.0	3.0		1.0		-4.0	2.0	4.0	1.0		174	arginine 1
104						1.0	3.0		1.0		-4.0	2.0	5.0	1.0		174	" 2
105		1.0					1.0		1.0		-1.0	1.0	1.0	1.0		133	aspartic ac. 1
106		1.0					1.0		1.0		-1.0	1.0		1.0		133	" 2
107		2.0					1.0		-2.0		2.0	-4.0		1.0		133	" 3
108						1.0			-1.0		2.0	-3.0		1.0		133	" 4
109					1.0		1.0				-1.0		2.0	1.0		132	asparagine
110						1.0	2.0		1.0		-3.0	2.0	3.0	1.0		175	citrulline 1
111						1.0	2.0		1.0		-3.0	2.0	2.0	1.0		175	" 2
112				1.0				1.0			-1.0			1.0		121	cysteine
113				2.0				2.0			-2.0	-1.0		1.0		240	cystine
114		2.0					1.0		-1.0			-1.0	1.0	1.0		147	glutamic ac. 1
115		3.0					1.0		-4.0		3.0	-6.0		1.0		147	" 2
116		1.0			1.0				-2.0		1.0	-2.0		1.0		147	" 3
117						1.0	1.0				-1.0		1.0	1.0		146	glutamine
118				1.0							-1.0			1.0	1.1	75	glycine 1
119		1.0					1.0		-1.0		1.0	-2.0	-1.0	1.0	10.	75	" 2
120	1.0						3.0		-1.0		-3.0	-3.0	6.0	1.0	-3.1	155	histidine 1
121	1.5						3.0		-3.0		-1.0	-8.5	4.0	1.0	11.	155	" 2
122					1.0						-1.0	2.0	1.0	1.0		119	homoserine
123						1.0				0.5	-2.0	2.0	2.0	1.0		131	hyd.proline 1
124						1.0				0.5	-2.0	2.0	1.0	1.0		131	" 2
125		1.0			1.0				-1.0		-3.0	4.0	4.0	1.0		131	iso-leucine
126		3.0					1.0		-3.0		-1.0			1.0		131	leucine
127		1.0			1.0		1.0		-1.0		-3.0	3.0	3.0	1.0		146	lysine 1
128		1.0				1.0	1.0		-2.0		-1.0		3.0	1.0		146	" 2
129					1.0			1.0			-2.0	2.0	2.0	1.0	-2.1	149	methionine
130						1.0	1.0				-2.0	2.0	2.0	1.0		132	ornithine 1
131						1.0	1.0				-2.0	2.0	1.0	1.0		132	" 2
132	2.0						1.0		-3.0		-4.0	-4.0	2.0	1.0		165	phenylalanine
133						1.0					-2.0	2.0	2.0	1.0		115	proline 1
134						1.0					-2.0	2.0	1.0	1.0		115	" 2
135	0.5						1.0					-1.0		1.0		105	serine
136					1.0						-1.0	2.0	3.0	1.0		119	threonine
137	2.5						2.0		-4.0		-5.0	-7.0	5.0	1.0		204	tryptophane
138	2.0						1.0		-3.0	1.0	-5.0	-3.0	3.0	1.0		181	tyrosine
139		2.0					1.0		-1.0		-2.0	2.0		1.0		117	valine
140	0.5											-1.0	-1.0	1.0	12.	88	pyruvic ac.
141	5.0				2.0		13.0		-2.0	1.0	-26.0	-13.0	36.4	1.0	-3.2	1285	RNA
142	5.0				2.0		13.0		-2.0	1.0	-31.0	-7.0	38.4	1.0	-3.3	1235	DNA
201	1.0								-2.0		2.0	-4.0	-3.0	1.0		120	erythrose
202	1.0								-1.0		1.0	-2.0	-1.0	1.0		150	ribose
203	1.0								-1.0			-1.0		1.0	11.	134	deoxy ribose
204	1.0													1.0		180	glucose
205	1.0												1.0	1.0		180	fructose
206	1.0												1.0	1.0		180	mannose
207	1.0												2.0	1.0		180	galactose
208	2.0										-1.0		3.0	1.0		342	sucrose
209	2.0										-1.0		2.0	1.0		342	lactose
210	1.0										-1.0		2.0	1.0	13.	162	cellulose
211	1.0										-1.0		2.0	1.0	11.	162	fructosan
212	2.0								-1.0		-2.0	-2.0	4.0	1.0	14.	276	hemicellulose
213	1.0											-2.0	3.0	1.0		176	pectin
214	1.0	1.0					1.0		-1.0		-2.0	-1.0	3.0	1.0	15.	203	chitin
301			4.0								-2.0	6.0	9.0	1.0		144	caprylic ac.
302			5.0								-3.0	8.0	12.0	1.0		172	capric ac.
303			6.0								-4.0	10.0	15.0	1.0		200	lauric ac.
304			7.0								-5.0	12.0	18.0	1.0		228	myristic ac.
305			8.0								-6.0	14.0	21.0	1.0		256	palmitic ac.
306			9.0								-7.0	16.0	24.0	1.0		284	stearic ac.
307			9.0								-7.0	15.0	23.0	1.0		282	oleic ac.
308			9.0								-7.0	14.0	22.0	1.0		280	linolic ac.
309			9.0								-7.0	13.0	21.0	1.0		278	linoleic ac.
310		18.0							-24.0	1.0	-7.0	-4.0	26.0	1.0		426	lanosterol
311	0.5											1.0	1.0	1.0		92	glycerol
312	1.0								-3.0			-5.0	-1.0	1.0		92	"
401	8.0								-8.0	2.0	-25.0	-3.0	12.0	1.0	16.	696	lignin
501			1.0								3.0	-3.0		1.0		90	oxalic ac. 1
502		1.0							-1.0		3.0	-4.0		1.0		90	" 2
503			1.0								2.0	-2.0		1.0		74	glyoxalic ac. 1
504		1.0							-1.0		2.0	-3.0		1.0		74	" 2
505		1.0							1.0				1.0	1.0		132	oxaloac. ac. 1
506		1.0							1.0					1.0		132	" 2
507			2.0								3.0	-3.0	1.0	1.0		132	" 3
508		2.0							-2.0		3.0	-5.0		1.0		132	" 4
509					1.0		-1.0				1.0	-1.0		1.0		132	" 5
510		1.0							1.0			1.0	1.0	1.0		134	malic ac. 1
511					1.0		-1.0				1.0			1.0		134	" 2
512			2.0								3.0	-2.0		1.0		134	" 3
513		2.0									1.0	-1.0	1.0	1.0		192	citric ac. 1
514			3.0								4.0	-3.0		1.0		192	" 2
515		3.0							-3.0		4.0	-6.0		1.0		192	" 3
516		1.0	1.0						1.0		1.0		1.0	1.0		192	" 4
517		2.0										-1.0	1.0	1.0		174	aconitic ac. 1
518			3.0								3.0	-3.0		1.0		174	" 2
519		3.0							-3.0		3.0	-6.0		1.0		174	" 3
520		1.0	1.0						1.0				1.0	1.0		174	" 4

Single-carbon-groups are transferred by tetra-hydro-folic acid (THF). These groups are indicated in column 16 of table 2 and explained in the table caption. Their production may occur according to



$$1 \text{ glucose} + 2 \text{ THF} \rightarrow 2 \text{ THF-C} + 1 \text{ pyruvic acid} + 1\ CO_2 + 1\ H_2O + 3\ NADH_2 \quad \text{(Eq. 4)}$$

and all overall excess is essentially removed by the reaction

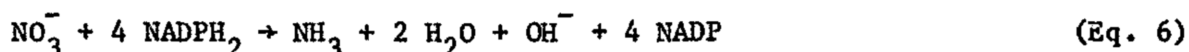
$$1 \text{ THF-C} + 2\ H_2O \rightarrow CO_2 + 2\ NADH_2 + \text{THF} \quad \text{(Eq. 5)}$$

(Dagley and Nicholson, 1970)

It should be noted that the majority of pathways have been established in micro-organisms and in specialized animal tissues. However, it may be assumed (Dagley and Nicholson, 1970) that other organisms do not have metabolic pathways deviating much from the known ones.

3.2. INORGANIC MOLECULES

Biomass often contains inorganic atoms and molecules, some of which are incorporated in organic molecules. The incorporation reactions occur spontaneously . Nitrate and sulphate are taken up and subsequently reduced. According to Bandurski (1965) nitrate reduction can be summarized by the equation



$$NO_3^- + 4\ NADPH_2 \rightarrow NH_3 + 2\ H_2O + OH^- + 4\ NADP \quad \text{(Eq. 6)}$$

Reduction of one sulphate molecule requires 4 ATP molecules more. Reduction of one mole of nitrate or sulphate consumes the hydrogen and energy obtained from about 0.35 mole of glucose and appears to be an expensive process. Feeding yeast with carbohydrate and nitrate yields 0.35 gram yeast per gram carbohydrate, and more (0.45 gram), if ammonia is the source of nitrogen (Terroine and Wurmser, 1922). Wesselius (1973) observed a yield decrease of 30 % if photosynthesizing algae were fed with nitrate instead of ammonia. A similar trend can be seen in fig. 2, 3 and 4. In higher plants reduction of nitrate and subsequent incorporation of nitrogen into amino acids occur during photosynthesis in leaves under conditions of adequate nitrogen supply (Beevers and Hageman, 1969; Bornkam, 1970). Thus the assimilate consists of sucrose and amino acids, and crop plants supplied with nitrate do not show a drop in yield per gram of assimilates as compared to ammonia fed plants. These and other aspects of the relations between processes occurring during photosynthesis were elaborated and evaluated experimentally elsewhere (Penning de Vries, 1974a).

3.3. SYNTHESIS OF POLYMERS AND FORMATION OF CELLULAR STRUCTURES

3.3.1. Polymerization

Most of the cell plasma and the cell wall are polymers. Polymerization of monomers requires 3 or 4 molecules of ATP per amino acid (one ATP → AMP conversion and, according to Lucas-Lenard and Lipmann (1971) one or two GTP → GDP conversions in ribosomal action), 2 per nucleotide monosphosphate and also for most of the (non phosphorylated) carbohydrate monomers. The lower value for amino acid polymerization was used for calculation in "standard conditions".

The energy content of the hydrogen bonds, which with sulfide bonds are responsible for the secondary and tertiary structure of polymers and polymer aggregates, is low compared with the bonds within and between monomers. These are assumed to evolve spontaneously, or to require only a negligible amount of energy. Formation of sulfide bonds yields $NADH_2$. It is generally assumed that the secondary and tertiary structure of proteins is determined by the amino acid sequence, and the kinetics of folding of the amino acid chain during its assembly on the ribosomes.

During polymerization water is split off, mostly one molecule per monomer. Accounting for such changes in the overall reaction equation completes the calculation of the direct requirements for biomass synthesis.

3.3.2. Tool maintenance

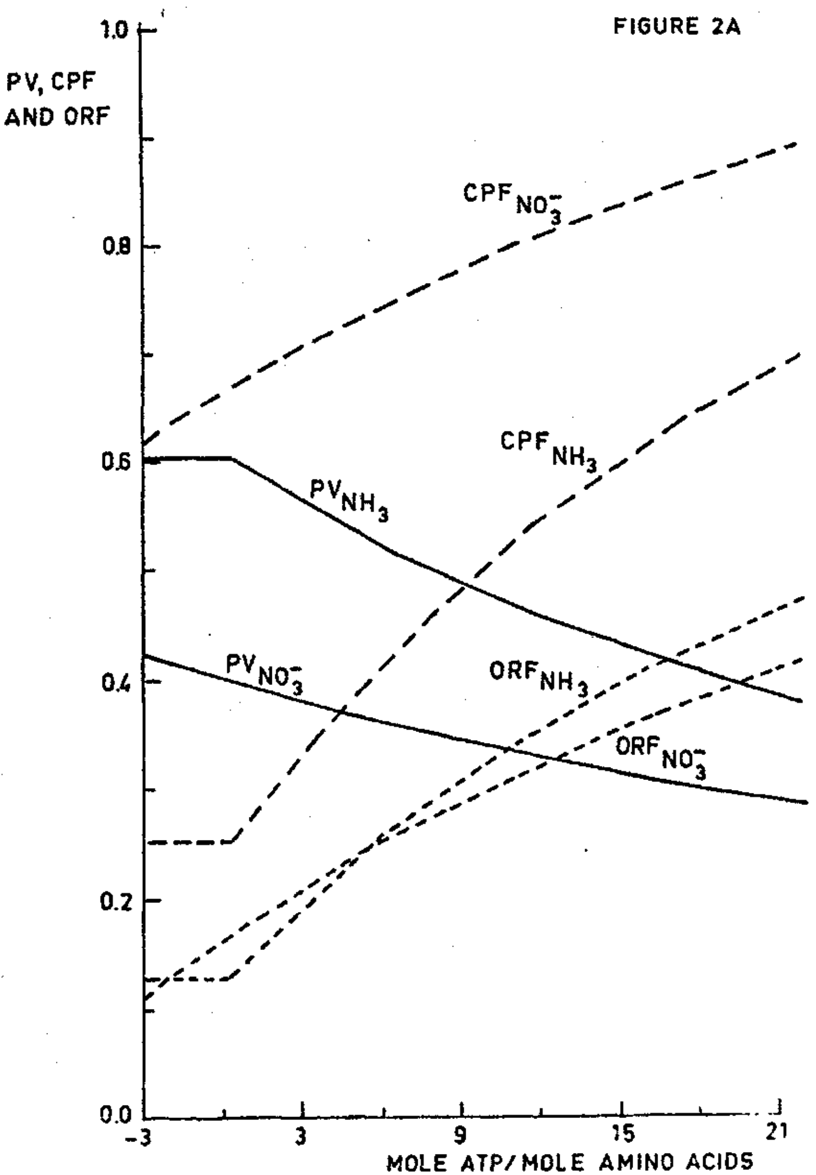
The rate energy expenditure for maintenance of the tools for biosynthesis, RNA and enzymes, is treated independently and separately from other maintenance processes; the latter will be called "structure maintenance". The rate of "tool maintenance" depends on the amount of tools and on their stability. If both are unaffected by the rate of biosynthesis, tool maintenance is constant and can be determined together with structure maintenance processes. However, if the amount of mRNA (the most unstable fraction of RNA) controls the rate of protein synthesis (as suggested by Goodwin, 1963; Lavallé and De Hamer, 1970, and others), and if enzyme activity is regulated according to cell needs, it is likely that these quantities are related to the rate of biosynthesis. No indication of a relationship between the stability of enzymes and RNA and growth rate in eukaryotes was found, although there may be such a relationship for RNA in bacteria (Salser et al., 1968; Norris and Koch, 1972). From Salser et al. (1968) and Geiduschek and Haselkorn (1969) it can be derived, that mRNA

molecules in bacteria are used about 20 times before they are degraded, and it is assumed that this is a fairly constant value. For this assumption is little evidence (Bielka, 1969), but counter-evidence was not found. Since (re)synthesis of a codon from monomers requires 6 ATP molecules, 0.3 ATP molecule per peptide bond is needed for mRNA maintenance. Other RNA fractions are known to be much more stable (Geiduschek and Haselkorn, 1969), so that their turnover does not double the RNA maintenance cost per peptide bond, in spite of their larger amounts. There is no indication that higher cost is incurred in higher organisms.

Amount and half-life or most enzymes involved in biosynthesis and related activities are unknown, except in some specialized mammalian tissues. Synthesis of ribosomal proteins may be related closely to RNA synthesis (Schweet and Heinz, 1966). From data of Strehler (1963) and Schimke and Doyle (1970) it is estimated that in slowly growing tissues 5 to 50 % of protein synthesis is resynthesis of hydrolized proteins. On the basis of this information it was assumed, somewhat arbitrarely, that the processes of tool maintenance can be accounted for by increasing the cost of polymerization of amino acids and nucleotides by 1 ATP molecule per monomer. Quantitatively, the turnover of other cell substances is much less important, as was shown for instance in experiments of Bieleski (1972) for phospholipids. Due to the more complex nature of synthesis of nitrogenous compounds than that of others, it may be expected that tool maintenance is more important for synthesis of the former compounds than to synthesis of nitrogen free compounds. For purposes of calculation, it is assumed that the rate of tool maintenance is proportional to the rate of synthesis of amino acids and nucleic acids. Thus, this process is accounted for as part of the biosynthesis of nitrogenous compounds.

Maintenance of enzymes and RNA was estimated on the basis of experiments to require about 5 mole ATP per mole monomer for milk protein production in cows and about 20 during animal growth (Van Es, 1971). Estimates on the basis of theoretical speculations are 2 ATP per monomer for protein synthesis in higher animals (Baldwin, 1968) and 3 in microbes (Woldendorp, 1971). Forrest and Walker (1971) used Baldwin's estimate.

The effect of various values of tool maintenance on the yield of conversion of 1 g glucose into "nitrogenous compounds" (for its chemical composition, see table 3) and the amounts of oxygen and carbon dioxide involved in this process are given in fig. 2a. Fig. 2b is a similar figure for synthesis of maize plant biomass. These figures present results of



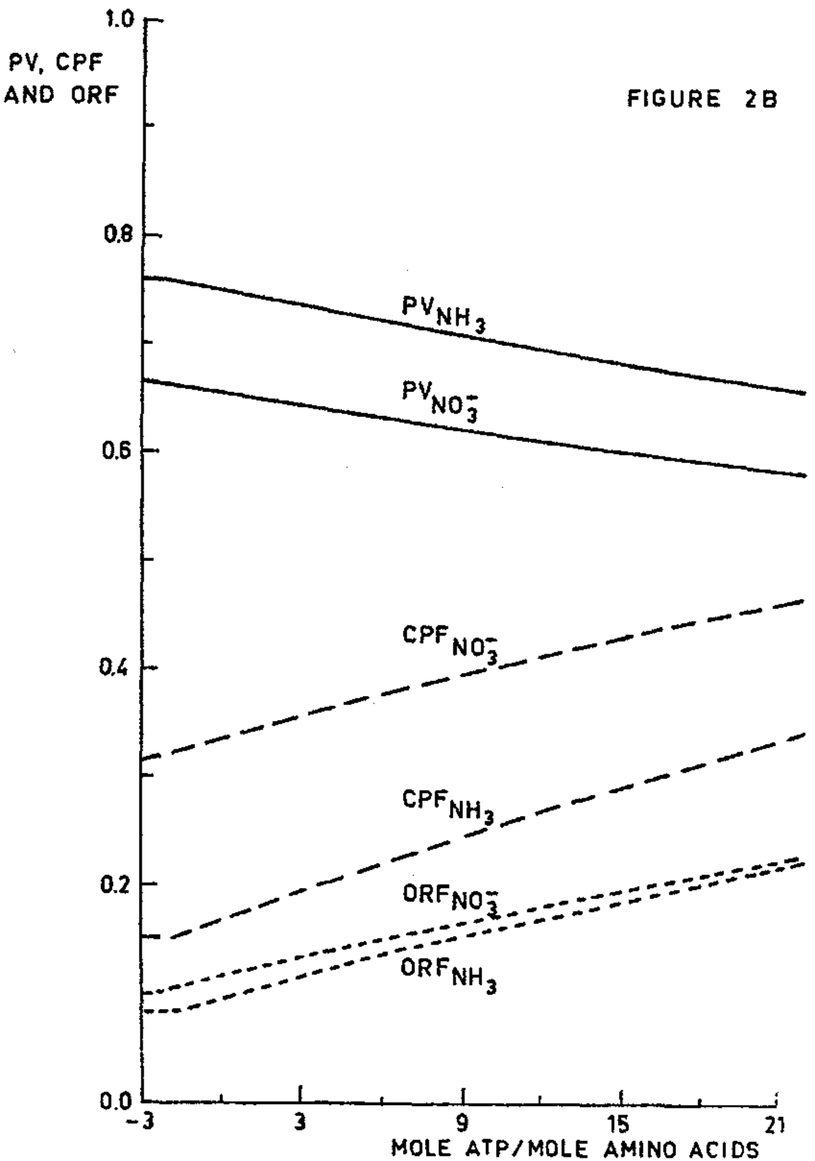


Figure 2 a and b. The conversion characteristics for protein (2a) and biomass (2b) biosynthesis at various cost of "tool" maintenance. Tool maintenance is expressed in mole ATP per mole amino acid. The cost of amino acid polymerization per bond is 3 ATP molecules.

of calculations, for which there is as yet no means of experimental validation. It is not suggested that the rate of tool maintenance varies, but the effect which different values would have is shown.

3.3.3. Biomass synthesis from polymers

Considering finally the synthesis of living biomass the question arises whether chemical energy is required to construct from polymers the macromolecular aggregates that together make up the living biomass. It is generally, and tacitly, assumed that biomass evolves spontaneously when its precursors, the macromolecules, are present (e.g. Lehninger, 1965; Forrest and Walker, 1971). No data have been found in literature on the heat of combustion of biomass, being different from the sum of the heats of combustion its contributing polymers separately. Meyerhoff (1924) found that the heats of combustion of living and quickly killed erythrocytes are exactly equal. Although dead, cell organelles will still have been present and this experiment therefore does not give exactly the evidence required. Krebs and Kornberg (1957) remark that enzymatic breakdown of biomass into monomers does not yield ATP and suggest implicitly that most, if not all, energy released in breakdown to monomers must be ascribed to polymer hydrolysis. Breakdown of macro molecular structures or polymers into monomers does not require chemical energy to disrupt bonds between polymers or monomers, some reactions require a catalyst. The conclusion may therefore be drawn that the free energy of formation of biomass is not noticably different from the sum of the free energies of its polymers separately, and that hardly any chemical energy is stored in the specific macromolecular structures.

Theoretical evidence for this statement is presented by Morowitz (1968, pg. 98) who calculated that the change in heat of formation for polymerization plus synthesis of biomass from polymers equals 16 cal per gram biomass and the change in free energy of formation 78 cal per gram biomass, the difference being the heat given up in these processes. This heat, divided by the absolute temperature at which the reaction occurs, represents the entropy increase of biomass and environment. Most of this change is brought about in polymerization. These values are small compared to the total free energy and heat of formation of the biomass (5500 and 5400 cal per gram respectively). The efficiency of transfer of chemical energy from one molecule to another is ususaly high (over 50 %) and although the energy efficiency of the polymerization process is already much less

(about 20 %), it is assumed that no more than a negligible amount of ATP is spent in the last step of biomass synthesis, if any. This conclusion is extrapolated to formation of multicellular organisms from cells. Gorski (1966) ascribes to the specific arrangement of atoms in a mycelium a decrease in entropy with respect to the unorganized state of these molecules, which is small compared with the total entropy production during aerobic growth on a glucose substrate.

It may seem remarkable that organized biomass, containing much more biologically relevant information than a similar amount of unorganized polymers, has about the same free energy and heat of formation as the same polymers in an unorganized state. It should, however, be realized that the thermodynamical concept "order" refers to arrangements of atoms and molecules in a system, and is different from the "meaning" (or "information") which the cell and the biologist attributes to the same entity (Makkink, 1971). This is easily seen by comparing the invariant entropy of equal amounts of DNA of two different bacteria species with its information for the biochemical machinery, which produces two different organisms.

3.4. NON SYNTHETIC ACTIVITIES DURING BIOSYNTHESIS

The bulk flow of water through the plant is a passive process. Cell elongation and the build-up or maintenance of a turgor pressure is not accomplished by active water transport, but in response to active ion uptake, so that plant growth in this study can be limited to dry matter accumulation. Mechanisms for active transport of ions across cell membranes are not yet understood (Kaback, 1970). Data collected by Beevers (1961), Stein (1967) and Schoffeniels (1967) demonstrate that the passage of the outer cell membrane by one cation requires the energy of approximately 0.3 ATP molecule, while the anion follows passively. On the basis of the Mitchell chemi-osmotic hypothesis it may be expected (Lehninger, 1971) that per pair of protons transferred from $NADH_2$ to oxygen six positive charges can be imported into mitochondria. Experiments by Mitchell and Moyle (1968) with rat liver mitochondria support this hypothesis. It is likely, however, that in plasmalemma and tonoplast a different mechanim for ion uptake is active, since proton transfer to oxygen occurs only in mitochondria. The experimental value of 0.3 mole ATP per mole of cations imported will therefore be used rather than the theoretical value of 0.5 mole ATP per mole cations. Translocation into the vacuole is assumed to require an equal amount of energy.

Inorganic molecules are treated as one group, nitrate and sulphate excluded, of which the average molecular weight is 75. A more detailed consideration is not useful because of the small amount of energy involved in translocation and lack of information about the underlying processes.

Most of the volume of mature plant cells is in the vacuole and most of the ions are present in this cell organelle. It is assumed that 70 % of the inorganic molecules is located in the vacuole and 10 % in the plasma. An arbitrary figure of 20 % is taken to represent the inorganic cell wall incrustrations. However, the effect of this assumption on the result of the computations is very small.

Most of the import of organic molecules into cells is active (Kaback, 1970; Höfer, 1971; Payne and Gilvarg, 1971). The mechanisms of these processes are still uncertain. It is estimated from data of Beevers (1961) and Albers (1967) that the uptake of one carbohydrate molecule requires the energy of 1 molecule of ATP. This is in agreement with uptake of carbohydrates through temporary phosphorylation of the molecule, as suggested by Kaback (1970) and Oxender (1972).

Phagocytosis and pinocytosis are not likely to be a cheaper alternative for single molecule uptake, as the membrane enveloping the material transported has to be "digested" and replaced. But sometimes it is the only way for a cell to obtain its substrate, as for example for Paramecia consuming bacteria. In higher plants this uptake mechanism is probably not utilized because substrate molecules are always of low molecular weight. Here, substrates are often transported over many centimeters. Energy cost for this process will not be considered.

Heat is very seldom a main product of substrate degradation.

4. VARIABLES CHARACTERIZING BIOSYNTHETIS

Growth results from synthesis of mixtures of compounds. The amount of glucose and intermediates required for biosynthesis of plant dry matter may be calculated from its chemical composition, an example of which is given in table 3, using data of table 2. Table 4 represents the assumptions and conditions used for standardized calculations. Aromatic, secondary plant substances, hormones etc. are, depending on their chemical properties and analytical method used, found in one of the major fractions. This simplification causes only small errors due to the low quantities involved. The type and amount of excreted compounds should also be considered if micro-organisms are studied.

Table 3. The chemical composition of a young and vegetative maize plant. All fractions are expressed on a dry weight basis.

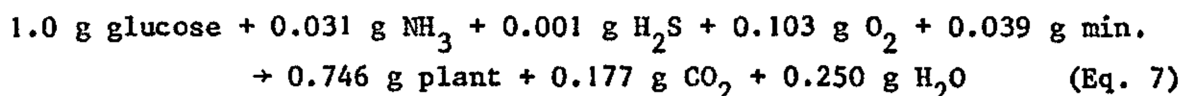
main fraction		subfractions	note
nitrogenous compounds	23 %	amino acids 10 % proteins 87 % nucleic acids 3 %	amino acid composition of zei (Handbook of Biological Data, 1956)
carbohydrates	56,5 %	ribose 1 % glucose 5 % fructose 2 % mannose 1 % galactose 1 % sucrose 5 % cellulose 40 % hemicellulose 40 % pectin 5 %	
lipids	2,5 %	glycerol-tripalmitate 12 % glycerol-tristearate 3 % glycerol-trioleate 47 % glycerol-trilinolate 33 % glycerol-trilinoleate 5 %	composition of maize oil (Winton and Winton, 1950)
lignin (nitrogen-free)	8 %		poly-coniferylalcohol, percentage based on Muller et.al. (1970)
organic acids	5 %	oxalic acid 5 % glyoxalic acid 5 % oxaloacetic acid 20 % malic acid 10 % citric acid 30 % aconitic acid 30 %	
minerals	5 %	potassium 80 % chloride 20 %	

Table 4. Standard conditions and assumptions used in computations

- chemical composition of biomass as given in table 3.
- all processes occur under fully aerobic conditions.
- polymerization of amino acids requires 3 ATP equivalents per peptide bond.
- enzyme and RNA maintenance during protein synthesis requires 1 molecule ATP per monomer. Other maintenance processes (maintenance of structures) are not considered.
- transport across the outer cell membrane requires 1 mole ATP per mole organic molecules and0.3 mole ATP per mole of inorganic molecules.
- intra-cellular translocation occurs spontaneously, except passage of the vacuole and mitochondria membranes; transport across these membranes is active and requires 1 mole ATP per mole organic molecules and 0.3 mole ATP per mole inorganic molecules.
- spatial compartmentation of only fatty acid synthesis (in mitochondria instead of hyaloplasm) and lignin precursors (formed in hyaloplasm but incorporated in cell wall); no temporal compartmentation of synthetic processes.
- glycolysis, tricarboxylic acid cycle and oxidative phosphorylation are used for $NADH_2$ and ATP production.
- P/O ratio for $NADH_2$ oxidation is 3.

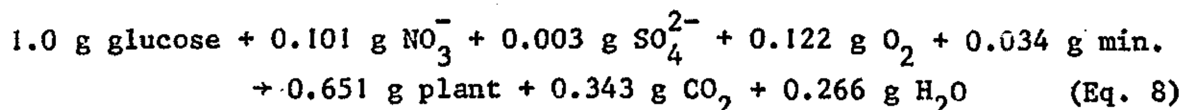
The reaction equation resulting from summation of the individual balances for all compounds may show an ATP and $NADH_2$ excess or shortage. Overproduction of $NADH_2$, which is often found, is met by its oxidation, usually generating ATP. A calculated excess of $NADH_2$ must be eliminated by its oxidation with oxygen, and not be reducing other compounds, because the experimentally determined chemical composition must be achieved. $NADH_2$ or ATP shortage is eliminated by additional substrate degradation. Only an excess of ATP can be eliminated without changing the reaction equation: the cell may hydrolyze the excess, or prevent the excess formation by uncoupling phosphorylation. Excessive formation of ATP may occur, but in most cases the ATP formed in synthetic processes is consumed completely in polymerization and translocation processes. The values characterizing a conversion process (pv, orf and cpf) can be applied fruitfully only to the total of processes, the total being defined as the sum of those processes which do not exchange ATP, $NADH_2$ or intermediates with their environment. Fig. 2a and b show that biosynthesis of protein or biomass from glucose with NH_3 causes an excess of energy, which evolved due to oxidation of the remaining $NADH_2$: at the (unrealistic) low values of energy consumption during polymerization the production value of the process does not decrease at increasing cost of polymerization.

The result of a synthetic process in which glucose with NH_3 and H_2S is converted into plant dry matter, with a chemical composition as given in table 3, can be described as



$$1.0 \text{ g glucose} + 0.031 \text{ g } NH_3 + 0.001 \text{ g } H_2S + 0.103 \text{ g } O_2 + 0.039 \text{ g min.} \rightarrow 0.746 \text{ g plant} + 0.177 \text{ g } CO_2 + 0.250 \text{ g } H_2O \qquad \text{(Eq. 7)}$$

With nitrate and sulphate the equation is

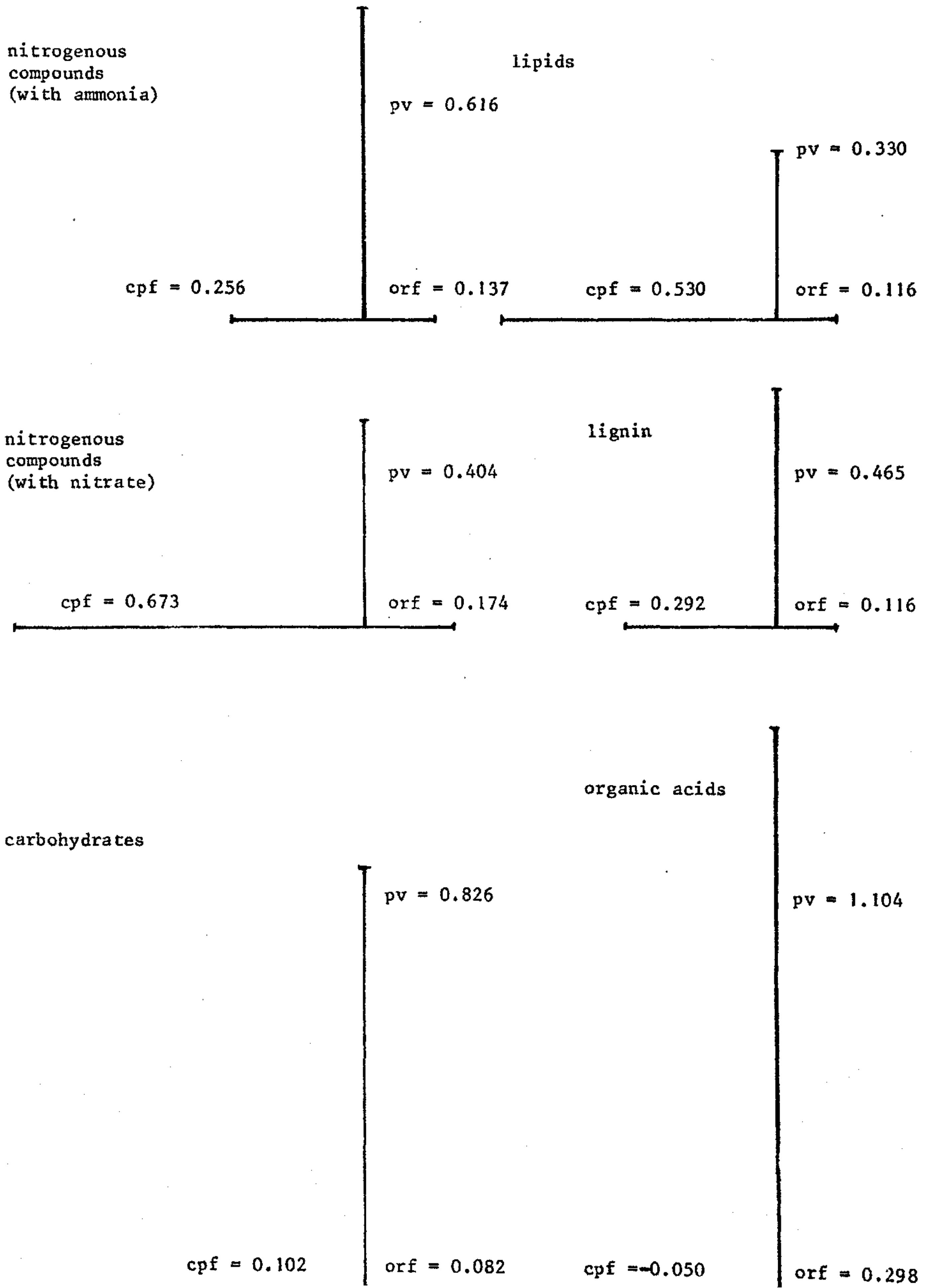


$$1.0 \text{ g glucose} + 0.101 \text{ g } NO_3^- + 0.003 \text{ g } SO_4^{2-} + 0.122 \text{ g } O_2 + 0.034 \text{ g min.} \rightarrow 0.651 \text{ g plant} + 0.343 \text{ g } CO_2 + 0.266 \text{ g } H_2O \qquad \text{(Eq. 8)}$$

Standard conditions, given in table 4, are used in calculations, unless specified otherwise. From these reaction balances it follows that pv for synthesis of plant material from glucose and NH_3 and H_2S is 0.746, and 0.651 if nitrogen and sulphur are supplied as NO_3^- and SO_4^{2-}. The values of orf and cpf are 0.103, 0.122, 0.177 and 0.343 respectively. In the conversion equations the sum of the weights of the substrates is, of course, equal to the sum of the weights of product and by-products.

In fig. 3 are represented the variables characterizing biosynthesis, pv, orf and cpf, of nitrogenous compounds, carbohydrates, lipids, lignin

Figure 3. The variables characterizing biosynthesis of nitrogenous compounds with ammonia and with nitrate, of carbohydrates, of lipids, of lignine and of organic acids.

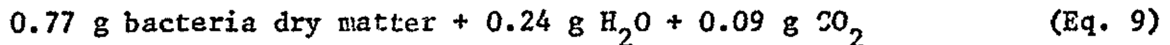


and organic acids as formed from glucose. It is not surprisingly that important differences exist between these fractions. The slightly negative value of cpf during formation of organic acids is caused by carboxylation of pyruvic acid.

Morowitz (1968) calculated that if

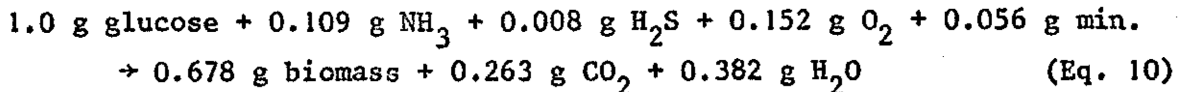
$$1.00 \text{ g glucose} + 0.12 \text{ g } NH_3 + 0.02 \text{ g } H_2SO_4 + 0.05 \text{ g } H_3PO_4$$

were converted into



$$0.77 \text{ g bacteria dry matter} + 0.24 \text{ g } H_2O + 0.09 \text{ g } CO_2 \qquad \text{(Eq. 9)}$$

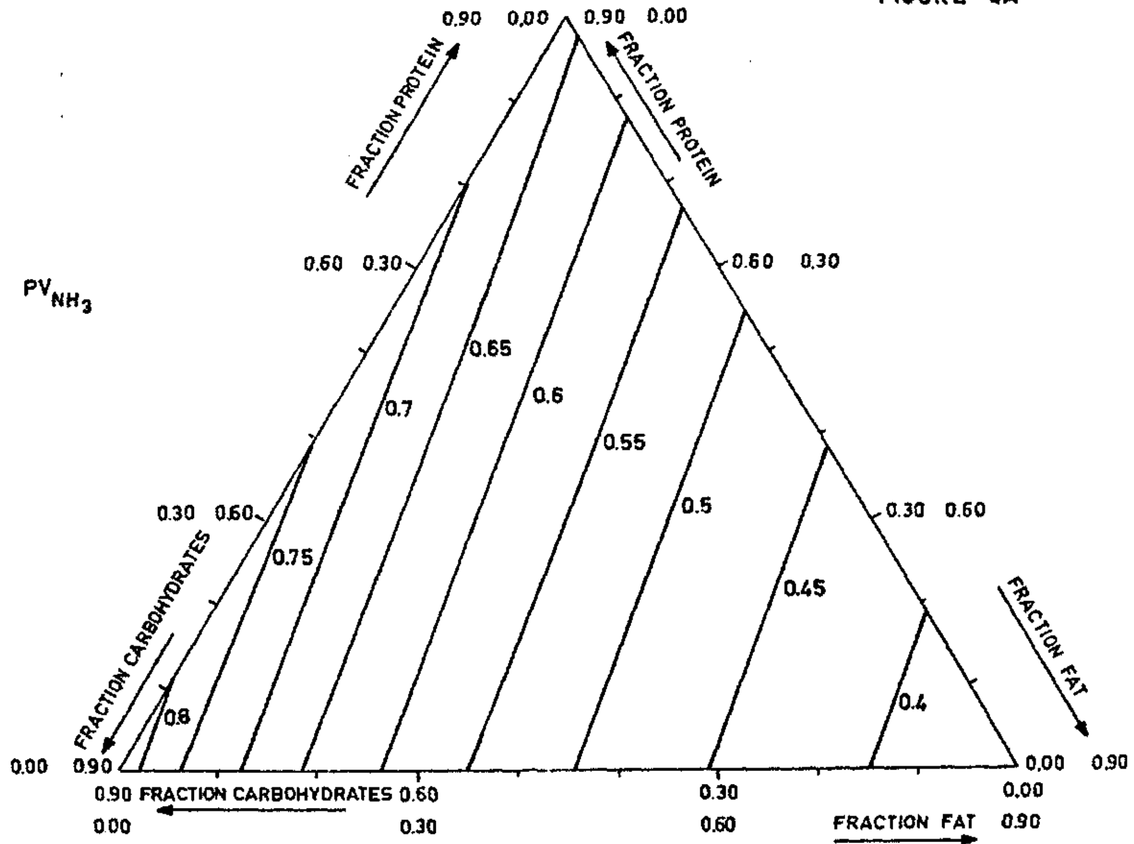
both the change in free energy of formation and the change in heat of formation of this system would be negative, showing that there is heat and entropy production. Since Morowitz's calculations neglect the energy spent in non-synthetic processes, his equation overestimates the conversion efficiency. The biosynthesis equation computed according to the procedure described above for bacteria with a similar elementary composition is



$$1.0 \text{ g glucose} + 0.109 \text{ g } NH_3 + 0.008 \text{ g } H_2S + 0.152 \text{ g } O_2 + 0.056 \text{ g min.}$$
$$\rightarrow 0.678 \text{ g biomass} + 0.263 \text{ g } CO_2 + 0.382 \text{ g } H_2O \qquad \text{(Eq. 10)}$$

Equation (10) yields less biomass than (9), thus more substrate is oxidized to produce energy and more heat is lost. It can be concluded that the result of the computation presented is not in conflict with the laws of thermodynamics. The difference between equations (10) and (7) is mainly caused by the high content of nitrogenous compounds in these bacteria (64 %).

The figures 4 a-f demonstrate the effect of various chemical compositions of synthesized dry matter on the values characterizing the conversion. In these triangular plots all combinations of three components can be indicated. Because the organism consists of six main fractions, a simplification is necessary to plot in this format. The fractions of nitrogenous compounds, fats and carbohydrates will be considered; 10 % of the weight of the carbohydrate fraction is taken to be lignin and organic acids and minerals are each assumed to be 5 % of the total biomass weight in all cases. In this way these triangles roughly cover many of the chemical compositions found in tissues. Figures 4 a-c show the conversion characteristics when ammonia and hydrogen sulfide are supplied with glucose, and figures 4 d-f when nitrate and sulphate are given. The lines in figures 4a and d, b and e, and c and f connect points representing those chemical compositions which have equal values of pv, cpf and orf, respectively. It can be seen from these figures that fats are the most expensive compounds to produce from



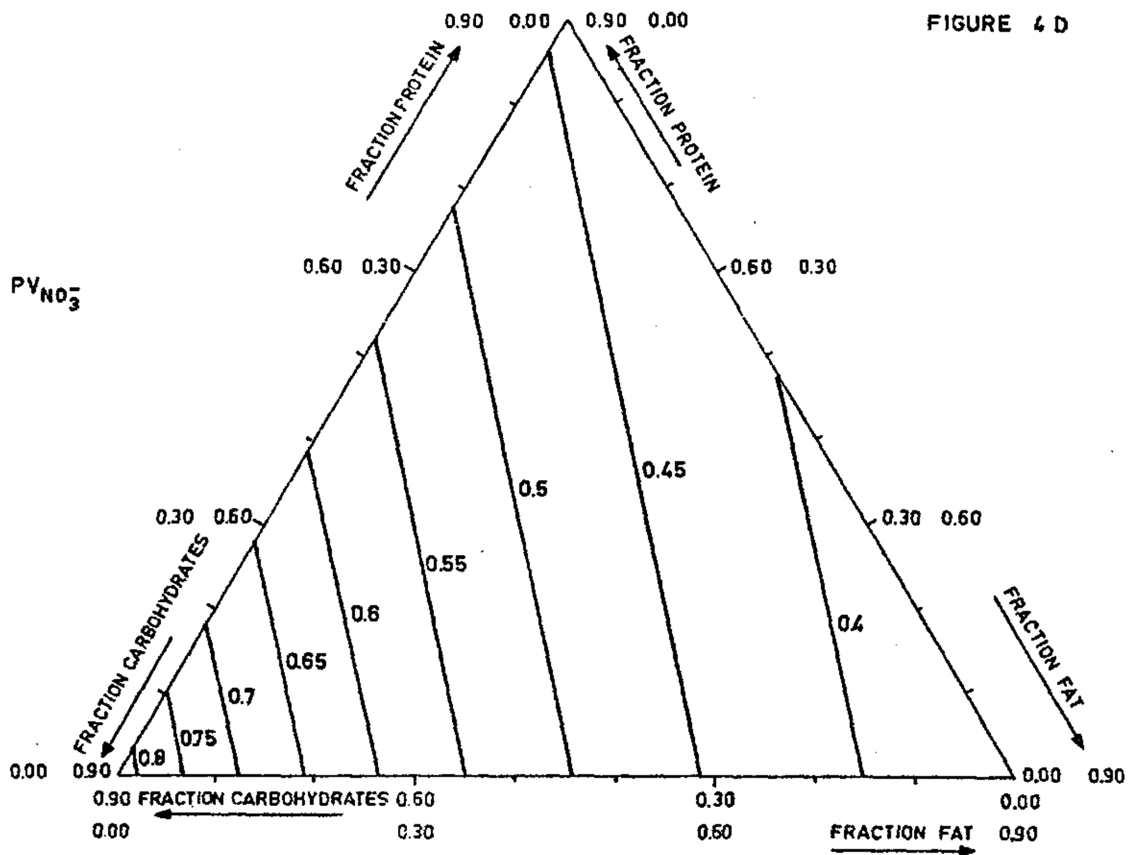
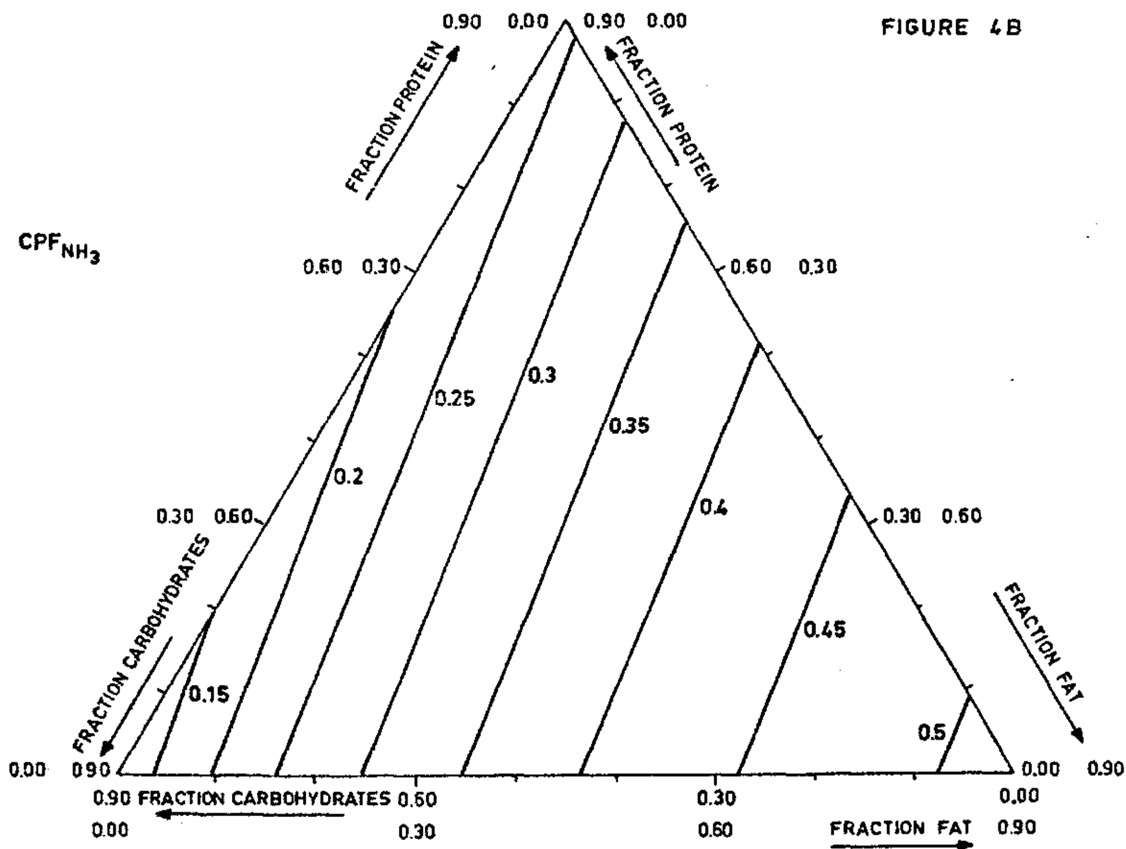


Figure 4 a and d. The production values (pv) of biosynthesis of biomass with various chemical compositions from glucose with ammonia (4a) and nitrate (4d). For further explanation see text.



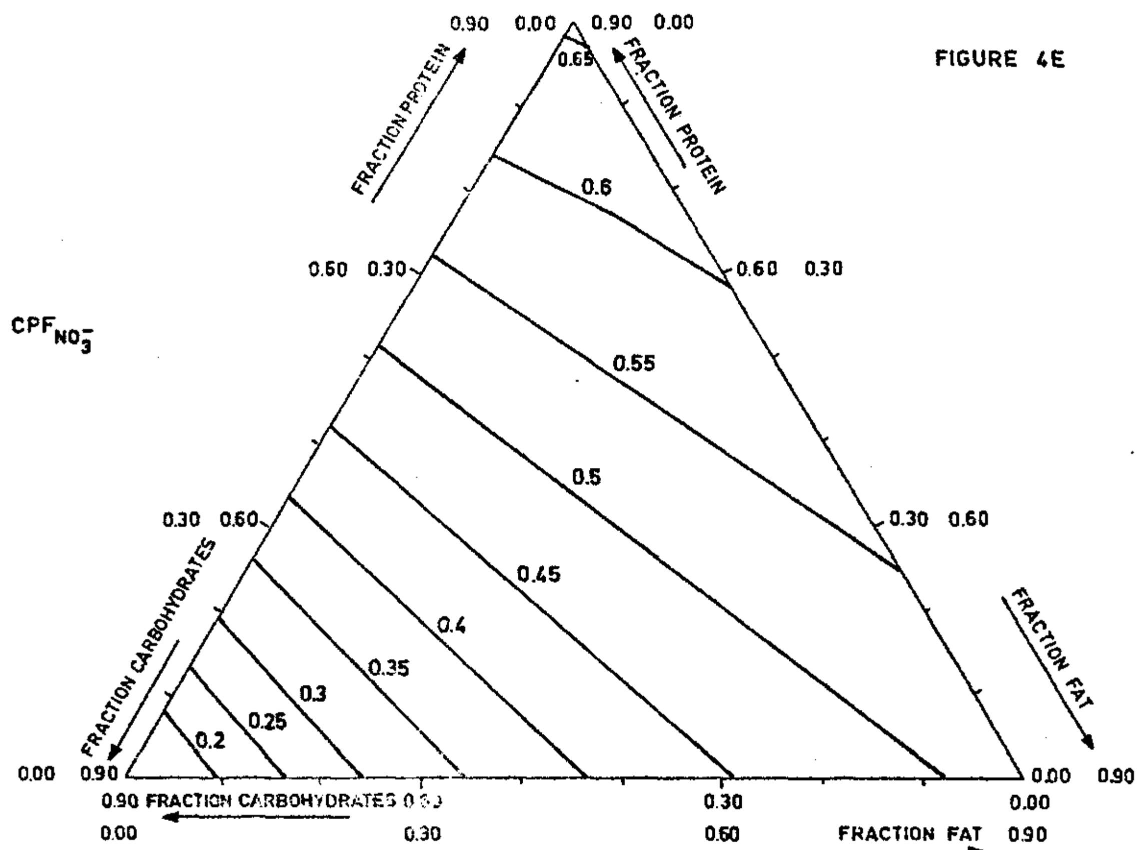
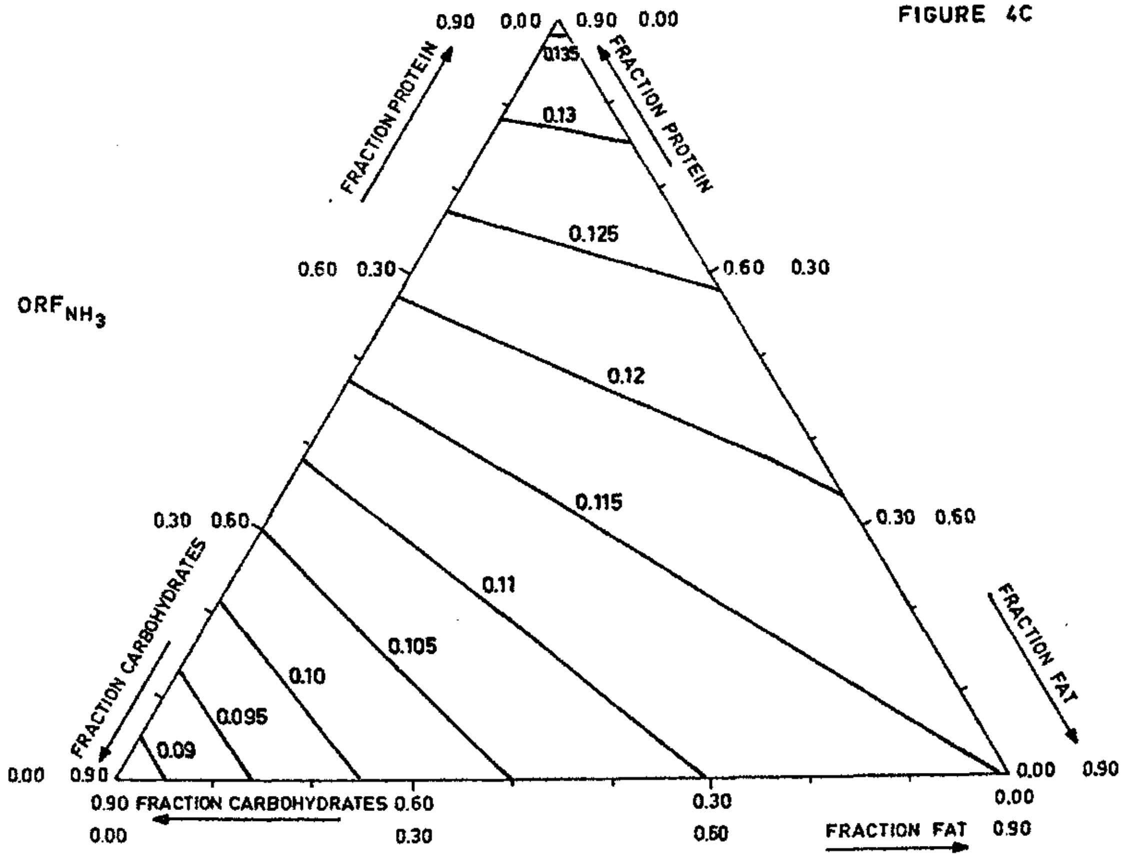


Figure 4 b and e. The carbon dioxide production factors (cpf) of biosynthesis of biomass with various chemical compositions from glucose with ammonia (4b) and nitrate (4e). For further explanation see text.



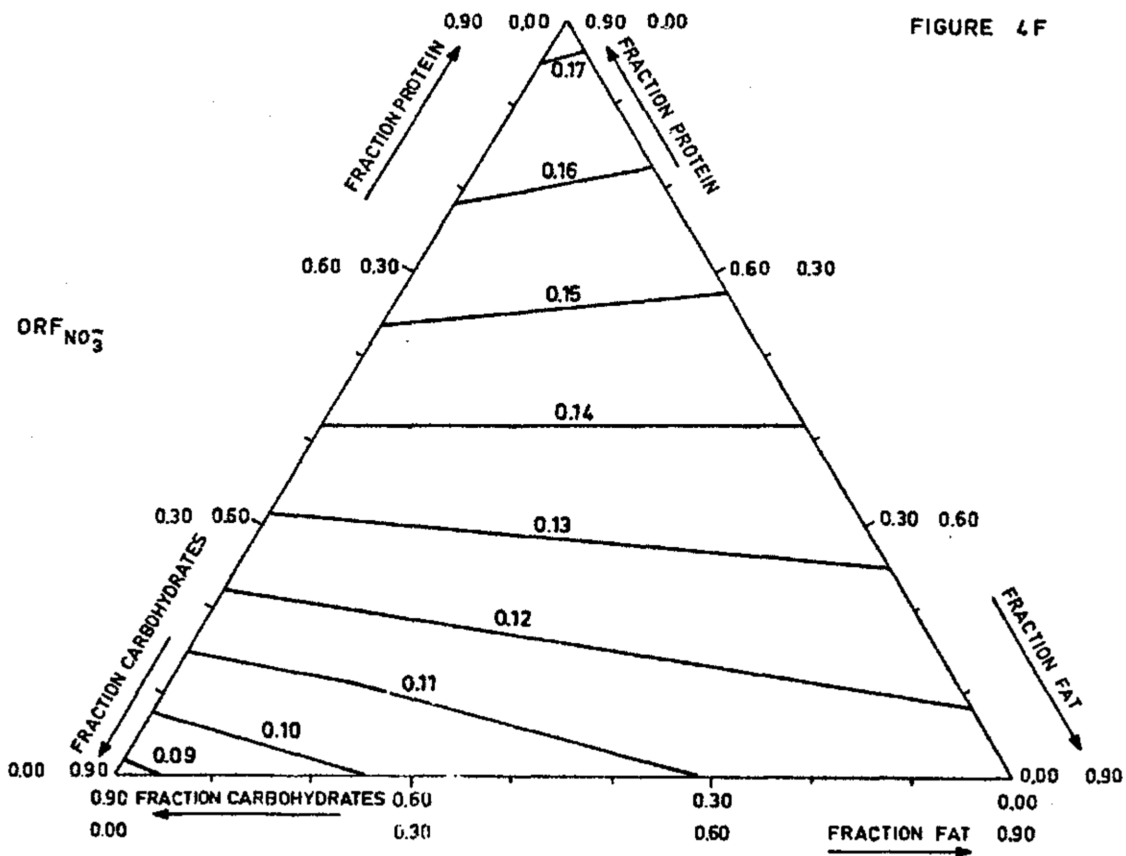
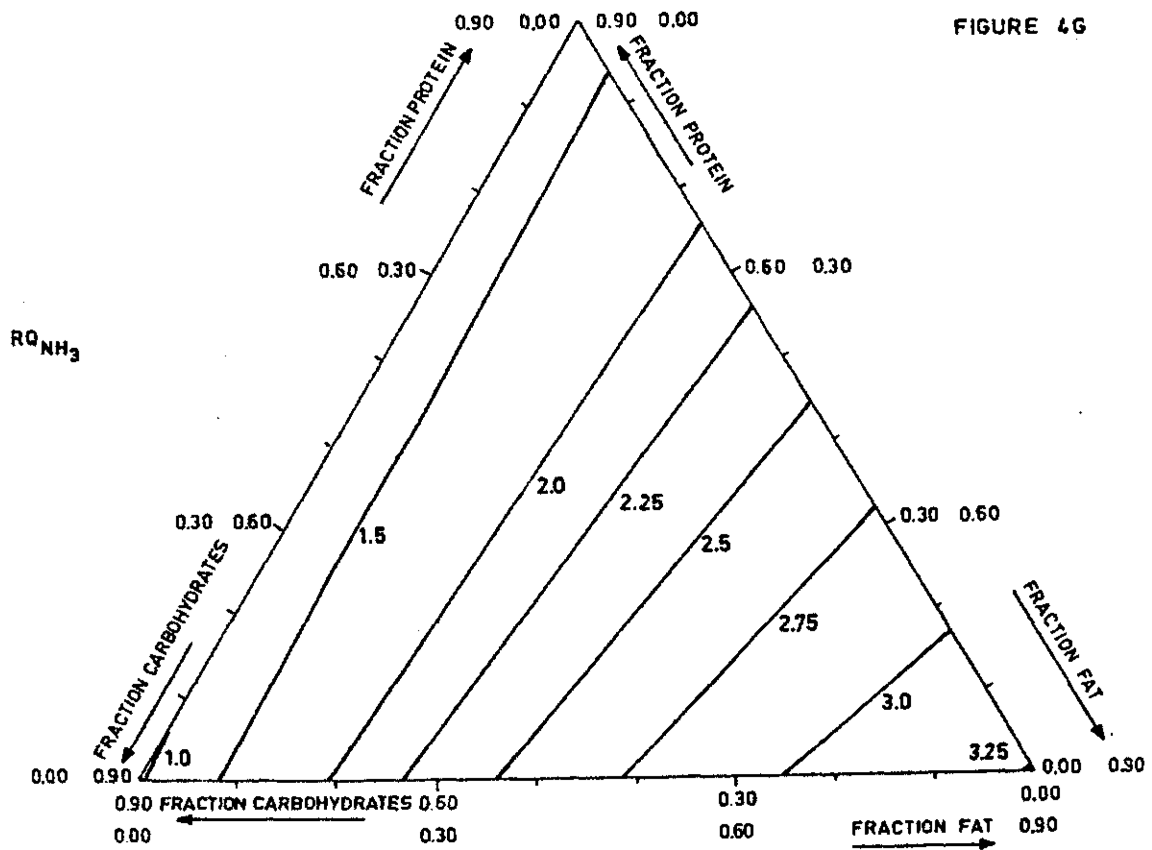


Figure 4 c and f. The oxygen requirement factors (orf) of biosynthesis of biomass with various chemical compositions from glucose with ammonia (4c) and nitrate (4f). For further explanation see text.



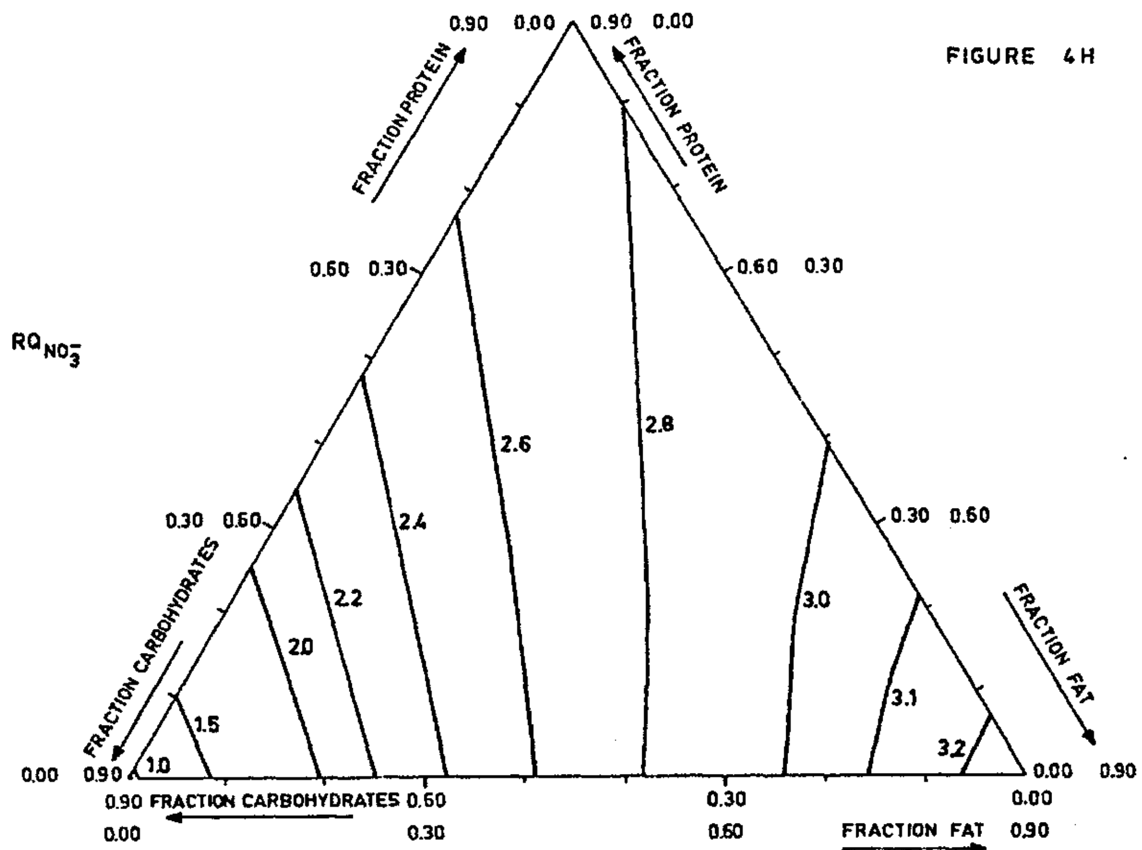


Figure 4 g and h. The respiratory quotients (RQ) of biosynthesis of biomass from glucose with ammonia (4g) and nitrate (4h). For further explanation see text.

glucose, and that protein synthesis yields only slightly more when nitrate reduction occurs. If reduced nitrogen is available pv for protein synthesis is about 50 % higher. The carbohydrate-lignin mixture is relatively "cheap" to synthesize. Fig. 4 g and h show the respiratory quotient (moles CO_2 produced per mole O_2 consumed) for these conversion processes.

Fig. 5 indicates the area of chemical compositions usually found in vegetative plant tissue, reproductive plant tissue, animals, and micro-organisms and can be used in combination with fig. 4 a-h.

It is evident that pv, cpf and orf for those combinations of fractions in which no nitrogen is incorporated must have similar points in both sets of figures. As a result of this, the lines in the fig. 4 d, e, f and h representing the values for the glucose plus nitrate substrate, are turned to the left around their fixed points on the horizontal axis, as compared to the glucose plus ammonia substrate values (fig. 4 a, b, c and g). Most lines in the figures prove to be nearly parallel and approximately straight. Straight lines indicate that there are no intermediates formed during synthesis of one compound and utilized in the synthesis process of another compound. Such a form of "biochemical symbiosis" would increase the efficiency of the overall process and cause the lines to curve: it increases pv for a given chemical composition above that what might be expected in using straight lines, and similarly decreases orf and cpf.

Applying the detailed computation described above to tissues with different chemical compositions is very laborious and difficult to transmit. A computer program has therefore been developed. It is written in the simulation language Continuous System Modeling Program and was also translated into FORTRAN. A copy is available on request.

Because of lack of data, the chemical composition within each of the major fractions (table 3) is often taken to be constant and only the relative contribution of each fraction to the total varying. Fortunately the results of the calculations are affected only to a small extend by changes in composition within these fractions. It was calculated for example that the variables characterizing the conversion of glucose into 12 different proteins show a much narrower range (table 5) than the variables calculated for the major fractions (fig. 3). Therefore, a much simpler way of calculating the variables characterizing the conversion can often be used. The process of converting glucose into one of the fractions may then be characterized by pv', cpf', orf' and two additional factors: the hydrogen requirement factor (hrf) and the energy requirement factor (erf). The first

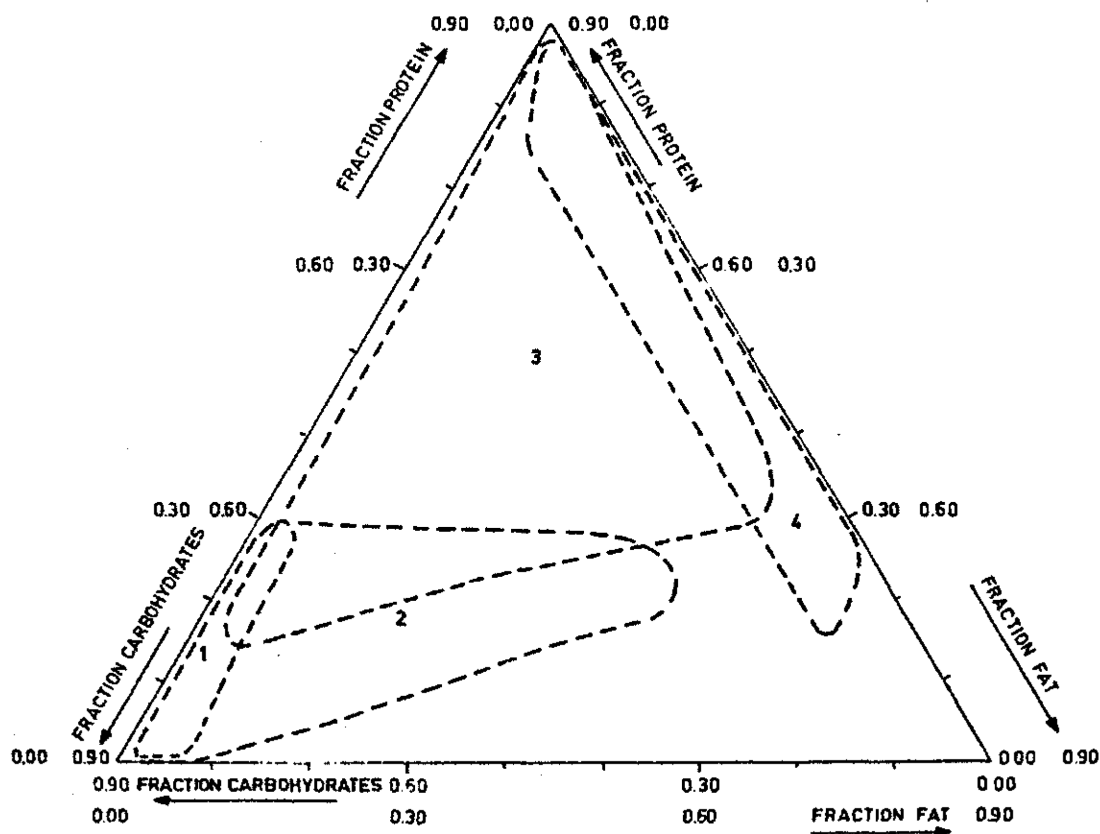


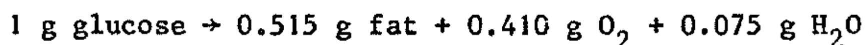
Figure 5. The variation in chemical composition within and between vegetative, non storage tissues of higher plants (area 1), seeds and storage tissues of higher plants (2), micro-organisms (3) and animals (4). 10 % of the biomass is assumed to consist of minerals plus organic acids. The composition of individual organs can vary over wider ranges. Based on data from the Handbook of Biological Data (1956) and Morowitz (1968).

Table 5. Values characterizing the conversion process of glucose with ammonia or nitrate into proteins with various amino acid compositions; the compositions were taken from the Handbook of Biological Data, 1956.

protein	with ammonia			with nitrate		
	pv	orf	cpf	pv	orf	cpf
albumin, egg	0.621	0.157	0.260	0.398	0.190	0.694
" , human serum	0.623	0.152	0.252	0.398	0.187	0.691
arachin	0.611	0.163	0.263	0.386	0.192	0.705
bacteriophage of E. coli	0.636	0.172	0.253	0.399	0.198	0.705
edestin	0.656	0.168	0.228	0.400	0.198	0.712
gliadin	0.638	0.158	0.233	0.431	0.184	0.633
gluten, corn	0.588	0.146	0.284	0.404	0.174	0.653
" , wheat	0.664	0.163	0.217	0.426	0.194	0.665
insulin	0.626	0.164	0.247	0.402	0.196	0.683
papilloma, shope (virus)	0.635	0.166	0.254	0.397	0.198	0.707
ribonuclease	0.673	0.183	0.233	0.413	0.210	0.710
zein	0.594	0.144	0.271	0.393	0.179	0.676

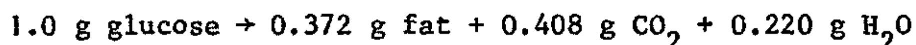
three were defined earlier (table 1); the prime indicates that the values refer to weight ratios at the stage at which all material is formed from glucose, but at which excess or shortage of $NADH_2$ and ATP has not yet been removed. Hrf and erf represent the number of moles of $NADH_2$ and ATP per gram of product, respectively, that have to be formed (value positive), or are released in excess during synthesis. Hrf and erf can link synthetic processes of different fractions and must be made zero at the end of the calculation: pv', cpf' and orf' therefore are intermediate values. Table 6 presents their values for the major fractions. They must be multiplied by the part that their fraction forms of the total, and then added together. The energy required for the non-synthetic activities must be added to erf, and the added values of the energy and hydrogen requirement (erf and hrf) are adjusted to zero as described above. Table 7 gives an example of this calculation. Slight mistakes are introduced because C_1-fragments and other intermediates may be exchanged between the synthetic processes of different chemical fractions. This is ignored in the simpler approach.

From fig. 3 it follows that synthesis of fat, composed as indicated in table 3, from glucose can be characterized by a production value of 0.330. From the "molecular formula" of this fat, $C_{94}H_{165}O_{10}$, the equation



$$1 \text{ g glucose} \rightarrow 0.515 \text{ g fat} + 0.410 \text{ g } O_2 + 0.075 \text{ g } H_2O$$

can be derived (James, 1953) assuming that oxygen is formed. If it is assumed that oxygen is neither released nor consumed the equation becomes



$$1.0 \text{ g glucose} \rightarrow 0.372 \text{ g fat} + 0.408 \text{ g } CO_2 + 0.220 \text{ g } H_2O$$

It is obvious that these very simple calculations ignore much of what biochemistry teaches, and gives errorreously high estimates of conversion efficiencies. On the other hand it appears that application of only superficial knowledge of end-product and substrate composition can provide a useful first estimate of the yield of conversion processes.

5. MODIFICATIONS OF VARIABLES CHARACTERIZING A BIOSYNTHETIC PROCESS INDUCED BY CHANGES IN CONDITIONS

Aspects of biosynthesis requiring additional consideration are the compartmentation of biochemical processes, the fact that different species can utilize different biochemical pathways to achieve the same end-product, and the possible effects of changes in internal variables such as the P/O ratio, or in external variables like temperature.

Table 6. Auxillary values for characterization of a conversion process, excluding cost of substrate intake from the environment. For the composition of the fraction "nitrogenous compounds" see table 3.

	pv'	orf'	cpf'	hrf	erf
amino acids with ammonia	0.700	0.0054	0.254	-0.01122	-0.00139
amino acids with nitrate	0.700	0.0054	0.254	0.02674	0.03899
protein with ammonia	0.604	0.0052	0.252	-0.01285	0.03492
protein with nitrate	0.604	0.0052	0.252	0.03140	0.08197
nucleic acids with ammonia	1.072	0.0270	0.043	-0.01242	0.02793
nucleic acids with nitrate	1.072	0.0270	0.043	0.03484	0.07732
nitrogenous compounds with ammonia	0.620	0.0056	0.249	-0.01267	0.03108
nitrogenous compounds with nitrate	0.620	0.0056	0.249	0.03104	0.07754
carbohydrates	0.853	0.0	0.057	-0.00360	0.01224
lipids	0.351	0.0	0.471	-0.01010	0.05097
lignin	0.483	0.0444	0.244	-0.00431	0.01868
organic acids	1.104	0.0	-0.050	-0.01686	-0.00452

Table 7. Example of a simplified calculation of the variables characterizing biosynthesis of biomass.
Substrate: glucose plus nitrate plus minerals.

compound	g per g biomass	pv'	gram glucose required for synthesis	orf'	gram oxygen required for synthesis	cpf'	gram CO_2 produced during synthesis	hrf	moles $NADH_2$ required for synthesis	erf	moles ATP required for synthesis
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
nitrogenous compounds	0.23	0.620	0.371	0.0056	0.00208	0.249	0.0924	0.03104	0.00714	0.07754	0.01783
carbohydrates	0.565	0.853	0.662	0.0	0.0	0.057	0.0377	-0.00360	-0.00203	0.01224	0.00692
lipids	0.025	0.351	0.071	0.0	0.0	0.471	0.0335	-0.01010	-0.00025	0.05097	0.00127
lignin	0.08	0.483	0.166	0.0444	0.00735	0.244	0.0404	-0.00431	-0.00034	0.01868	0.00150
organic acids	0.05	1.104	0.045	0.0	0.0	-0.050	-0.0023	-0.01686	-0.00084	-0.00452	-0.00023
minerals	0.05	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0
total	1.00		1.315		0.00943		0.2017		0.00368		0.02729

0.0552 g glucose $\rightarrow$ 0.00368 mole $NADH_2$ + 0.00061 mole ATP + 0.0810 g CO_2

(1.315 + 0.0552) g glucose and 0.05 g minerals must be imported, which requires 0.00761 + 0.00032 = 0.00793 mole ATP

0.168 g glucose** + 0.180 g O_2 $\rightarrow$ (0.02729 + 0.00793 - 0.00061) mole ATP + 0.247 g CO_2

Total glucose requirement: 1.315 + 0.0552 + 0.168 = 1.539 — pv = 0.650

Total oxygen requirement : 0.00943 + 0.180 = 0.188 — orf = 0.122

Total carbon dioxide production: 0.2017 + 0.0810 + 0.247 = 0.530 — cpf = 0.344

column (4) = (2) / (3)
(6) = (2) x (5) / (3)
(8) = (2) x (7) / (3)
(10) = (2) x (9)
(12) = (2) x (11)

** assuming that 1 mole glucose produces 38 mole ATP, of which 1 is used in its own uptake

5.1. COMPARTMENTATION

Compartmentation has two aspects: the place of synthesis of a compound may be different from the place of "destination", and an excess of ATP, $NADH_2$ or an intermediate that is produced in excess in one place may not be available for a demand elsewhere. Two examples of the first aspect can be given. The cell wall consists mainly of polysaccharides, but often also includes lignin and proteins. The cell wall is situated outside the cytoplasm, so that its building blocks are either excreted, or activated and polymerized on the outside of the membrane enveloping the cytoplasm. It is assumed that all lignin molecules are synthesized in the cytoplasm and excreted at a cost of 1 ATP molecule per coniferyl-alcohol monomer; that no protein is excreted, and that 50 % of the cell polysaccharides is formed from glucose that did not enter the cytoplasm, while the other 50 % is formed and remains in the cytoplasm.

The majority of synthetic processes occur in the hyaloplasm (the cell plasma, embedding the cell organelles), while some occur in the mitochondria, most of which is fatty acids synthesis (Bielka, 1969). Without much evidence it is assumed that only fatty acids are synthesized in mitochondria and are translocated into the hyaloplasm, and that all other events occur in the hyaloplasm. 1 ATP molecule may be required to translocate 1 fatty acid molecule from the mitochondrion into the plasma. Only these two cases of compartmentation are included in the set of standard conditions for calculations (table 4). It must be realized, however, that these refinements decrease pv only by about 1 %.

The second aspect of compartmentation concerns synthesis in isolated cell compartments or at different times, so that exchange of intermediates with other processes or transport of the end-product does not occur. If synthesis of different compounds occurs with spatial or temporal separation while no exchange of energy of hydrogen takes place the values of erf and hrf must be made zero before adding pv', orf' and cpf', thereby decreasing the yield and efficiency of the overall process.

Separation of the $NADH_2$ pool in hyaloplasm and mitochondria is known to exist and to be effective. However, by transferring H_2 from $NADH_2$ to oxalo-acetate in mitochondria a compound is formed (malate) which passes the membrane easily, while in the hyaloplasm the hydrogen can be transferred to NAD and the oxalo-acetate formed diffuses back to the mitochondria (Bielka, 1969). A system where exchange is facilitated by carriers without supply of additional energy can be regarded as free exchange. Compartmentation

in this context must therefore be defined as the separation of spaces between which no passive transport takes place. The size of compartments is not equal for all molecules. For water molecules the compartment exceeds the cell, for pyruvate and malate the hyaloplasm and mitochondria are equivalent, while for many molecules the hyaloplasm, vacuole and mitochondria are separate compartments.

Separation of processes in time occurs commonly. Secundary cell wall thickening in plant cells, for instance, occurs in cells with "fully grown" protoplasma, and also lipids or aromatics are synthesized mainly in mature cells.

It has been suggested (Lardy and Ferguson, 1969) that cells store energy as osmotic energy by accumulation of ions in mitochondria and that this may become available as ATP in a reverse process. Such a process can link energy-yielding and energy-consuming reactions separated in time, but because of the small volume of mitochondria, it is probably unimportant for the yield of biosynthetic processes.

The values of pv, orf and cpf for synthesis of standard plant biomass and bacteria are shown in table 8, assuming that the six major fractions either do or do not exchange energy and hydrogen during their synthesis. This aspect of compartmentation proves to be unimportant for biosynthesis of plants and bacteria when nitrate is the nitrogen source, but noticable if ammonia is supplied.

5.2. ALTERNATIVE PATHWAYS

The similarity between anabolic processes in organisms is appreciable and facilitates calculations enormously. In some cases, however, different species use different pathways to degrade or synthesize the same product, (Dagley and Nicholson, 1970). The difference between both may be small, as for ornithine (table 2) or large. For an example of the latter, oxalo-acetic acid, an intermediate in many reactions, may be formed by glycolysis and the glyoxylate cycle, yielding:

1 glucose + 3 H_2O → 1 oxalo acetic acid + 2 CO_2 + 2 ATP + 7 $NADH_2$

(table 2, pathways 140 and 508), or by the carboxylation of pyruvate, yielding:

0.5 glucose + CO_2 → 1 oxalo acetic acid + 1 $NADH_2$

(table 2, pathways 140 and 505). The amount of substrates differ by a factor 2. Synthesis via carboxylation releases little $NADH_2$, whereas synthesis via other pathways yields considerably more. The excess of $NADH_2$ will generally

Table 8. The effect of compartmentation of biosynthesis of plant dry matter and bacteria biomass. For the chemical composition of plant biomass and of the fractions see table 3. The composition of bacteria was assumed to be 0.61 g/g proteins, 0.17 g/g nucleic acic 0.12 g/g carbohydrates, 0.07 g/g lipids and 0.03 g/g minerals. For further explanation see text.

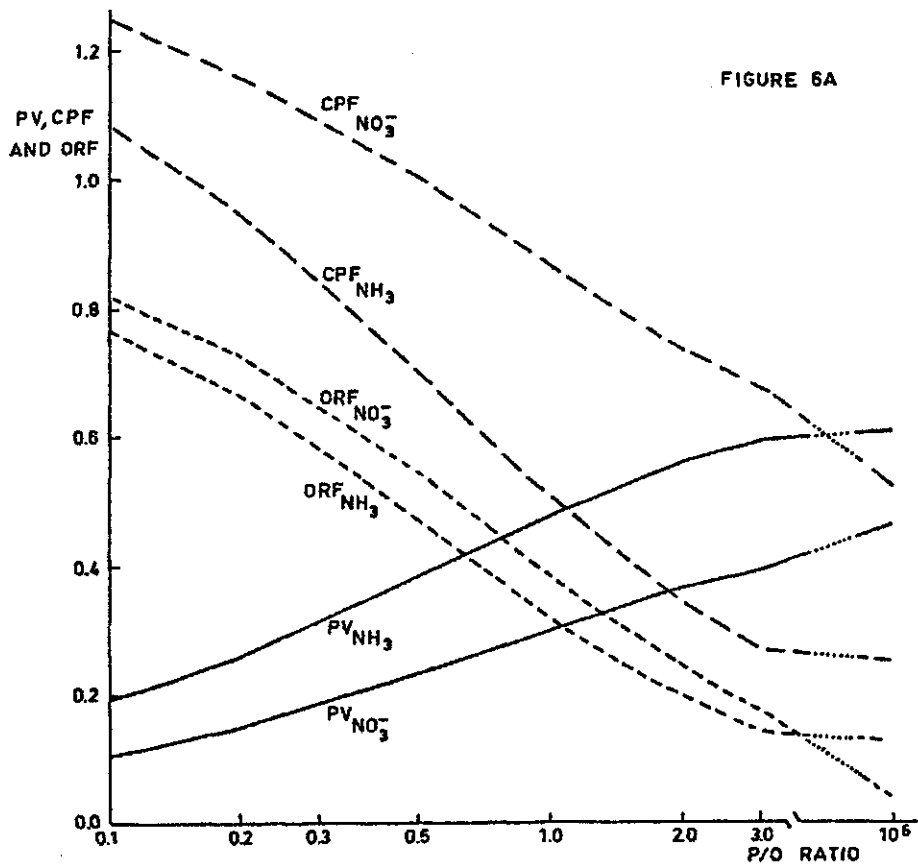
	pv	orf	cpf	compartmentation
glucose + ammonia → plant biomass	0.746	0.103	0.179	no
glucose + ammonia → plant biomass	0.735	0.116	0.197	yes
glucose + nitrate → plant biomass	0.650	0.123	0.343	no
glucose + nitrate → plant biomass	0.645	0.130	0.352	yes
glucose + ammonia → bacteria biomass	0.651	0.143	0.266	no
glucose + ammonia → bacteria biomass	0.644	0.154	0.280	yes
glucose + nitrate → bacteria biomass	0.449	0.177	0.637	no
glucose + nitrate → bacteria biomass	0.448	0.177	0.638	yes

be used in other reactions, which spares other substrate molecules from being oxidized. 0.5 glucose molecule releases in combustion (glycolysis and TCA cycle) 6 $NADH_2$ and 1 ATP molecules, so that the difference between both pathways is only 1 ATP. Thus, if the overall process requires more ATP or $NADH_2$ than is released during synthesis the choice between pathways 508 and 506 (table 2) hardly affects the pv of the process. If, however, all oxalo-acetic acid is formed via the first pathway and much of it is synthesized an $NADH_2$ overproduction may occur. Without further evidence it is assumed that the cell regulates its metabolism in such a way that it switches processes to the "cheapest pathway", if only little of the energy excess can be used elsewhere. A regulation of the activity of synthetic pathways according to the needs of the cell was suggested by experiments of Brown and Wittenberger (1971) and may operate via the "energy charge" of the cell (the sum of the high energy phosphate bonds: 2 in ATP and 1 in ADP, see Atkinson, 1968) or the $NADH_2$/NAD ratio. On the assumption that a mechanism of this kind exists, the simplest way to account for it is to calculate as though the "cheapest" pathway is always taken, unless there is counter evidence.

In conclusion it can be stated that the effects of using alternative pathways on the values characterizing the conversion are generally small. Because of the similarity of metabolism in various kinds of living organisms it is likely that the conversion characterizing values do not depend on the species considered.

5.3. EFFECT OF THE P/O RATIO ON PV, CPF AND ORF

For aerobic growth, with which this paper is concerned, the number of ATP molecules formed per molecule $NADH_2$ oxidized (the P/O ratio) and per molecule of glucose is important. These values have been taken to be 3 and 38, respectively. When the P/O ratio is altered the amount of substrate required for ATP production changes. Fig. 6a represents the relationship between the values characterizing the conversion of glucose with nitrate or ammonia into "standard protein" with the P/O ratio as variable, and fig. 6b represents the same for growth of "standard plant dry matter". A semi logarithmic scale was used to obtain higher legibility. Values at the unrealistic P/O ratio of 10^6 are included to show how much substrate is used for energy production at the various P/O ratios.



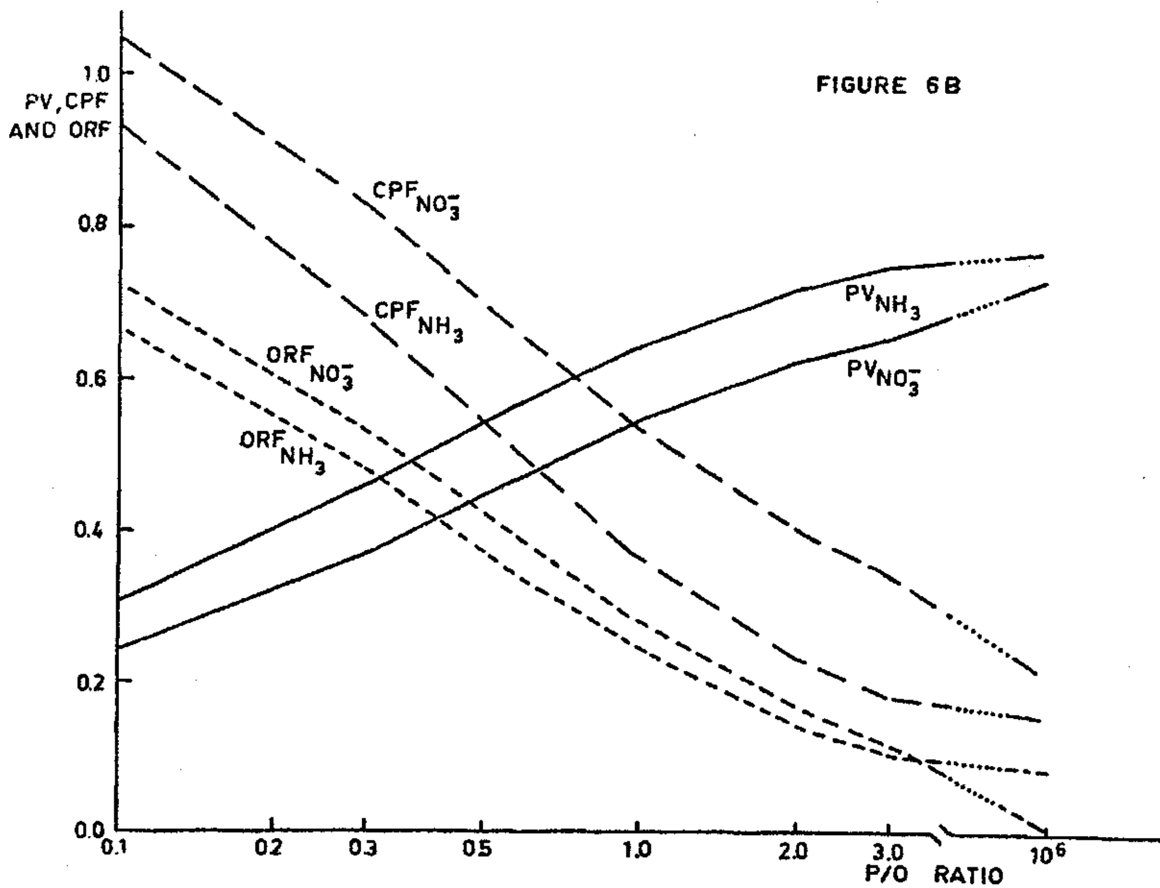


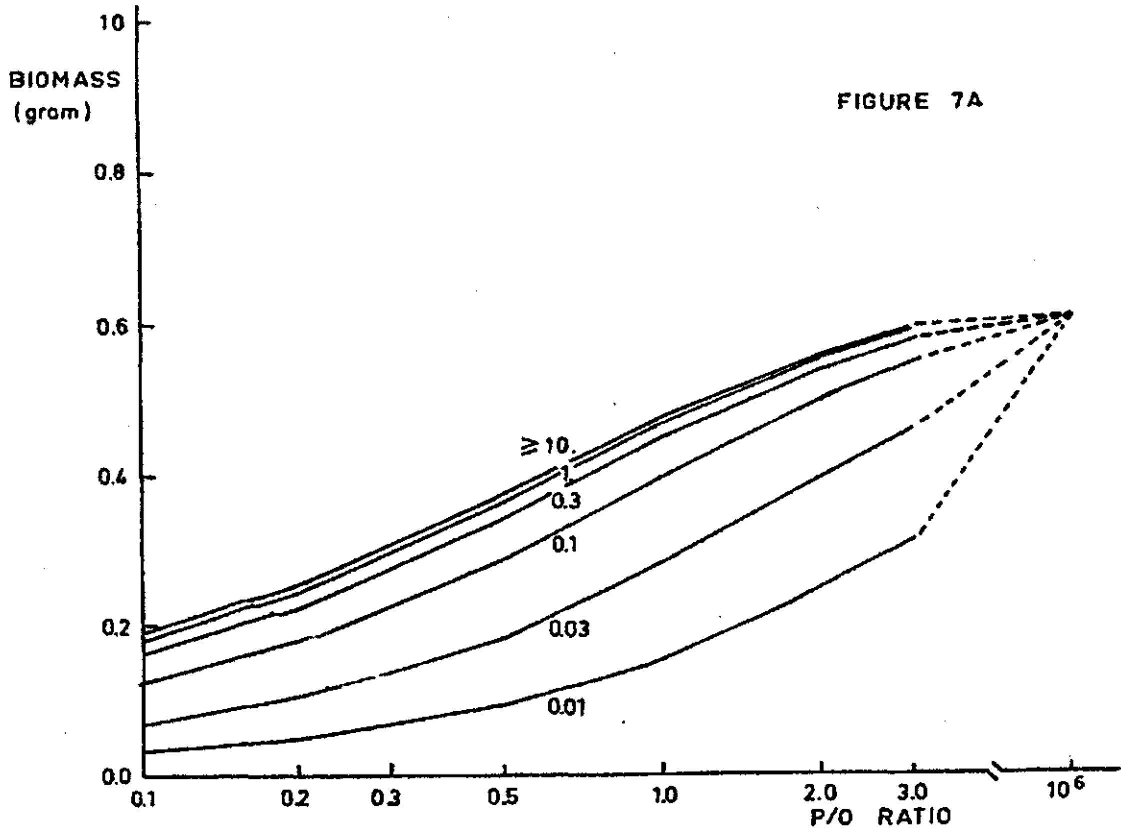
Figure 6 a and b. Conversion characteristics for protein biosynthesis (6a) and biomass biosynthesis (6b) from glucose at various P/O ratios.

From fig. 6b it appears that the pv of biosynthesis of "standard plant biomass" is hardly affected by the P/O ratio between 2 and 3. The efficiency of substrate consumption for processes like transport and maintenance is proportional to the P/O ratio. It may therefore be expected that although in rapidly growing plants the effect of a change in the P/O ratio is small, it is of increasing importance with decreasing relative growth rates. Fig. 7a represents the effect of the P/O ratio on the amount of protein, and fig. 7b the effect on the amount of "standard plant dry matter" formed at various relative growth rates, assuming an energy consumption of 0.00317 gmol ATP (0.015 g glucose) per gram dry matter per day for structure maintenance (cf. Penning de Vries, 1974b). McDaniel (1969) and others attributed a large heterosis effect in seedlings to an improvement of the P/O ratio from 2.0 to 2.5. This increase was questioned by Ellis et al. (1973), but even if it is real, fig. 7b indicates that it is improbable that such a small increase in P/O ratio is directly responsible for the yield increase observed.

Low P/O ratios are commonly reported for cell free extracts of micro-organisms (Stouthamer, 1969; Van Meyenburg, 1969), but measurements in living cells yields values of approximately 3 (Hadjipetrou et al., 1964; Hempfling, 1970; De Vries et al., 1970). In higher animals and in plant tissues the P/O ratio is mostly found to be 3 (Beevers, 1961; Pullman and Schatz, 1967). Under certain conditions cells can produce more ATP or $NADH_2$ than they consume, for instance during rapid breakdown of fatty acids in cotyledons of germinating peanuts. Under these conditions the cell must limit ATP production, resulting in a lowering of the P/O ratio.

5.4. TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS

There is no evidence that the P/O ratio is changed or that alternative synthetic pathways are utilized at different temperatures, or under different levels of water stress. Thus pv, cpf and orf are taken as independent of temperature and water stress over the range of temperature and tissue water potentials normally encountered. Experimental evidence has been presented (Penning de Vries, 1972, 1974a) that, in germinating seeds and growing plants, temperature does not affect the rate of conversion of substrate into end-product, and thus the rate of respiration, but the relation of growth to respiration and substrate consumption remains unaffected.



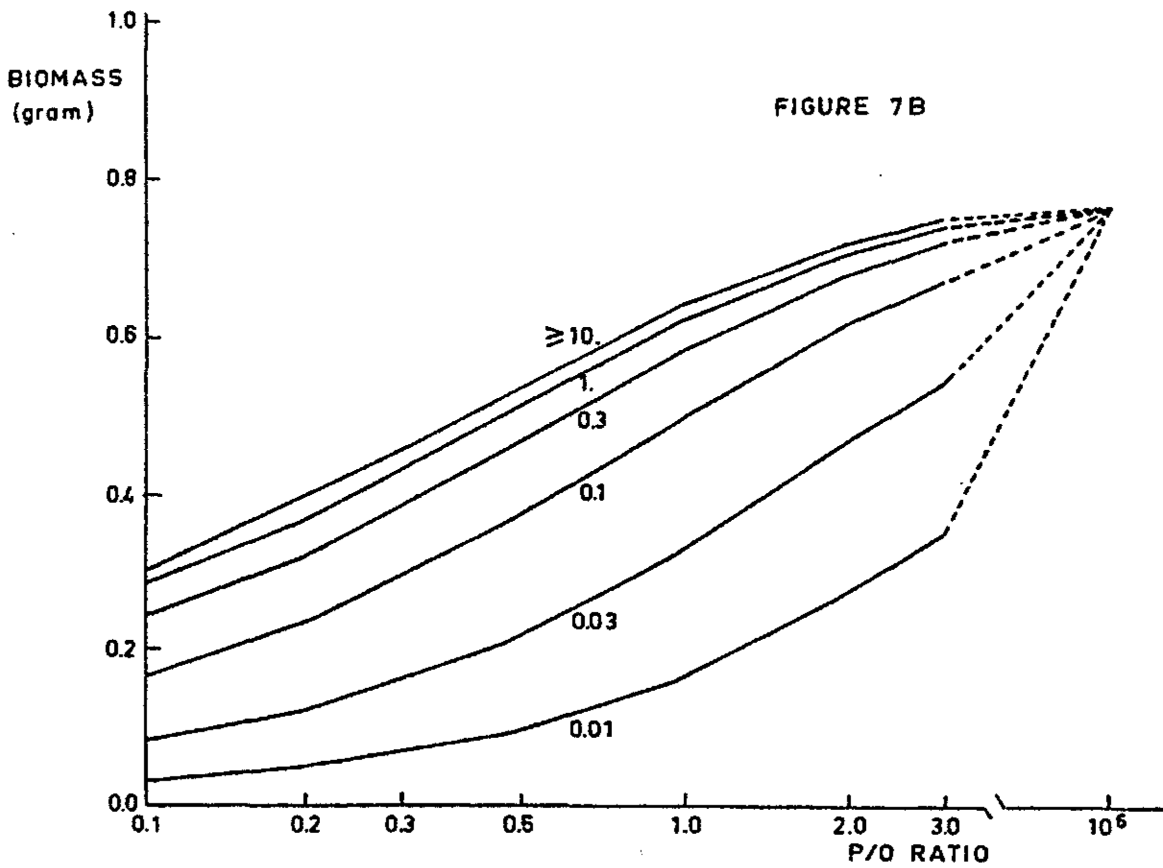


Figure 7 a and b. The yields of growth processes in g protein (7a) or g biomass (7b) per g glucose (with ammonia, hydrogen sulfide and minerals) at various P/O ratios and relative growth rates (units g g^{-1} day^{-1}).

Due to temperature changes the chemical composition of growing material may change (Udaka and Horiutchi, 1965; Jones and Hough, 1970; Schweyen and Kaudewitz, 1971) which changes the pv. This is an indirect effect of temperature, and is small in the cases reported. Type and quantity of substrate influence strongly the nitrogen content of Aspergillus niger (Terroine et al., 1922). Growth conditions affect the chemical composition of algae considerably (Van Oorschot, 1955), and the salinity of the medium may change the composition to a small extent (Reistad, 1970).

6. MAINTENANCE OF CELLULAR STRUCTURES AND OTHER RESPIRATION PROCESSES

Although it is common knowledge that living cells have a minimal rate of metabolism which is necessary to maintain their structure in the actual condition, little is known about the type and rate of maintenance processes. The rate of these processes was estimated in higher plants to require 1 - 4 % of the dry matter per day to be oxidized (Penning de Vries, 1972, 1974b).

Idling respiration has been suggested to be a sink for assimilates in plant tissue (Beevers, 1970; Tanaka, 1972). When it exists it is substrate oxidation useless to the cell, unless it indicates the presence of an aspect of respiration that has been overlooked in this approach. It was shown (Penning de Vries, 1972, 1974a) that experimental results with higher plants can be explained completely without assuming the presence of idling respiration. A comparison of accurate respiration measurements with theoretical rates of respiration caused by conversion, transport and maintenance is one means of detecting the existence of idling respiration.

Photorespiration of green leaves in the light diminishes the net rate of assimilate production, but does not interfere directly with the conversion processes of assimilates into biomass. Thus, photorespiration may be regarded as a process that only decreases the efficiency of light utilization for production of assimilates (Penning de Vries, 1974a).

7. CONCLUSIONS

It is possible to compute from biochemical data the amount of substrate required to synthesize 1.00 gram biomass of a specified chemical composition and the simultaneous oxygen consumption and carbon dioxide production. This approach of biosynthesis and growth is very powerful, as the chemical composition of substrate and end-product, the particular biochemistry of the organism and also the non-synthetic activities during biosynthesis can

be taken into account in predicting the dry matter production from a given amount of substrate. It gives an insight into quantitative aspects of growth processes, since it considers all important parts of the total separately, and in relation to each other.

Numerous biochemical pathways are described in micro-organisms, but not many have been determined in higher plants. However, it is not expected that very different synthetic pathways will be discovered (Dagley and Nicholson, 1970). As differences between pathways to obtain a particular product from a given substrate are usually small, unfamiliarity with specific aspects of plant biochemistry seems to be a small handicap. Much more important is the lack of knowledge about the energy requirements for cellular processes, such as membrane transport, compartmentation and enzyme maintenance. In the calculations, membrane transport was found to be relatively important, and the energy requirement for "tool maintenance" less important (fig. 2a and b); compartmentation has its largest effect when nitrogen is supplied as ammonia (table 8). Few useful basic data in these fields could be collected from literature. The inaccuracy of the numerical estimates for the cost of these processes contribute considerably to the inaccuracy of the end results of the computations. Often, the P/O ratio is another unknown. Whereas it is generally agreed that the P/O ratio in higher organisms is 3 or close to this value, this ratio can be considerably reduced by uncoupling agents. It is shown (fig. 6 and 7) that a reduction in P/O ratio from 3 to 2 causes a yield reduction of about 5 % at high relative growth rates. The smaller the relative yield growth rate, the more important the P/O ratio becomes.

Suitable determinations of biomass components, including nitrogenous compounds, carbohydrates, fats, lignin, organic acids and minerals, are scarce and standard methods to determine these figures are not available. This is even more true for the amino acid composition of the proteins, the different sugars in the carbohydrate fraction, etc. But knowledge of the amounts of the main fractions is of major importance, as values characterizing a conversion are quite different for the main chemical fractions (fig. 3, table 6), and much more similar within these (table 5). Lack of information on the chemical composition of the end product is often the major cause of inaccuracy of the end result of the computations.

An attractive feature of this approach to biochemical production processes is that the weights of the required co-substrates and by-products are obtained, as well as the weights of the main product and principal

substrate. It was shown that biosynthesis and respiration are related in a predictable manner. Respiration measurements can be performed without disturbing the growing organism, and the rate of growth can thus be determined from the rate of respiration. For many purposes this method is more suitable than the method for monitoring plant dry weight increase now commonly used, which is based upon destructive determination of the weights of essentially unrelated samples at successive moments in time. Hence studies on growth and respiration can profit from this approach.

This approach to biosynthesis may be called simulation, defining simulation as the imitation of real processes using some kind of a model (cf De Wit, 1970). Simulation by this definition comprises a numeric imitation of the types and quantities of the materials converted and of the energetic aspects of all relevant processes. Dynamic simulation of growth on a biochemical level must include the concentration of ATP in cells, which controls the rate of reactions and is itself changed by these reactions, and must account for the inhibiting or stimulating action of substrate, end-product and regulating molecules. All relevant concentrations are then continuously computed. It is easy to conceive that dynamic simulation requires far more detailed knowledge than is available with respect to such relationships as the activity of individual enzymes and the manner in which enzyme activity varies with changes in ATP concentration, end-product level and environmental factors, but knowledge about regulatory mechanisms is rapidly accumulating. Attempts of a limited scope may be very useful, since they indicate gaps in our knowledge and specify the type of data required for a better understanding of the whole system. In completed models, systems behaviour can be studied and the importance of particular elements in the total system recognized. For small subsystems like mitochondria or the Krebs-cycle, enough information has been collected and dynamic simulation reported (Garfinkel, 1970; Garfinkel et al., 1970). The simulation model discussed here is not a model for dynamic simulation. The chemical composition of the end-product is, therefore, not a result of this simulation, but an input for the calculations. However, it seems as though a first attempt can be made to construct a complete model for simulating the conversion of all types of organic substrates into each product required; the model presented does this only for the substrate glucose.

ACKNOWLEDGEMENTS

The author thanks Dr. W. Dijkshoorn, Dr. A.J.H. van Es, Dr. M.G. Huck, Dr. A.H. Stouthamer, Dr. J.H.M. Thornley, Drs. N. Vertregt and Dr. C.T. de Wit for the valuable discussions and their positive criticism on this manuscript, and Miss A.H. van Rossem for correcting the English text. Miss C.G. van Gulijk typed the manuscript many times, and Ir C. de Jonge translated the CSMP program into a FORTRAN program.

8. REFERENCES

Albers, R.W.: Biochemical aspects of active transport. Ann. Rev. Bioch. 36(2), 727-756, 1967.

Atkinson, D.E.: The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry, 7(11), 4030-4034, 1968.

Baldwin, R.L.: Estimation of theoretical calorific relationship as a teaching technique. A review. J. Dairy Science, 51, 104-111, 1968.

Bandurski, R.S.: Biological reduction of sulphate and nitrate. In: Plant Biochemistry, Bonner and Varner ed. pg. 467-496, Academic Press, London, 1965.

Bauchop, T. and S.R. Elsden: The growth of micro-organisms in relation to their energy supply. J. gen. Microbiol. 23, 457-469, 1960.

Beevers, H.: Respiration metabolism in plants. Harper and Row, New York, 1961.

Beevers, H.: Respiration in plants and its regulation. In: Prediction and measurement of photosynthetic productivity, pg. 209-214, Pudoc, Wageningen, The Netherlands, 1970.

Beevers, L. and R.H. Hageman: Nitrate reduction in higher plants. Ann. Rev. Plant Physiol., 20, 495-522, 1969.

Bieleski, R.L.: Turnover of phospholipids in normal and phosphorus deficient Spirodela. Plant Physiol., 49, 740-745, 1972.

Bielka, H.: Molekulare Biologie der Zelle. Gustav Fischer Verlag, Jena, 1969.

Blaxter, K.L.: The energy metabolism of ruminants. Hutchinson & Co. Ltd., London, 1962.

Bornkamm, R.: Dunkel Assimilation von Nitrat bei Lemna minor L.: Planta, 92, 50-56, 1970.

Brody, S.: Bioenergetics and growth. Reinhold Publishing Corporation, New York, 1945.

Brown, A.T. and C.L. Wittenberger: Mechanism for regulating the distribution of glucose carbon between the Embden-Meyerhof and Hexose Monosphosphate Pathway in Streptococcus feacalis. J. Bact., 106(2), 456-467, 1971.

Chen, S.L.: Energy requirement for microbial growth. Nature, 4937, 1135-1136, 1964.

Dagley, S. and D.E. Nicholson: An introduction to metabolic pathways. Blackwell Scientific Publications, Oxford, 1970.

DeMoss, R.D., R.C. Bard and I.C. Gunsalus: The mechanism of heterolactic fermentation, a new route of ethanol formation. J. Bact., 62, 499-511, 1951.

Ellis, J.R.S., C.J. Brunton and J.M. Palmer: Can mitochondrial complementation be used as a tool in breeding hybrid cereals? Nature 241(5384), 45-47, 1973.

Es, A.J.H. van: Der Energieaufwant bei der Eiweissbildung der Haustiere. Hülsenberger Gespräche, Verlagsgesellschaft für Tierzüchterische Arbeiten, Hamburg, 1971.

Forrest, W.W. and D.J. Walker: The generation and utilization of energy during growth. Adv. Microbiol. Physiol. 5, 213-274, Academic Press, 1971.

Garfinkel, D.: Simulation of the kinetics of ^{14}C in the Krebs Cycle in liver and its application to the design of experiments. J. Theoretical Biology, 29, 113-130, 1970.

Garfinkel, D., L. Garfinkel, M. Pring, S.B. Green and B. Chanche: Computer applications to biochemical kinetics. Ann. Rev. Biochem. 39, 473-498, 1970.

Geiduschek, E.P. and R. Haselkorn: Messenger RNA. Ann. Rev. Bioch., 38, 647-676, 1969.

Goddard, D.R. and B.J.D. Meeuse: Respiration of higher plants. Ann. Rev. Plant Physiol., 1, 207-232, 1950.

Goodwin, B.C.: Temporal organization in cells. Academic Press, London, 1963.

Gorski, F.: Plant growth and entropy production. Zaklad Fizjologii Roslin Pan, Krakow, 1966.

Gunsalus, I.C. and C.W. Shuster: Energy yielding metabolism in bacteria. In: The Bacteria, ed. Gunsalus and Stanier, Academic Press, London, vol. 2, 1-58, 1961.

Hadjipetrou, L.P., J.P. Gerrits, F.A.G. Teulings and A.H. Stouthamer: Relation between energy production and growth of Aerobacter aerogenes. J. Gen. Microbiol., 36, 139-150, 1964.

Handbook of biological data. Ed. Spector, Saunders Company, Philadelphia, 1956.

Hempfling, W.P.: Studies of the efficiency of oxidative phosphorylation in intact Escherichia coli B. Biochim. Biophys. Acta, 205, 169-182, 1970.

Höfer, M.: Transport of monosaccharides in Rhodotorula gracilis in the absence of metabolic energy. Archiv Microbiol., 80, 50-61, 1971.

James, W.P.: Plant Respiration. Oxford Clarendon Press, 1953.

Jones, R.C. and J.T. Hough: The effect of temperature on the metabolism of Bakers yeast on continuous cultures. J. Gen. Microbiol., 60, 107-116, 1970.

Kaback, H.R.: Transport. Ann. Rev. Biochem., 39, 561-598, 1970.

Kleiber, M.: The fire of life. Wiley and Sons, New York, 1961.

Krebs, H.A. and M.L. Kornberg: A survey of energy transformations in living matter. In: Ergebnisse der Physiologie, Biologischen Chemie und Experimentelle Pharmacologie. Bd. 49, 213-298, 1957. Ed. Krayer, Lehnartz, Muralt und Weber. Springer Verlag, Berlin.

Lardy, H.A. and S.M. Ferguson: Oxidative phosphorylation in mitochondria. Ann. Rev. Bioch., 38, 991-1034, 1969.

Lavallé, R. and G. De Hamer: Tryptophane messenger translation in E. coli. J. Mol. Biol., 51, 435-447, 1970.

Lehninger, A.L.: Bio-energetics. W.A. Benjamin Inc., New York, 1965.

Lehninger, A.L.: Biochemistry. Worth Publishers, New York, 1971.

Lucas-Lenard, J. and F. Lipmann: Protein biosynthesis. Ann. Rev. Bioch., 40, 409-488, 1971.

Makkink, G.F.: De theoretische bovenbouw van de oecologie. Contactblad voor Oecologen, 7(1), 14-36, 1971.

McCree, K.: An equation for the rate of respiration of white clover plants under controlled conditions. In: Prediction and measurement of photosynthetic productivity, pg. 221-230, 1971, Pudoc, Wageningen, The Netherlands.

McDaniel, R.G.: Mitochondrial heterosis in barley. Proc. 2nd Int. Barley Genetics Symposium, 323-337, 1969. Washington State University Press.

Meyenburg, K. von: Energetics of the budding cycle of Saccharomyces cerevisiae during glucose limited aerobic growth. Arch. Mikrobiol., 66, 289-303, 1969.

Meyerhof, O.: Chemical dynamics of life phenomena. Philadelphia, 1924.

Mitchell, P. and J. Moyle: Protein translocation coupled to ATP hydrolysis in rat liver mitochondria. European J. Biochemistry, 4, 530-539, 1968.

Monod, J.: Recherches sur la croissance des cultures bacterienne. Hermann et Cie., Paris, 1942.

Morowitz, H.J.: Energy flow in biology. Academic Press, London, 1968.

Muller, F.M., J.G. Dijkhuis and S. Heida: On the relationship between chemical composition and digestability in vivo of roughage. Agric. Res. Rep. 736, Pudoc, Wageningen, The Netherlands, 1970.

Needham, A.E.: The growth process in animals. Pitmann and Sons ltd., 1964.

Norris, T.E. and A.L. Koch: Effect of growth rate on the relative rates of synthesis of messenger, ribosomal and transfer RNA in Escherichia coli. J. Mol. Biol., 64, 633-649, 1972.

Oorschot, J.L.P. van: Conversion of light energy in algal cultures. Meded. Landbouwhogeschool 55, 225-276, 1955.

Oxender, D.L.: Membrane transport. Ann. Rev. Biochem., 41, 777-814, 1972.

Payne, W.J.: Energy yields and growth in heterotrophs. Ann. Rev. Microbiol., 24, 17-52, 1970.

Payne, J.W. and Ch. Gilvarg: Peptide transport: Advances in enzymology 35, 187-244, 1971.

Penning de Vries, F.W.T.: Respiration and growth. In: Crop processes in controlled environments, pg. 327-347, ed. Rees, Cockshull, Hand and Hurd. Academic Press, 1972.

Penning de Vries, F.W.T.: Use of assimilates in higher plants. In: Photosynthesis and productivity in different environments. Cambridge University Press. 1974a (in press).

Penning de Vries, F.W.T.: The cost of maintenance processes in plant cells. (In prep.).

Pullman, M.E. and G. Schatz: Mitochondrial oxidation and energy coupling. Ann. Rev. Bioch. 36, 539-610, 1967.

Reistad, R.: On the composition and nature of the bulk protein of extremely halophytic bacteria. Archiv Microbiol., 71, 353-360, 1970.

Rippel-Baldes, A.: Die Energieausnützung durch Microorganismen in quantitativer Hinsicht. Arch. für Microbiol., 17, 166-188, 1952.

Rubner, M.: Energieverbrauch im Leben der Microorganismen. Arch. für Hygiene, 48, 260-311, 1904.

Salser, W., J. Janin and C. Levinthal: Measurement of the unstable RNA in exponentially growing cultures of Bacillus subtillus and Escherichia coli. J. Mol. Biol., 31, 237-266, 1968.

Schimke, R.T. and D. Doyle: Control of enzyme levels in animal tissue. Ann. Rev. Bioch., 39, 929-976, 1970.

Schoffeniels, E.: Cellular aspects of membrane permeability. Pergamon Press, London, 1967.

Schweet, R. and R. Heintz: Protein synthesis. Ann. Rev. Bioch., 35, 723-758, 1966.

Schweyen, R.J. and F. Kaudewitz: Differentiation between mitochondrial and cytoplasmatic protein synthesis in vivo by use of a temperature sensitive mutant of Saccharomyces cerevisiae. Bioch. and Biophys. Res. Comm., 44, (6), 1351-1355, 1971.

Siegel, B.V. and C.E. Clifton: Energy relationships in carbohydrate assimilation in Escherichia coli. J. Bact., 60, 573-583, 1950.

Stein, W.D.: The movement of molecules across membranes. Academic Press, London, 1967.

Stouthamer, A.H.: Determination and significance of molar growth yields. In: Methods in Microbiology, 1, 629-663, Academic Press, London, 1969.

Stouthamer, A.H. and C. Bettenhaussen:Utilization of energy for growth and maintenance in continuous and batch cultures of micro-organisms. Biochim. Biophys. Acta, 301, 53-70, 1971.

Strehler, H.: Time, cells and aging. Academic Press, London, 1963.

Tamiya, H.: Zur Energetik des Wachstums. Beiträge zur Atmungsphysiologie der Schimmelpilze. II. Acta Phytochimica 6(2), 265-304, 1932.

Tamiya, H. and S. Yamagutchi: Ueber die Aufbau- und die Erhaltungsatmung. Beiträge zur Atmungsphysiologie der Schimmelpilze. III. Acta Phytochimica, 7(1), 43-64, 1933.

Tanaka, A.: Efficiency of respiration. Rice Breeding. International Rice Research Institute, Los Banos, Philippines, 483-498, 1972.

Terroine, E.F. et R. Wurmser: L'energie de la croissance. I. Le developpement de l'Aspergillus niger. Bull. Soc. Chimie Biologique, 4, 519-567, 1922.

Terroine, E.F., R.W. Wurmser et J. Montané: Influence de la constitution des milieu nutritifs sur la composition de l'Aspergillus niger. Bull. Soc. Chimie Biologique 4, 623-643, 1922.

Thornley, J.H.M.: Respiration, growth and maintenance in plants. Nature, 227(5255), 304-305, 1970.

Thornley, J.H.M. and J.D. Hesketh: Growth and respiration in cotton balls. J. appl. Ecol., 9, 315-317, 1972.

Udaka, S. and T. Horiutchi: Mutants of E. coli having temperature sensitive regulatory mechanism in the formation of arginine biosynthetic enzymes. Bioch. Biophys. Res. Comm., 19(2), 156-160; 1965.

Vries, W. de, W.M.C. Kapteijn, E.G. van der Beek and A.H. Stouthamer: Molar growth yields and fermentation balances of Lactobacillus casei L3 in Batch cultures and continuous cultures. J. Gen. Microbiol., 63, 333-345, 1970.

Wesselius, J.C.: Influences of external factors on the energy conversion and productivity of Scenedesmus sp. in mass culture. Communication Agricultural University, Wageningen, The Netherlands, 73-6, 1973.

Winton, A.L. and K.B. Winton: The structure and composition of foods. Wiley and Sons, New York, 1950.

Wit, C.T. de: Dynamic concepts in biology. In: Prediction and measurement of photosynthetic productivity, pg. 17-23, Pudoc, Wageningen The Netherlands, 1970.

Woldendorp, J.W.: Energieverbruik bij biosynthese. Chemisch Weekblad 50, 11-14, 1971.

USE OF ASSIMILATES IN HIGHER PLANTS

F.W.T. Penning de Vries

Department of Theoretical Production Ecology
Agricultural University
Wageningen

In: Photosynthesis and productivity
in different environments,
(IBP Photosynthesis Meeting
Aberystwyth, April 1973),
Cambridge University Press, 1974.

Summary and introduction

It is explained briefly how to compute the quantitative relation between substrate consumption, dry matter production and respiration when substrate and product are chemically well defined. To compute dry matter increase of plants, the chemical composition of the end product must be determined analytically and the quality and amount of substrate specified. Analysis of phloem contents shows that the organic substrate for growth consists mainly of sucrose and amino acids, whose biosynthesis is closely linked. Thus, total plant CO_2-assimilation is, alone, not a sufficient base from which to compute growth, and nitrate reduction, amino acid synthesis and other processes must also be considered: neglecting these may underestimate the yield from a given amount of CO_2 assimilated up to 30 %. These subjects are elaborated, and the consequences for the interpretation of CO_2-assimilation light response curves are discussed. Maintenance of biomass and translocation of assimilates through phloem vessels also use assimilates, but will not be considered here, mainly for reasons of uncertainty about the underlying mechanisms.

An experimental approach to the relation of CO_2-assimilation to growth

From the "molecular formula" of the biomass produced the relation of net CO_2-assimilation to biomass increase is easily obtained: when the "molecular formula" is $C_{86}H_{160}O_{45}N_7$ it follows from the "molecular weight" and the fraction of carbon that 1.00 gram biomass is formed from 1.88 gram CO_2. The net carbon assimilation corresponds with the dry weight increase, irrespectively of the nature and efficiency of the processes that occur. The efficiency of carbon utilization seems 100 %, because CO_2 losses are masked.

This procedure is very simple in case of algae continuously exposed to sufficient light. When periods of light and darkness alternate, the CO_2-assimilation in the light and dissimilation in darkness must be measured to determine the daily net CO_2-uptake. Again, from this value and the elementary

composition the weight of the biomass produced can be calculated. In higher plants the site of CO_2-assimilation is removed from the sites where growth occurs, and the period for substrate production (CO_2-assimilation) is shorter than that for substrate consumption in growth. Nevertheless the rate of dry matter increase can be calculated from the daily net CO_2-uptake and the elementary composition, but this knowledge is of small practical value. Empirical equations may be used to calculate the biomass yield and respiration from the gross assimilation, but since the underlying mechanism is not known such equations cannot be applied in other conditions or to other species (McCree, 1970). To obtain an insight into the relation between gross assimilation and biomass yield and respiration in different situations a more fundamental approach is required.

The term "biosynthesis" will be used to refer to formation of dry matter, and "growth" to total dry weight increase, including biosynthesis and maintenance.

A biochemical approach to plant biosynthesis

Detailed analysis of the biochemical and cellular processes occurring in growing cells enables computation of yield and gas exchange for the conversion of glucose into plant dry matter in darkness. Both yield and gas exchange depend on the chemical composition of the end product (Table 1). Energy requirements for maintenance of enzyme activity and uptake of molecules through membranes are not well known, but results of such computations are often little affected by rough estimates of these costs (Penning de Vries et al., 1973).

The substrate for growth in plants consists of mono- and disaccharides, amino acids, organic acids and other specific compounds (e.g. Kursanov, 1963). This makes computation of the yield and gas exchange of growth more complicated, but does not add a major difficulty as long as the substrate

composition is known, and the most efficient use of the substrate is made. For biosynthesis of 1.00 gram leaf dry matter 1.36 gram of a mixed organic substrate is required; formation of organs with different chemical compositions requires other amounts, examples of which are given in Table 2. It is important to note that the numbers given in Tables 1 and 2 are independent of temperature and species, and are determined only by the compositions of substrate and end product.

Translocation of substrate in the phloem is an active process. Only a negligible fraction of the translocated assimilates is consumed to provide energy for translocation over short distances (Kursanov, 1963; Weatherley & Johnson, 1968; Aikman & Anderson, 1971), but the integrated costs for transport over meters may not be negligible. Costs of translocation within the phloem are not considered here. Loading and unloading the phloem will be treated separately.

It is concluded that the rate of biosynthesis can be predicted from the rate of substrate supply to growing points and the chemical composition of the biomass formed. The rate of substrate production by leaves is easily obtained from the CO_2-assimilation light response curve and incident light intensity at a 10 % accuracy level, but a precise calculation contains some pitfalls.

A plant physiological-biochemical approach to biosynthesis

a. CO_2-assimilation and photosynthesis

In growth simulation models it is generally assumed that all assimilated CO_2-molecules are converted into glucose, and that glucose plus minerals are the only substrates for growth. This is an over-simplification, because in irradiated leaves very often other energy consuming processes occur simultaneously with CO_2-reduction. The reduction of NO_3^-, the subsequent formation of amino acids, and the loading the phloem are the most important

of these processes, which are not detected by measuring CO_2-uptake. Most of the NO_3^--reduction of agricultural crop plants occurs in the light in green leaves (Beevers & Hageman, 1969; Bornkamm, 1970; Hewitt, 1970). In these cases photosynthesizing cells consume more energy than is calculated from reduction of the assimilated CO_2 to glucose. Still, not all assimilated CO_2 is reduced: for each molecule of reduced NO_3^- one organic acid molecule is formed by carboxylating pyruvate. The salts of these acids remain in the leaves in some species, but about half is transported to the roots in others (Ben Zioni et al., 1971; Dijkshoorn & Ismunadji, 1972), where the organic acid is converted into pyruvate and the CO_2 reformed is exchanged with NO_3^- from the root medium, NO_3^- reduction being accompanied by a CO_2 flux through the plant.

Determination of energy absorption by leaves via measurement of O_2 evolution is better than via CO_2-uptake, because it accounts for NO_3^--reduction and carboxylation. But, for instance, loading of the phloem consumes energy without exchange of molecules with the environment, and cannot be detected by measuring gas exchange (Ried, 1970). Even if it were possible to determine accurately leaf energy absorption in chemical processes (including transport processes) by measuring the total energy absorption and subtracting the energy lost by thermal reradiation and transfer of sensible and evaporative heat loss, the actual energy absorption would still not be measured. Firstly, because in primary chlorophyll reactions more energy is absorbed than is retained in glucose, but since glucose is the starting point for biochemical conversion calculations there is no need to consider energy lost before this point. Secondly, because the energy retained in processes that occur in addition to CO_2-reduction is smaller than the amount of energy required to execute them. Simultaneous energy consuming processes in the leaf are probably competitive, so that at low light intensities the rate of CO_2-reduction is reduced when the rate of NO_3^- reduction increases, as shown experimentally by Bongers (1956) with algae. At high light intensities, where the rate of CO_2-diffusion limits the assimilation rate, these additional

energy consuming processes occur free of cost for the plant. It was shown by Dijkshoorn & Ismunadji (1972) that rice plants supplied with NO_3^- at high light intensities grow as fast as with NH_3, but such experiments must be interpreted with care, since the plant composition may be changed, thereby changing the relation between CO_2 fixation and dry matter increase.

Such considerations indicate that photosynthesis, CO_2-assimilation and conversion processes must be considered in their physiological context. Only when the information is available as to which processes occur in the leaf and at what rate can the measured CO_2-assimilation light response curve be extrapolated to a range of conditions in which it was not established. CO_2-assimilation light response curves are fairly well known for many species, especially agricultural plants, but little information is available on the rate of NO_3^--reduction and substrate export from leaves throughout the day in field situations or in particular experiments. An average rate of NO_3^--reduction during the day can be obtained analytically, but the actual rate may vary with light intensity (e.g. Bongers, 1956) and incubation period (e.g. Travis et al., 1970). At present, only in steady state conditions when the rate of all processes can be derived from the CO_2-uptake rate, can calculations be performed with some accuracy.

Photorespiration decreases the net CO_2-reduction rate of an irradiated leaf. It does not contribute to any substrate production, and does not provide energy to any active process that cannot be otherwise performed (Beevers & Björkman, in Canvin, 1970). On a cellular level, photorespiration may be useful in providing reduction equivalents to the cytoplasm, where these are used instead of mitochondrial products (Tolbert, 1971). For growth predictions photorespiration can be seen as a factor that diminishes the rate of CO_2-assimilation, like low temperatures or low atmospheric CO_2-concentrations.

b. Assimilation and dissimilation in a steady state condition

A particular steady state condition may be considered in order to verify the predicted dissimilation rate against a measured rate of CO_2-assimilation (substrate production). Maize plants were grown on a nutrient solution at 20° to $25^{o}C$ and at a light level of 70 J m^{-2} sec^{-1}. Whole plants of 10 to 24 days age received 3 periods of 7 hours light of one intensity, each followed by 1 hour darkness at a relative humidity of 85 %. During 4 subsequent days light intensities were applied up to 300 J m^{-2} sec^{-1}. The rate of CO_2-assimilation was monitored continuously with the assembly described by Louwerse & Van Oorschot (1969). At the end of the third period the rate of net assimilation and dissimilation, measured in the next hour, are in "equilibrium", as was concluded from preliminary experiments, unless the rates in the old and new "steady state" are very different. In this "steady state" the amount of carbon assimilated in 7 hours is equal to the amount of carbon utilized in 8 hours for conversion, transport and maintenance. The relation between the amounts of CO_2 assimilated, dry matter produced and CO_2 formed in these processes for this particular "steady state" experiment will be computed below. Essentially similar experiments have been reported elsewhere (Penning de Vries, 1972), but some improvements have been introduced and the range of experiments extended. The biomass formed was composed as indicated in the small rectangles in Figure 1; the numbers refer to the weight of each fraction per 1000 gram of biomass. The arrows represent conversion and dissimilation processes, and the numbers beside them the amounts of glucose and CO_2 involved. O_2 was not considered, but the amounts involved can be found from data published elsewhere (Penning de Vries et al., 197). From Table 1 and other data the amounts of glucose needed to synthesize lipids, lignin and carbohydrates were derived. The latter fraction was assumed to consist of cellulose, while the other fractions consist of a natural mixture of molecules. It was also assumed that half of the lignin is formed in mature leaves. The amino acid composition of the protein was chosen to be that of zein (Handbook

of Biological Data, 1956). The composition of the transported amino acids, in Figure 1 characterized as $A.A._t$, was derived from zein by assuming that the amino acids that can be formed from aspartic and glutamic acid are formed from them, and that cysteine is transported as such. This composition was also used in Table 2. The other amino acids present were assumed to be synthesized from glucose, the ammonia being carried in amides (glutamine and asparagine). The resulting mixture consists of aspartic acid 41 % (by weight), glutamic acid 37 %, asparagine 7 %, glutamine 15 % and cysteine 0.6 %.

Uptake of minerals, glucose and amino acids are active processes. On the basis of little quantitative information (Kaback, 1970; Oxender, 1972) it is estimated that both uptake of 1 mole of carbohydrates and 3 moles of salts or amino acids requires the energy of 1 mole ATP per membrane passage. Minerals are taken up from the xylem through at least one membrane, and pass two membranes of the root endodermis. Between the sieve tubes and the cytoplasm of other cells are at least two membranes; here too the minimal number will be used. Energy for uptake processes is provided by glucose. Export costs are expected to be similar to import costs. But while sucrose is taken up, glucose is exported and transferred into sucrose. Loading the phloem with sucrose is therefore more expensive (eq. 4 below) than unloading.

To illustrate the amount of reduction equivalents and energy consumed for NO_3^--reduction, glucose was taken to be required. But if $NADPH_2$ from chloroplasts is used instead, less glucose is required, and less CO_2 is assimilated and released. Thus this notation affects only the internal CO_2 turnover rate. Mutatis mutandis this is true for other processes where glucose may not be the intermediate. Synthesis of amino acids and organic acids (OA^-) is closely linked with NO_3^--reduction (De Wit et al., 1963). The weight of the organic acids formed is found by assuming creation of one gram equivalent oxaloacetic acid per grammolecule NO_3^- reduced. Some organic acids remain in the leaves and the rest are transported to the roots where they enter the cell metabolism. For simplicity it is assumed that the carbon skeletons

yield glucose. Although glucose breakdown and gluconeogenesis do not occur in one cell at the same time, a precise calculation probably hardly changes the picture. The amount of minerals in the mature leaf is related to the organic acid content. Also for simplicity circulation of K^+ between leaf and roots was not included.

The glucose required for all processes results from photosynthetic CO_2-reduction. The CO_2 formed during and due to NO_3^--reduction evolves in light only, but transport and biosynthetic processes continue in darkness. Assuming that these continue in darkness at the same rate as in the light, according to Figure 1 the dark respiration for the whole plant is (353+63+93+22=) 531 gram CO_2 per 1000 gram end product. The net CO_2-assimilation rate in an equilibrium situation must be (2330-125-531x21 (hours in light)/ 24 (hours of biosynthesis) = 1741 gram CO_2. The ratio of net CO_2-assimilation to dissimilation is predicted to be 3.75 in this particular experiment.

The experiment was repeated with sunflower (Helianthus annuus). Unlike maize leaves, growing sunflower leaves use both their own assimilates and those supplied by other leaves. Less transportation costs are incurred, and the synthesis of some proteins does not require $A.A._t$ as intermediates. The chemical composition of the fractions was assumed to be identical to those of maize. The computations were performed on a similar basis and are depicted in Figure 2. Mainly because transportation costs are smaller, the rate of respiration of whole plants at a given net assimilation rate is smaller than in maize.

Figures 3 a-b show that "steady state" rates of assimilation and dissimilation in H. annuus at 25° and 18°C. The predicted ratio between assimilation and dissimilation is given by the slope of the solid line; its position is chosen between the actual values. The intercept with the z-axis, which has a 1:1 relation to x and y axis, represents the rate of maintenance respiration. There is a good agreement between the position of the measured points and the computed slope over a wide range of assimilation rates, and

there is no indication that the lower temperature decreases the slope. Treatment with dilute nutrient solution decreased the nitrogen content of the biomass produced to 2.4 %. A calculation similar to previous ones showed that this should increase the slope by 28 %. Figure 3c shows that under these conditions this efficiency is maintained, and that the rate of maintenance respiration was not increased (Semikhatova, 1970). The maize experiments were performed at 25°, 18° and 33°C, and the results presented in Figures 4 a-c. Except at the lowest light intensities there is a good agreement between the position of the points and the calculated slope of the line at the three temperatures. It is concluded that these experiments confirm the value of this approach and support the hypothesis that the efficiency of biochemical processes in higher plants is independent of temperature in the range normally encountered. It was suggested earlier (Penning de Vries, 1972) that a low relative humidity could induce some waterstress in the light period, eliminating the "steady state" character of the experiment. To check this the experiment at 25°C was repeated at a relative humidity of about 50 %. The results are included in Figure 4a and demonstrate that no waterstress developped. McCree (1970) obtained results similar to those in the Figures 3 and 4 by plotting daily totals of dissimilation versus gross assimilation of white clover plants.

The dashed lines in the Figure 4 indicate the ratios of net assimilation of the photosynthesizing leaves to their dissimilation, which were not measured. Their intercepts with the z-axis are found by multiplying the plant maintenance respiration rate by the dry weight of the mature leaves over the total dry weight. The solid lines in Figures 3 a-b and 4 a-c have different intercepts with the z-axis, indicating that the rate of maintenance respiration depends upon temperature, The intercept with the z-axis in H. annuus suggests that its maintenance respiration is about three times larger than that in maize, which is an unexpected result. The rate for maize compares well with

other observations (McCree, 1970; Penning de Vries, 1972).

In maize the rate of leaf appearance does not depend on light intensity above 50-70 J $m^{-2}sec^{-1}$ (Grobbelaar, 1962; Gallagher and Lof, unpublished results). A minimal specific leaf weight combined with a constant rate of leaf production leads to a minimal rate of formation of biomass. It is suggested that at light levels below 60 J $m^{-2}sec^{-1}$ this minimal rate of about 0.15 gram leaves per gram plant per day could not be supported by photosynthesis, but is sustained by break down of biomass and translocation from older organs, causing a relatively high respiration rate. This process continues for a few days at a diminishing rate. Figure 3a suggests that in sunflower, unlike maize, there is no minimal rate of formation of structural material. This was also found in leaves of lettuce (Bensink, 1971).

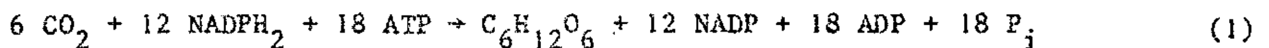
These experiments exclude the presence of wasteful respiration (Beevers, 1970; Tanaka, 1972), except one at a low and constant rate, which would appear similar to maintenance respiration. The scatter of the measurements in all experiments is fairly large, and may cover an error in the approach, but can also be interpreted as changes of the rate of biosynthesis. Research on rates of biosynthesis of plant biomass and its chemical fractions in relation to external and internal factors is still largely terra incognita. A key to one of the first problems of this field, the measurement of the rate of biosynthesis of an intact plant, may be the measurement of the rate of conversion respiration.

c. Interpretation of CO_2-assimilation light response curves of leaves

Steady state conditions are exceptional. The rate of CO_2-assimilation and other energy consuming processes changes continuously. To use a CO_2-assimilation light response curve (CO_2-a.l.c.) correctly for calculations of plant assimilate production, external conditions (CO_2-concentration, temperature) and internal conditions (rate of NO_3^--reduction and assimilate export) prevailing during the measurement and at the moment for which the

curve is used must be considered. To assess the relative importance of CO_2-assimilation and other energy consuming processes some widely different examples will be studied. That the CO_2-a.l.c. is not a constant but subject to stresses and adaptations is not important in this context, where the maximum rate of CO_2-assimilation is measured and used as input for further calculations.

<u>EXAMPLE 1</u>. DURING MEASUREMENTS OF THE CO_2-A.L.C. THE LEAF ONLY REDUCES THE ASSIMILATED CO_2 TO GLUCOSE. In this case photosynthesis in C_3 plants may be represented by



$$6\ CO_2 + 12\ NADPH_2 + 18\ ATP \rightarrow C_6H_{12}O_6 + 12\ NADP + 18\ ADP + 18\ P_i \qquad (1)$$

The energy absorption per assimilated C atom is 2 $NADPH_2$ molecules plus 3 ATP molecules, expressing energy absorption in reduction equivalents and ATP units. Slightly more ATP is used in C_4 plants (Mayne et al., 1971).

<u>EXAMPLE 2</u>. DURING MEASUREMENT OF THE CO_2-A.L.C. THE LEAF REDUCES THE ASSIMILATED CO_2 TO GLUCOSE AND FORMS CELLULOSE OR STARCH OR EXPORTS SUCROSE. Recognizing that glucose-6-P or fructose-6-P is the photosynthesis product, these processes can be characterized by

$$n\ \text{glucose-6-P} + 2n\ ATP \rightarrow \text{starch} \qquad (2)$$

$$n\ \text{glucose-6-P} + 3n\ ATP \rightarrow \text{cellulose} \qquad (3)$$

$$2\ \text{glucose-6-P}_{cell} + 4\ ATP \rightarrow \text{sucrose}_{phloem} \qquad (4)$$

Synthesis of cellulose is more expensive than starch synthesis since the monomers must be exported through one cell membrane. For eq. (4) it was assumed that export of each fructose and glucose molecule requires 1 ATP molecule, the coupling of both to sucrose a third, while another ATP molecule is required for uptake of sucrose into the phloem. Assuming that formation of 1 $NADPH_2$ molecule from NADP uses 7.5 times more light energy than 1 ATP molecule from ADP, it follows that in these cases 1.9 to 2.8 % more energy is absorbed per assimilated C atom than calculated according to eq. (1).

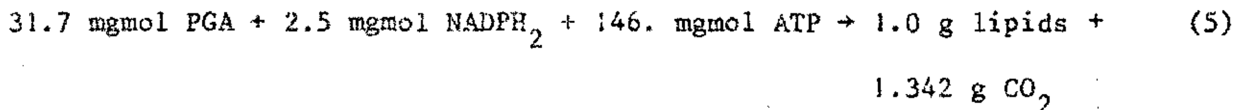
It is also assumed that the ratio of photosynthetically produced $NADPH_2$ and ATP can vary according to the requirements, as suggested by Ried (1970). Photorespiration may help to achieve this (Tolbert, 1971).

The cell cannot use energy stored in starch other than in glucose molecules, since its hydrolysis does not yield ATP. From the point of view of energy conservation starch and glucose formation are equal. The energy spent to export sucrose (eq. 4) corresponds with 1.9 % of the energy needed to synthesize glucose, but with 5.3 % of the energy stored in it (per glucose molecule 38 ATP molecules can be formed). Thus 5.6 % more glucose remains when the processes described in eq. (4) occur during photosynthesis, as compared with glucose formation in daylight and sucrose export in darkness. The latter percentage represents the undervaluation of the yield of biosynthesis when photosynthesis is considered to be merely glucose formation, and is given in Table 3.

These cases apply to many laboratory and field conditions: often the CO_2-a.l.c. is measured when NO_3^--reduction does not occur and in some field situations lack of NO_3^--reduction in leaves has been reported. When a leaf forms starch only and exports sucrose in darkness, the conversion calculations described above can be applied directly.

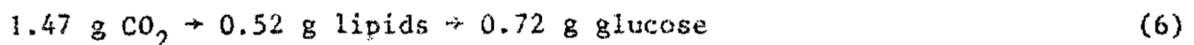
If the CO_2-a.l.c. is measured in conditions in which eq. (1) applies but sucrose is exported, the CO_2-a.l.c. can be constructed from an initial slope, equal to the slope of the original curve divided by the energy absorption in eq. (4) relative to eq. (1), and the maximum rate of CO_2 diffusion, causing a maximum rate of energy fixation.

EXAMPLE 3. DURING MEASUREMENT OF THE CO_2-A.L.C. THE LEAF STORES THE CO_2 ASSIMILATED AS LIPIDS. This occurs in algae under conditions where growth is restricted (Van Oorschot, 1955) and possibly in leaves of higher plants when oil droplets are formed. When the starting point is phosphoglyceric acid,(PGA) the equation can be derived

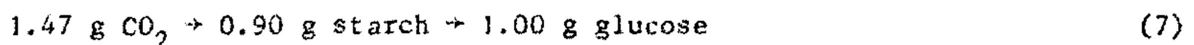


$$31.7 \text{ mgmol PGA} + 2.5 \text{ mgmol NADPH}_2 + 146. \text{ mgmol ATP} \rightarrow 1.0 \text{ g lipids} + 1.342 \text{ g } CO_2 \quad (5)$$

The energy consumed per C atom in the lipid fraction is about 46 % more than in eq. (1) and under these conditions the slope of the CO_2-a.l.c. is considerably lowered. Sucrose or glucose are used for transport of carbon and energy between cells, and although slightly more chemical energy per gram C is present in lipids (4.56 10^4 Joule) than in starch (3.86 10^4 Joule), in the conversion of lipids into glucose some 28 % of the C gets lost. This reduces the yield of lipids considerably:



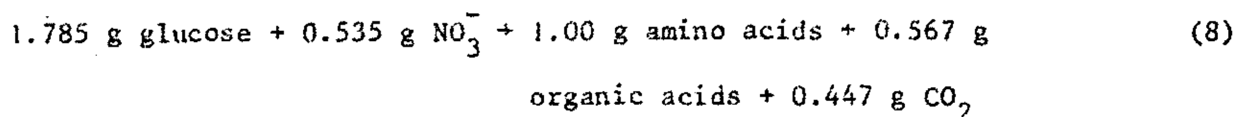
$$1.47 \text{ g } CO_2 \rightarrow 0.52 \text{ g lipids} \rightarrow 0.72 \text{ g glucose} \quad (6)$$



$$1.47 \text{ g } CO_2 \rightarrow 0.90 \text{ g starch} \rightarrow 1.00 \text{ g glucose} \quad (7)$$

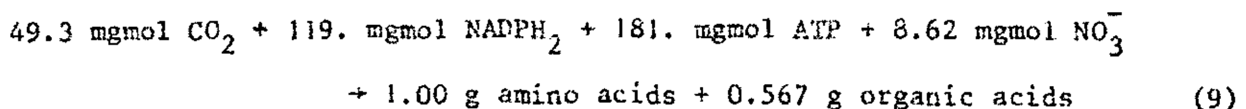
Storage of lipids may be advantageous when low weight is important, as in some seeds, or when the specific characteristics are useful. The high costs of glucose synthesis from lipids may be the major reason that lipids are not used for short term storage in leaves.

<u>EXAMPLE 4</u>. DURING THE MEASUREMENT OF THE CO_2-A.L.C. THE LEAF REDUCES NO_3^- AND FORMS AMINO ACIDS; CO_2 IS REDUCED ONLY TO PROVIDE CARBON SKELETONS FOR THE ACIDS. NO_3^--reduction, amino acid and organic acid formation can be described by



$$1.785 \text{ g glucose} + 0.535 \text{ g } NO_3^- \rightarrow 1.00 \text{ g amino acids} + 0.567 \text{ g organic acids} + 0.447 \text{ g } CO_2 \quad (8)$$

and by



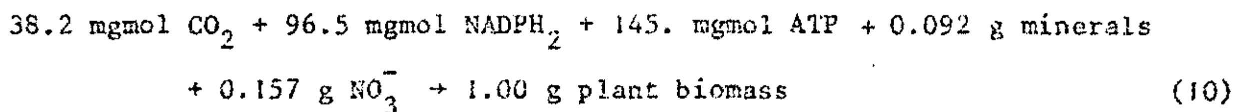
$$49.3 \text{ mgmol } CO_2 + 119. \text{ mgmol NADPH}_2 + 181. \text{ mgmol ATP} + 8.62 \text{ mgmol } NO_3^- \rightarrow 1.00 \text{ g amino acids} + 0.567 \text{ g organic acids} \quad (9)$$

In the processes described by eq. (9), 21.0 % more energy is consumed per assimilated C atom than in eq. (1). If it were supposed that only glucose was formed photosynthetically and amino acid synthesis occurred in darkness, the conversion calculations would underestimate the yield of the photosynthesis

process by 19.8 %, and 9 % of the assimilated CO_2 remains unreduced and is exported in organic acids to the roots and excreted.

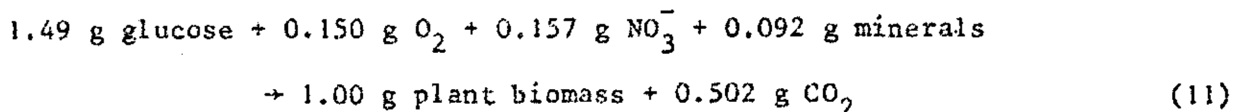
Like starch formation during photosynthesis, storage of amino acids as proteins increases the energy fixation per assimilated C atom (to 24.1 %) but this is lost during protein hydrolysis. Export of the amino acids is relatively cheap and when it occurs during photosynthesis the energy assimilatio per C atom is increased only to 21.3 %.

EXAMPLE 5. DURING THE MEASUREMENT OF THE CO_2-A.L.C. THE LEAF INCREASES IN BIOMASS, BUT DOES NOT EXPORT OR IMPORT ASSIMILATES. In this example photosynthesis can be described by



$$38.2 \text{ mgmol } CO_2 + 96.5 \text{ mgmol } NADPH_2 + 145. \text{ mgmol ATP} + 0.092 \text{ g minerals} + 0.157 \text{ g } NO_3^- \rightarrow 1.00 \text{ g plant biomass} \quad (10)$$

Eq. (10) was obtained by modifying Figure 2 such that all growth occurred in the photosynthesizing part, and it is concluded that the energy absorption per C atom is 26.7 % more than that calculated from eq. (1). This result depends on the chemical composition of the biomass, which in this example is very rich in minerals. For biosynthesis from glucose in darkness the equation is



$$1.49 \text{ g glucose} + 0.150 \text{ g } O_2 + 0.157 \text{ g } NO_3^- + 0.092 \text{ g minerals} \rightarrow 1.00 \text{ g plant biomass} + 0.502 \text{ g } CO_2 \quad (11)$$

Performing the processes described by eq. (10) during photosynthesis yields 30 % more dry matter than performing those of eq. (1), with those of eq. (11) occurring in darkness!

Leaves of bean and sunflower plants go through this stage, but generally most of the growth occurs in darkness or in organs that do not photosynthesize. For leaves growing from their own assimilates the efficiency of C utilization, expressed as the percentage of assimilated CO_2-molecules retained in the plant 24 hours after application, must be between 100 % (eq. 10) and 70 % (eq. 11 plus eq. 1). When leaves send their assimilates to mature organs, such as lower leaves to roots, these assimilates are used for

maintenance exclusively, and thus the C utilization is 0 %, although some labelled C may be retained due to exchange in turnover processes. It can be estimated that some 70 % of the assimilated C is retained in the plant permanently at relative growth rates of 0.3 $g.g^{-1}.day^{-1}$ and higher, about 50 % at a relative growth rate of 0.03, and about 30 % at a relative growth rate of 0.01. Estimating the fractions of substrate consumed for maintenance and biosynthesis gives the key for these predictions.

Acknowledgements

The author is indebted to Dr.Ir. C.T. de Wit for reading the manuscript critically and to Miss H.H. van Laar and Mr. W. van der Zweerde for carrying out the experiments. The chemical analyses were done by the IBS chemical laboratory.

Literature cited

Aikman, D.P. and W.P. Anderson: A quantitative investigation of peristaltic model for phloem translocation. Annals of Botany, 35(1971), 761-772.

Beevers, H.: Respiration in plants and its regulation. Proceedings IBP/PP Technical Meeting Trebon. Prediction and Measurement of Photosynthetic Productivity, 209-214, Pudoc, Wagennigen, 1970.

Beevers, L. and R.H. Hageman: Nitrate reduction in higher plants. Annual Rev. Plant Physiol. 20, 495-522, 1969.

Ben-Zioni, A., Y. Vaadia and S.H. Lips: Correlations between nitrate reduction, protein synthesis and malate accumulation. Physiologia Plantarum 23(1971), 1039-1047.

Bensink, J.: On morphogenesis of lettuce leaves in relation to light and temperature. Mededelingen Landbouwhogeschool Wageningen, 71(15), 1-93, 1971.

Bongers, L.H.J.: Aspects of nitrogen assimilation by cultures of green algae. Mededelingen Landbouwhogeschool, Wageningen 56(15), 1-52, 1956.

Bornkamm, R.: Dunkel-Assimilation von Nitrat bei Lemna Minor L. Planta, 92(1970), 50-56.

Canvin, D.T.: Losses in energy transformation in relation to the use of photosynthates for growth and maintenance of photosynthetic systems. Proceedings IBP/PP Technical Meeting Trebon, Prediction and measurement of photosynthetic productivity, 251-257, Pudoc, Wageningen, 1970.

Dijkshoorn, W. and M. Ismunadji: Nitrogen nutrition of rice plants measured by growth of nutrient content in pot experiments. 3. Change during growth. Netherlands Journal of Agricultural Science, 20(1972), 133-144.

Grobbelaar, W.P.: The growth of maize pretreated at various soil temperatures. Jaarboek Instituut voor Biologisch en Scheikundig Onderzoek van Landbouwgewassen, 1962, 33-38.

Ried, A.: Energetic aspects of the interaction between photosynthesis and respiration. In: Prediction and measurement of photosynthetic productivity. Proceedings IBP/PP Technical Meeting, Trebon, 231-246. Pudoc, Wageningen, 1970.

Semikhatova, O.A.: Energy efficiency of respiration under unfavorable conditions. In: Prediction and measurement of photosynthetic productivity. Proceedings IBP/PP Technical Meeting, Trebon, 247-250. Pudoc, Wageningen, 1970.

Tanaka, A.: Efficiency in respiration. Proceedings Symposium on Rice Breeding, I.R.R.I., Los Banos, Philippines, 483-498, 1972.

Tolbert, N.E.: Leaf peroxisomes and photorespiration. In: Photosynthesis and photorespiration. Ed. Hatch, Osmond and Slatyer. Wiley Interscience, New York, 1971, pg. 458-471.

Travis, R.L., R.C. Huffaker and J.L. Key: Light induced development of polyribosomes and the induction of nitrate reductase in corn leaves. Plant Physiology 46(1970), 400-405.

Weatherley, P.E. and R.P.C. Johnson: The form and function of the sieve tube; a problem in reconciliation. International Review of Cytology, 24(1968), 149-192.

Wit, C.T. de, W. Dijkshoorn and J.C. Noggle: Ionic balance and growth of plants. Agricultural Research Reports, 69-15, 1963, Pudoc, Wageningen.

Handbook of biological data, Ed. Spector, Saunders Company, Philadelphia, 1956.

Hewitt, E.J.: Physiological and Biochemical factors which control the assimilation of inorganic nitrogen supplies by plants. In: Nitrogen nutrition of the plant. Ed.: E.A. Kirkny, 78-103. The Waverley Press, Leeds, 1970.

Kaback, H.R.: Transport. Annual Review of Biochemistry, 39(1970), 561-598.

Kursanov, A.L.: Metabolism and transport of organic substances in the phloem. Advances in Botanical Research 1(1963), 209-279. Academic Press Inc., London.

Louwerse, W. and J.L.P. van Oorschot: An assembly for routine measurements of photosynthesis, respiration and transpiration of intact plants under controlled conditions. Photosynthetica, 3(1969), 305-315.

Mayne, B.C., G.E. Edwards and C.C. Black: Light relations in C_4 photosynthesis. In: Photosynthesis and photorespiration. Ed.: Hatch, Osmond and Slatyer. Wiley Interscience, New York, 1971, 361-371.

McCree, K.J.: An equation for the rate of respiration of white colver plants grown under controlled conditions. In: Prediction and measurement of photosynthetic productivity. Proceedings IBP/PP Technical Meeting, Trebon, 221-229, Pudoc, Wageningen, 1970.

Oorschot, J.L.P. van: Conversion of light energy in algal cultures. Mededelingen Landbouwhogeschool, 55(5) 1955, 225-276.

Oxender, D.L.: Membrane transport. Annual Review of Biochemistry, 41(1972), 777-814.

Penning de Vries, F.W.T.: Respiration and growth. Proceedings of Symposium "Crop processes in controlled environments", ed. Rees, Cockshull, Hand and Hurd, Academic Press, 1972, 327-347.

Penning de Vries, F.W.T., A. Brunsting and H.H. van Laar: Products, requirements and efficiency of biosynthesis, a quantitative approach. In Prep.

Table 1. Values characterizing conversion of glucose into the main chemical fractions of plant dry matter in darkness. Each fraction consists of a natural mixture of different molecules.

chemical fraction	yield (g product / g glucose)	oxygen consumed ($g\ O_2$ / g glucose)	carbon dioxide produced ($g\ CO_2$ / g glucose)	note
nitrogenous compounds (consisting of amino acids, proteins and nucleic acids)	0.616	0.137	0.256	$+ NH_3$
	0.404	0.174	0.673	$+ NO_3^-$
carbohydrates	0.826	0.082	0.102	
lipids	0.330	0.116	0.530	
lignin	0.465	0.116	0.292	
organic acids	1.104	0.298	-0.050	

Table 2. Requirements for biosynthesis of 1.00 gram dry matter of tissues with different chemical compositions. The composition used is given in percent of nitrogenous compounds, carbohydrates, lipids, lignin and minerals respectively.

nature of biomass	composition	sucrose (gram)	amino acids (gram)	CO_2 produced (gram)	O_2 consumed (gram)
leaves	25;66.5;2.5;4;2	1.055	0.305	0.333	0.150
non woody stem	12.5;74;2.5;8;2	1.153	0.153	0.278	0.135
woody stem	5;45;5;40;5	1.515	0.061	0.426	0.176
bean seeds	35;55;5;2;3	1.011	0.427	0.420	0.170
rice seeds	5;90;2;1;2	1.135	0.061	0.186	0.110
peanut seeds	20;21;50;6;3	1.915	0.245	1.017	0.266
bacteria	60;25;5;2;8	0.804	0.732	0.573	0.208

Table 3. The hydrogen and energy requirements during photosynthesis, the additional energy assimilation and the yield undervaluation in different examples explained in the text.

	requirements per assimilated CO_2 molecule		additional energy assimilation	undervaluation dry matter yield	calculated from equation
	$NADPH_2$	ATP			
example 1	2.00	3.00	0 %	0 %	-
example 2	2.00	3.33	2 %	0 %	2
example 2	2.00	3.33	2 %	6 %	4
example 3	2.74	5.65	46 %	-28 %	5.6+7
example 4	2.47	3.73	21 %	20 %	9.8
example 5	2.53	3.80	27 %	30 %	10,10+11

Legend of figures

Fig. 1. A schematic representation of assimilation of carbon and nitrogen and their utilization in maize plants. The rectangles indicate end products, the circles intermediates. Double lines indicate processes occurring during photosynthesis only, single lines conversions or translocations, and dashed lines CO_2-formation. The numbers give the corresponding weights.

Fig. 2. A schematic representation of assimilation of carbon and nitrogen and their utilization in sunflower plants. Half of the dry matter increase occurs in photosynthezing leaves. For explanation see fig. 1.

Fig. 3a. The relation between assimilation and dissimilation in a "steady state" situation in whole sunflower plants at 25°C.

3b. The relation between assimilation and dissimilation in a "steady state" situation in whole sunflower plants at 18°C.

3c. The relation between assimilation and dissimilation in a "steady state" situation in whole sunflower plants at 25°C. Plants were grown and measured at a diluted nutrient solution.

Fig. 4a. The relation between assimilation and dissimilation in a "steady state" situation in whole maize plants at 25°C, and at 85 % (crosses) and 50 % (dots) relative humidity.

4b. The relation between assimilation and dissimilation in a "steady state" situation in whole maize plants at 18°C.

Fig. 4c. The relation between assimilation and dissimilation in a "steady state" situation in whole maize plants at 33°C.

Desired sizes of figures, including legends:

figure	1	half page
"	2	half page
"	3a+3b+3c	half page
"	4a+4b+4c	half page

FIGURE 1 PATHWAYS OF CARBON FIXATION AND UTILIZATION IN MAIZE PLANTS

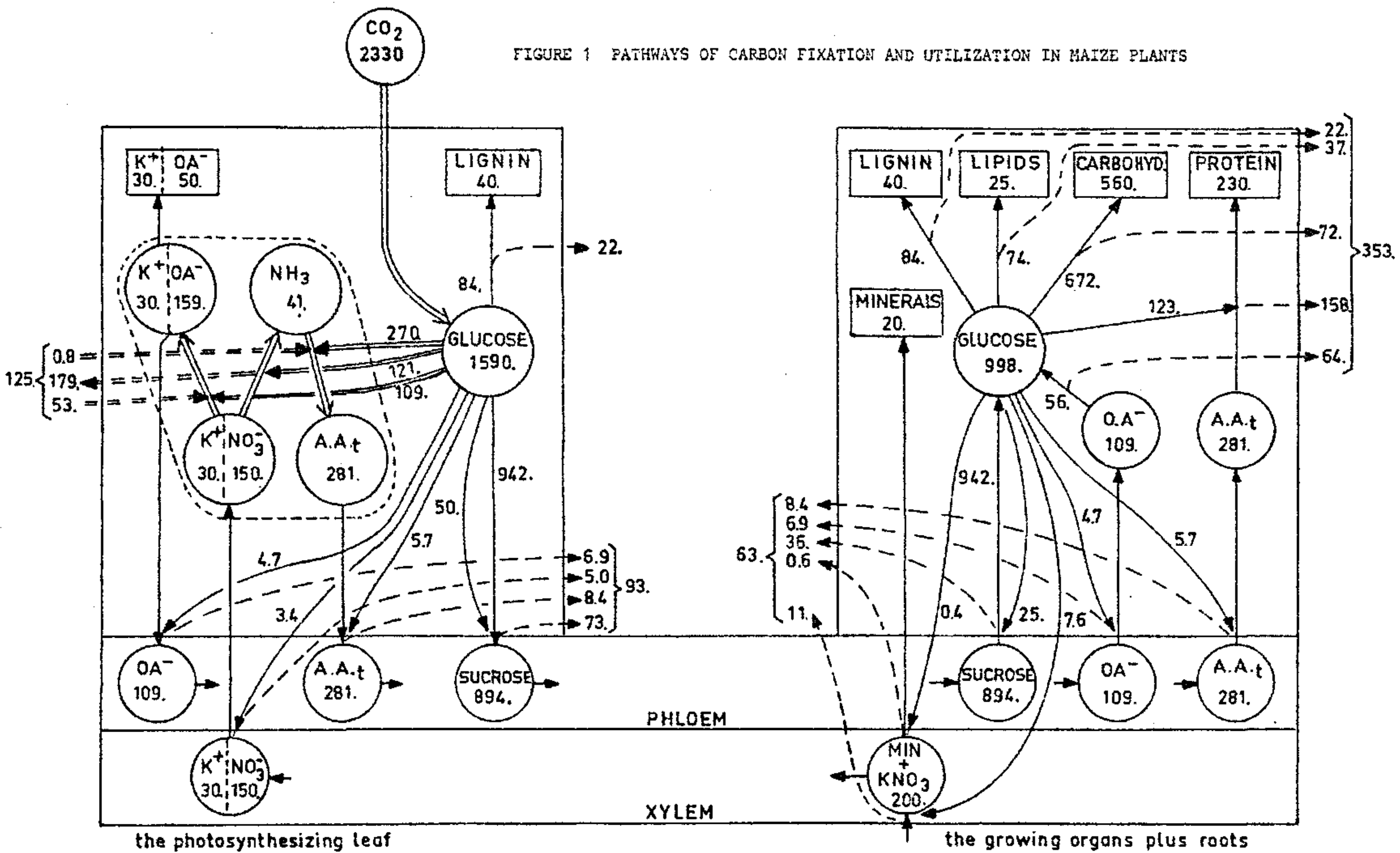
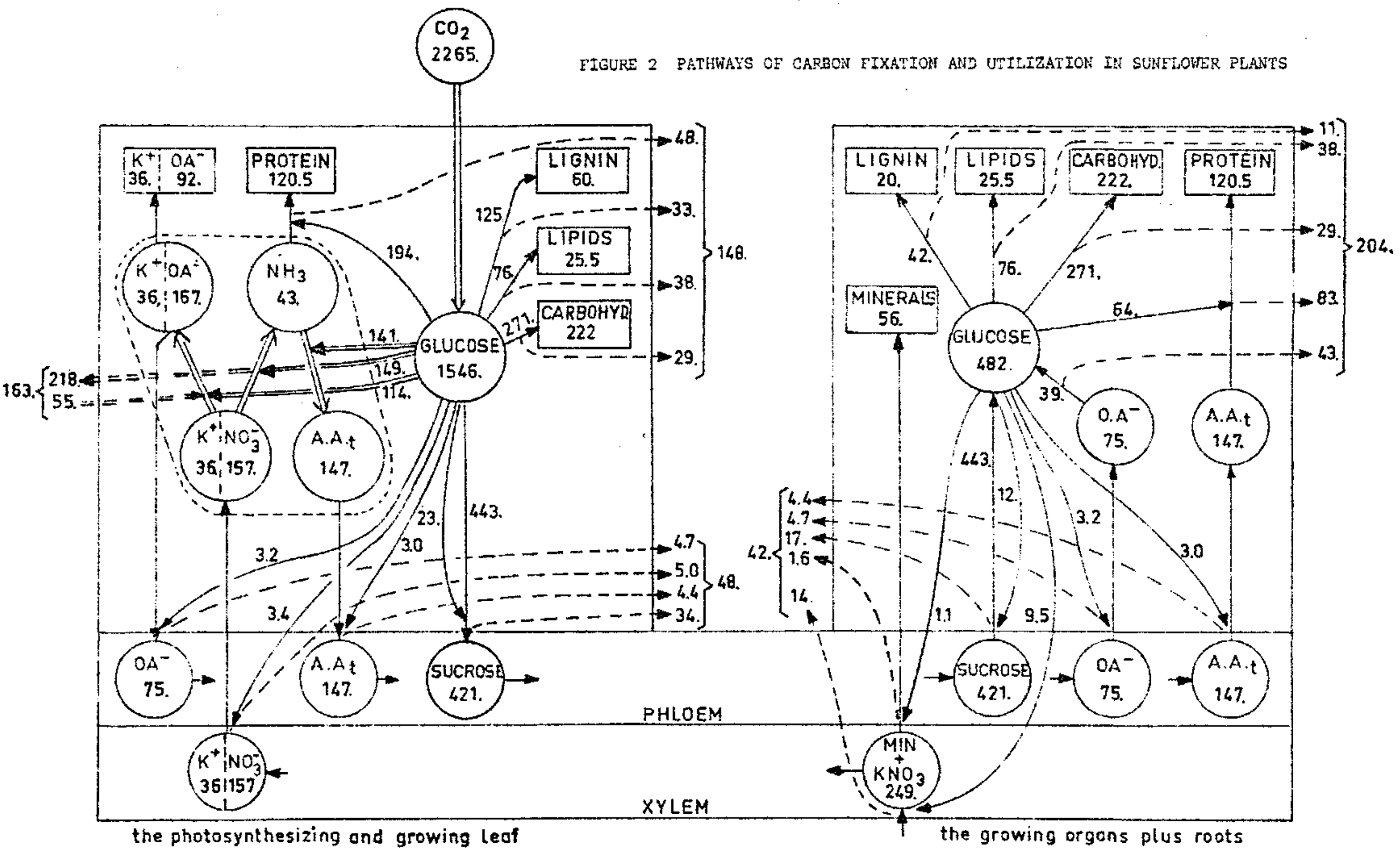


FIGURE 2 PATHWAYS OF CARBON FIXATION AND UTILIZATION IN SUNFLOWER PLANTS



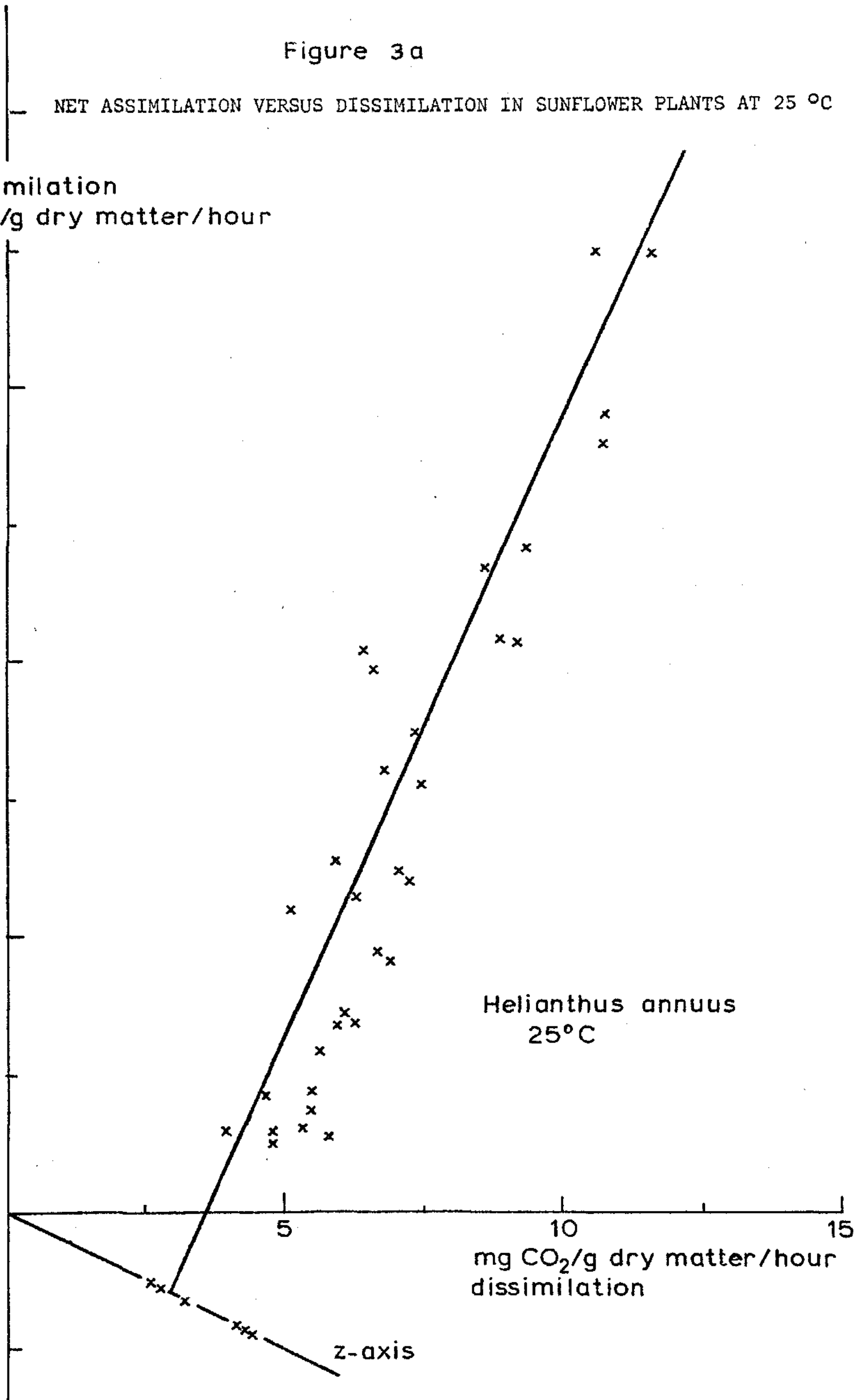


Figure 3a
NET ASSIMILATION VERSUS DISSIMILATION IN SUNFLOWER PLANTS AT 25 °C
40
net assimilation
mg CO2/g dry matter/hour
30
20
10
-5
5
10
15
mg CO2/g dry matter/hour
dissimilation
Helianthus annuus
25°C
z-axis

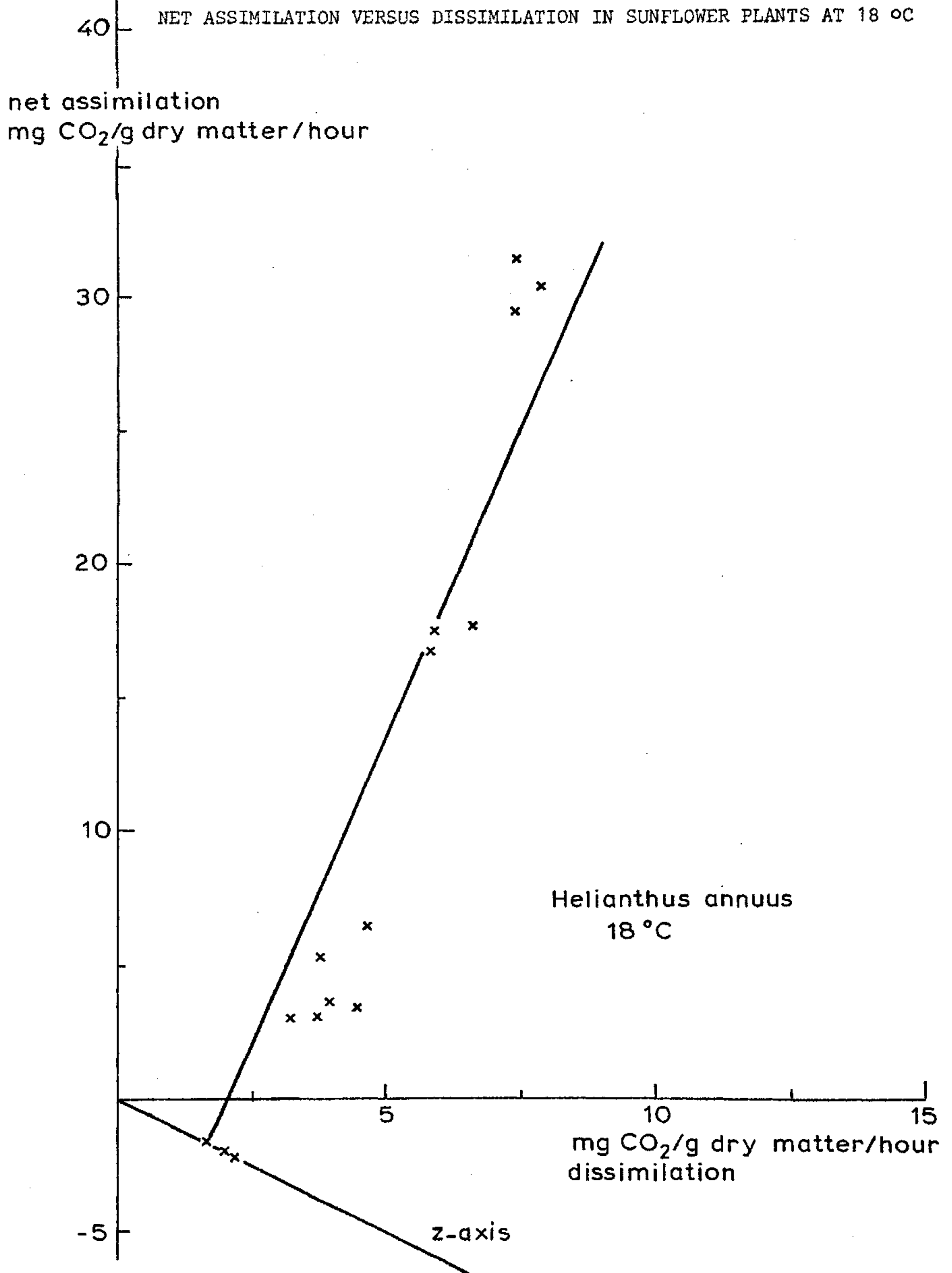


Figure 3b
NET ASSIMILATION VERSUS DISSIMILATION IN SUNFLOWER PLANTS AT 18 °C
40
net assimilation
mg CO2/g dry matter/hour
30
20
10
-5
Helianthus annuus
18 °C
5
10
15
mg CO2/g dry matter/hour
dissimilation
z-axis

FIGURE 3C

NET ASSIMILATION VERSUS DISSIMILATION IN SUNFLOWER PLANTS AT 25 °C

(DILUTED NUTRIENT SOLUTION)

40

net assimilation
mg CO_2/g dry matter/hour

30

20

10

Helianthus annuus,
25 °C, diluted
nutrient solution

5

10

15

mg CO_2/g dry matter/hour
dissimilation

-5

z-axis

FIGURE 4A NET ASSIMILATION VERSUS DISSIMILATION IN MAIZE PLANTS AT 25 °C

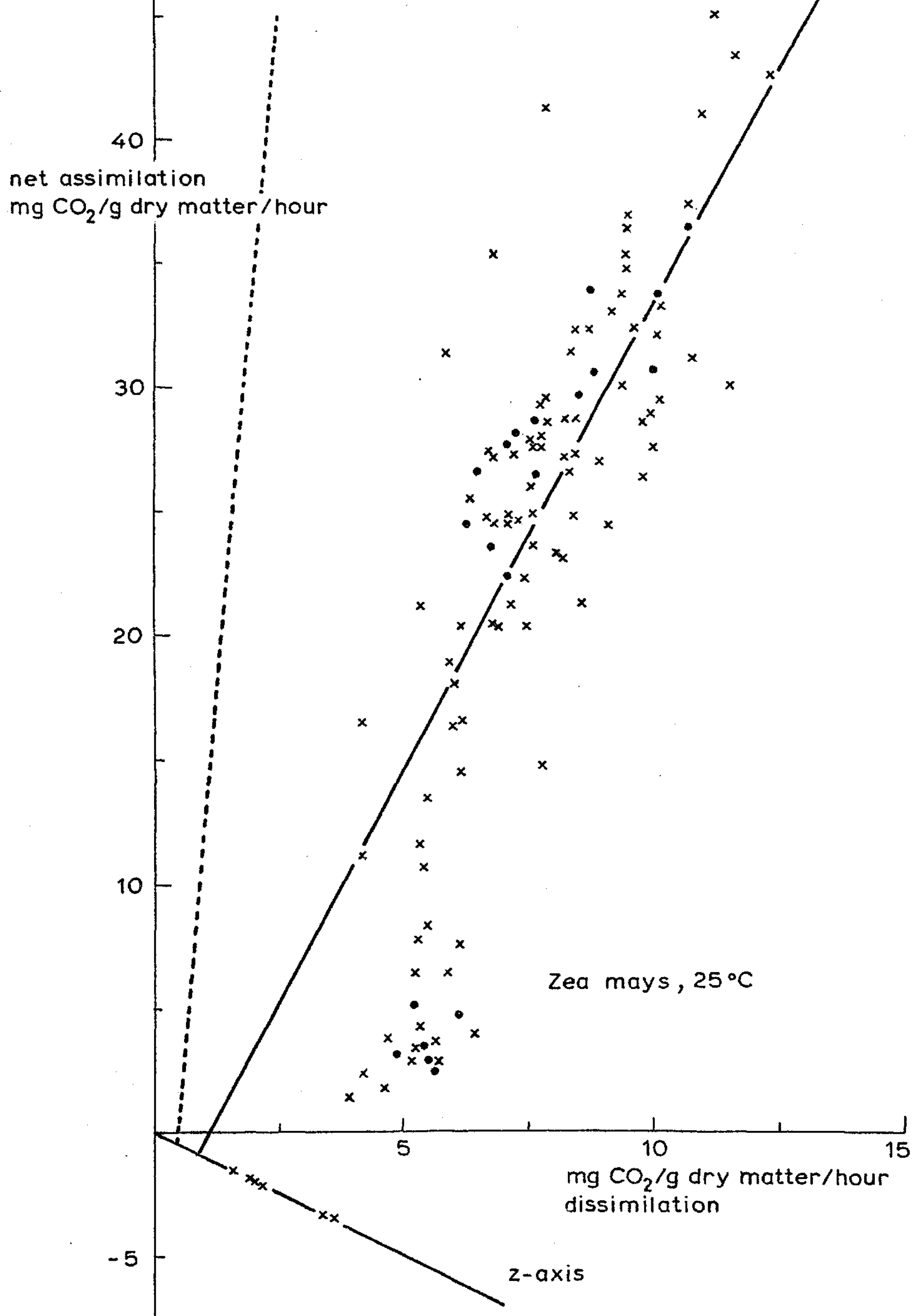


FIGURE 4B NET ASSIMILATION VERSUS DISSIMILATION IN MAIZE PLANTS AT 18 °C

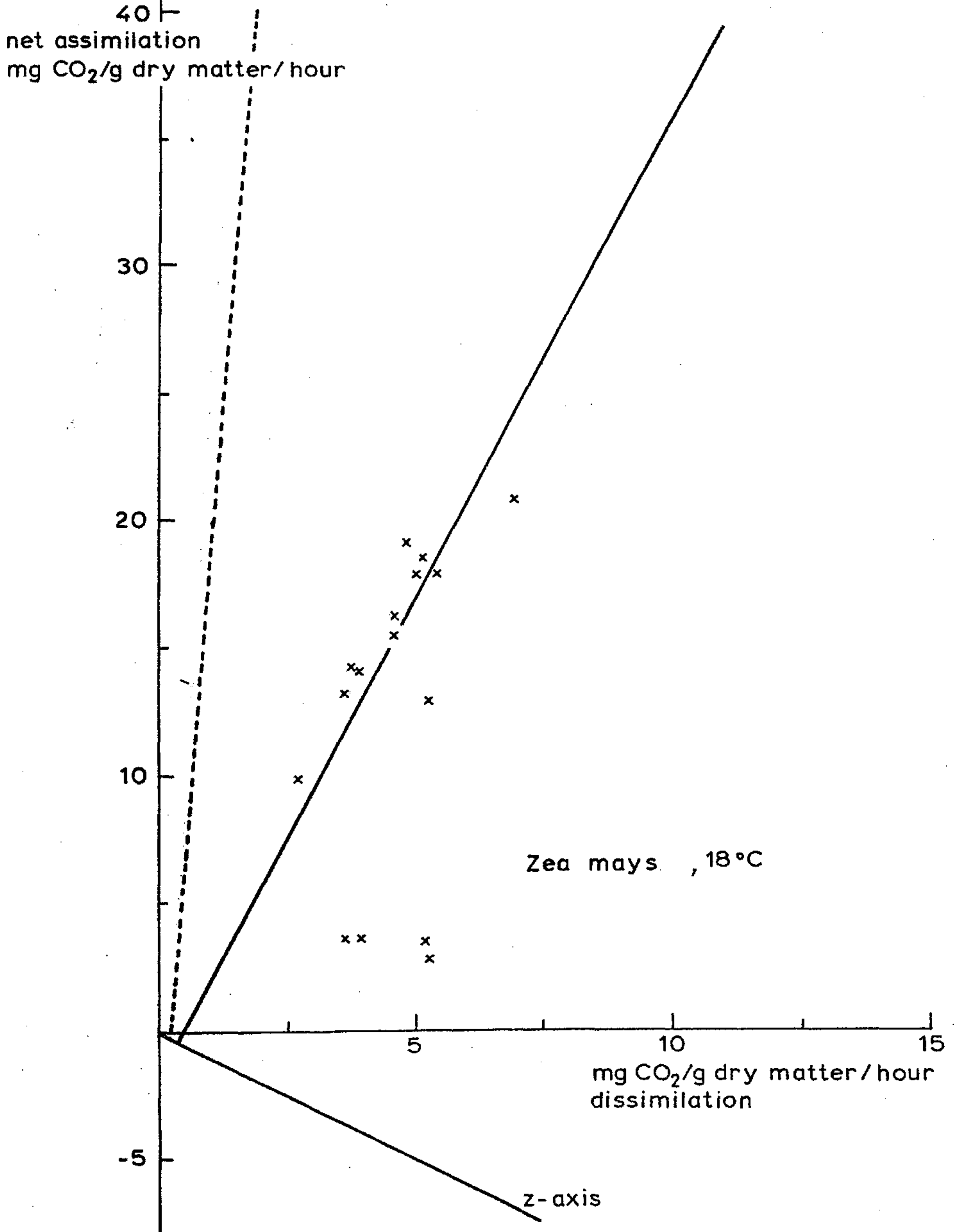
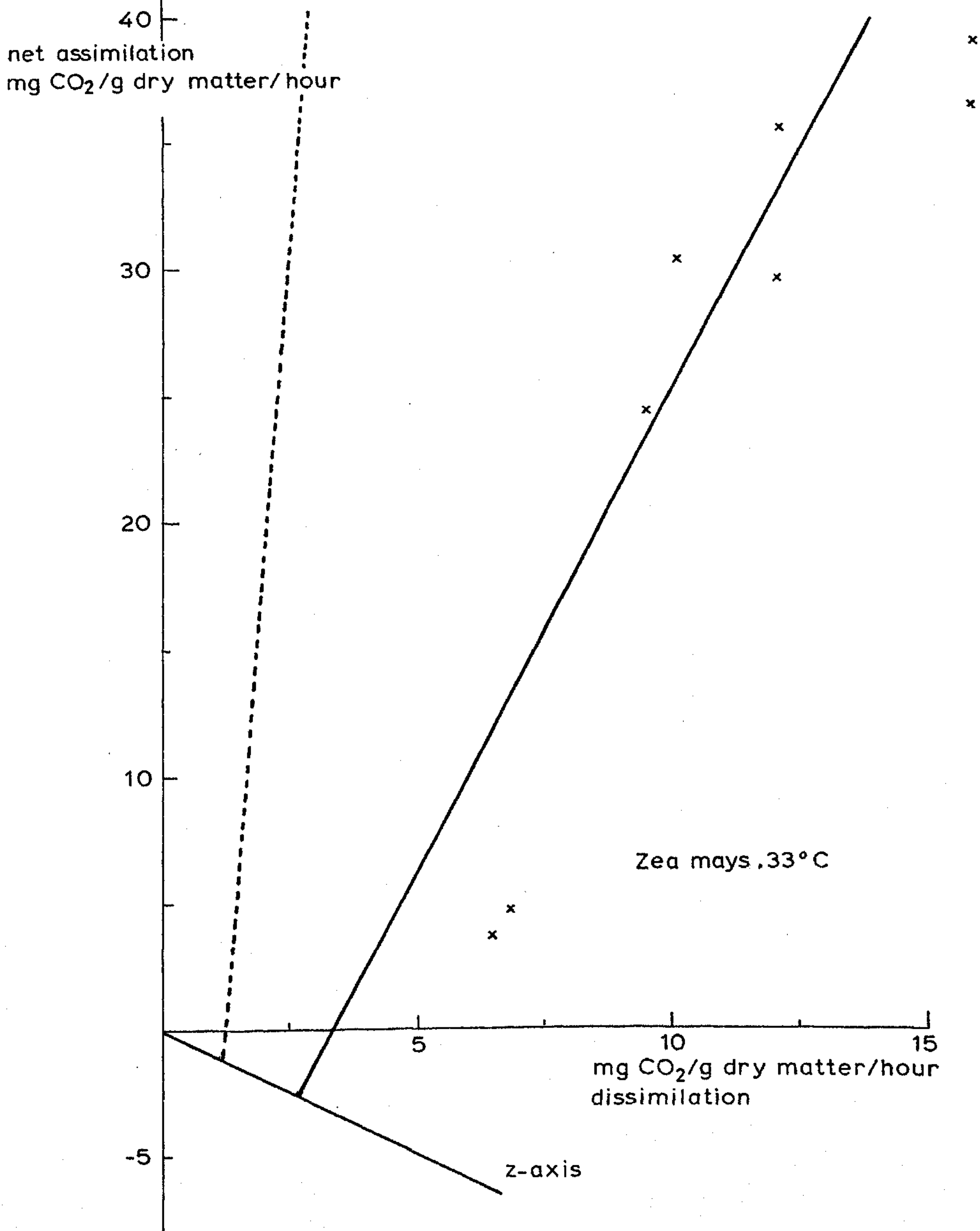


FIGURE 4C NET ASSIMILATION VERSUS DISSIMILATION IN MAIZE PLANTS AT 33 °C



THE COST OF MAINTENANCE PROCESSES IN PLANT CELLS

F.W.T. Penning de Vries

Department of Theoretical Production Ecology

Agricultural University, Wageningen

The Netherlands

Accepted for publication
in the Annals of Botany

THE COST OF MAINTENANCE PROCESSES IN PLANT CELLS

F.W.T. Penning de Vries
Department of Theoretical Production Ecology
Agricultural University, Wageningen

ABSTRACT

The most important maintenance processes in plant cells are protein turnover and active transport processes to maintain certain ion concentrations in the cells. In this paper an attempt is made to calculate the total energy cost of these processes from what is known about their specific costs and what has been observed about their rates. Mainly because of lack of sufficient and reliable data about rates of individual maintenance processes, only approximate values can be obtained as yet.

The average turnover rate of leaf proteins may be about 100 mg protein per g proteins per day at normal temperature in leaves assimilating at moderate light intensities. This process consumes 28-53 mg glucose per g protein per day, which equals 7-13 mg glucose per g dry weight per day in leaves. It is likely that the protein turnover rate and cell metabolic activity are related. The cost of maintaining ion concentrations is estimated to be about 6-10 mg glucose per g dry weight per day in leaves. This value is also an approximatior mainly because the intercellular ion concentrations are unknown. The sum of the figures is lower than what,inaccurate,measurements of maintenance respiration rates indicate. One reason for the underestimation may be that the protein turnover rates used in the calculations apply to plants that were less metabolically active than the plants of which the maintenance respiration was measured.

Effects of water stress and salinity, temperature and other environmental factors on the rate of maintenance processes are discussed, but at the present stage of physiology only a few observations of changed maintenance respiration

rates can be fully explained.

The consumption of assimilates for maintenance of plant cells is a significant, negative factor for plant productivity. A better understanding of maintenance respiration processes may give a clue to manipulating plant environment and plant characteristics for reducing the amount of assimilates consumed in these processes. It is suggested that an artificial reduction in protein turnover rates may be one of such manipulations, while possibly also the cost of maintaining ion concentrations can be lowered. However, a considerable number of well directed studies about rates of maintenance processes will have to be carried out first.

INTRODUCTION

All living cells expend energy for maintenance purposes. A few attempts have been made to establish experimentally the energy involved in plants and it was found that this can be an appreciable amount. In so far as the author is aware no study has been published on calculating the maintenance cost of plant cells from basic data. This paper presents an introductory calculation of the energy and substrate requirement for the maintenance of cells of higher plants, which is largely based on information from the literature on nature and rate of underlying processes. The present knowledge only allows to make a first estimate of maintenance cost, but does already indicate main areas for further research.

The term "maintenance" includes the processes for maintaining cellular structures and gradients of ions and metabolites, and physiological adaptation processes that maintain the cell as an active unit in a changing environment. Formation of new enzymes at the expense of others and salt accumulation in some stress conditions are examples of such adaptations. Hence, maintenance is not a process as conservative as its name suggests. When using the concepts "maintenance" and "growth" it has to be realized that these terms always concern a certain level of complexity: an ecologist may consider growth of

an organism as one of the processes maintaining the population size, while maintenance of the number of erytrocytes is a growth process to the histologist This study concentrates on maintenance of cells. Some redistribution processes in plants can be regarded as maintenance processes of the organism, but these are not studied in this paper. "Maintenance respiration" refers to the CO_2 that results from protein breakdown, plus the CO_2 produced in respiratory processes that provide energy for the maintenance processes.

In calculating the maintenance cost information is needed about the rates of processes, their specific costs and the efficiency of energy production. Derivation of rates of individual maintenance processes from more basic data is futile as yet, since little is known of their mechanisms and regulations. Thus, observations of such rates are indispensable. Mainly because their number is still very limited only some general conclusions can be drawn with respect to the magnitude of the cost of maintenance processes in plant cells. To find the specific cost of maintenance processes, the cost of maintaining concentrations of ions and metabolites, the biochemical cost of breakdown and resynthesis of molecules and the use to be made of breakdown products will be considered. Beevers (1961) collected evidence that in plants under normal conditions the efficiency of energy utilization from organic substrates, measured as the P:O ratio of $NADH_2$ oxidation, is close to the maximum value of 3, as in animals (Lehninger, 1970). Lower P:O ratios have often been observed, but may be attributed, at least partly, to the procedure by which mitochondria were isolated (Romani and Ozelkok, 1973). In the calculations it will therefore be assumed that mitochondrial phosphorylation (in non stressed conditions) is maximally efficient; for bacteria there is evidence that the P:O ratio may be considerably less than 3 (cf Stouthamer, 1973; Stouthamer and Bettenhausen, 1973). The substrate demand for maintenance processes increases inversely with this efficiency. Since glucose or other oligo-saccharides are consumed first for energy production, maintenance cost will be expressed in mg glucose per g dry matter per day. Because the ratio

of moles CO_2 produced to moles O_2 consumed in mature organs under normal conditions is mostly about unity (James, 1953) complete oxidation of glucose will be assumed.

RATES AND COST OF MAINTENANCE PROCESSES UNDER STANDARD CONDITIONS

Maintenance of molecular structures

Breakdown and resynthesis of nitrogenous compounds. Only a short account of the present state of research on protein turnover will be given, for details the reader is referred to reviews by Glasziou (1969), Schimke (1969), Schimke and Doyle (1970), Pine (1972) and Siekevitz (1972). In spite of much research in this field it is still difficult to construct more than a general picture of protein turnover in different organisms and in plant organs particularly.

Mostly protein breakdown is an enzymatic process (Travis, Huffaker and Key, 1969; Pine, 1972), but no energy seems required for peptide bond cleavage (Pine, 1972). Once degradation starts enzyme activity decreases exponentially. Schimke (1969) and Pine (1972) suggested that, as a general rule, enzymes may be automatically stabilized against proteolysis when interacting with their substrates. Both enzyme synthesis and breakdown can be regulated (Schimke and Doyle, 1970; Trewavas, 1972), but the control mechanism in eukaryotes is still largely unexplained. Long term changes of enzyme activity indicate mostly breakdown or de novo synthesis (Beevers and Hageman, 1969; Travis et al., 1969; Zucker, 1972; Oaks et al., 1972).

Information collected by Pine (1972) indicates that the bulk of bacterial protein (70 %) is stable. A small fraction (1-7 %) has a turnover rate constant (abbreviated to turnover rate) of 17. - 70. g protein per g protein per day (abbreviated to 17. - 70. day^{-1}; it represents the relative rate of decrease when exponential decay occurs). The other proteins are stable in growing bacteria, but have a turnover rate of about 2.4 day^{-1} in non growing conditions when these result from nitrogen starvation. Overall protein turnover

amounts to 0.6 - 0.7 day^{-1} in growing, and to 1.2 - 1.4 day^{-1} in non growing bacteria.

The literature about plant protein turnover is limited and concerns mainly leaves. Turnover rates of total leaf protein were reported to be about 0.10 day^{-1} in tobacco leaves (Holmsen and Koch, 1964), measured as the rate of incorporation of $^{14}CO_2$ into proteins. Since protein turnover is possible without incorporation of $^{14}CO_2$ into amino acids this method provides a minimum value. On the other hand, $^{14}CO_2$ is usually supplied at a high concentration in high light, and a resulting high assimilation rate may stimulate enzyme synthesis. Racusen and Foote (1962) determined a rate of loss of labelled protein-serine and protein-glycine of bean leaf discs and found about 0.22 day^{-1}. Hellebust and Bidwell (1964b) reported protein turnover rates of 0.11 day^{-1} in rapidly growing wheat leaves and 0.04 day^{-1} in expanding tobacco leaves, while that of non growing wheat leaves was 0.06 day^{-1}, and almost zero in non growing tobacco leaves. They measured the rate of loss of $^{14}CO_2$ from previously labelled proteins, which in fact reflects amino acid turnover rather than protein turnover. The decrease in turnover rate was ascribed to cell differentiation being finished. The rate of protein breakdown in growing Lemna minor under normal conditions is 0.096 $\pm$ 0.005 day^{-1} (Trewavas, 1972). Protein turnover in Chlorella was found to be as low as 0.01 - 0.02 day^{-1} (John,Thurston and Syrett, 1970). In these studies relatively rapid labelling or loss of label of a small fraction was not observed, suggesting that either a fraction with a high turnover rate was very small or absent, or that it had a separate amino acid pool. The previously labelled soluble protein fraction minus ribulose 1,5 diphosphate carboxydismutase (RuDPCase) of barley leaves comprises about half of the total leaf proteins and showed a loss of $^{14}CO_2$ of 0.14 day^{-1} at a constant light intensity of about 30 W m^{-2} (Peterson, Kleinkopf and Huffaker, 1973). Such protein turnover rates allow adaptation of the enzyme system capacity to environmental changes in 2 to 4

days, which is the correct order of magnitude.

More information about protein turnover can be found on the level of individual enzymes. However, seldom quantities of enzymes are reported, which is a great handicap in using these data. Some enzyme turnover rates have been collected in Table 1. The high rate of vacuolar invertase in sugar cane was questioned by Trewavas (1972) on the basis of the method used. Degradation and resynthesis of RuDPCase in expanded barley leaves was investigated by Peterson et al. (1973). In darkness the rate of degradation was about 0.06 day^{-1} initially, increasing to 0.38 day^{-1} after 30 hrs, and then decreasing. When the plants were returned to the light RuDPCase was resynthesized at a rate of 0.12 day^{-1} initially and of 0.55 day^{-1} after 20 hrs. Degradation of RuDPCase in constant light was not observed and its turnover within the chloroplasts is unlikely. Turnover of RuDPCase is particularly important, since it constitutes 30-70 % of the total leaf soluble proteins.

As in bacteria and yeasts (Mandelstam, 1960), DNA turnover in full grown leaves is very slow (Dyer and Osborne, 1971). Because of the small amount involved in plants (0.5 - 1.5 % of the weight of the nitrogenous compounds) and the low cost of resynthesis from monomers, DNA maintenance will be neglected.

The rate of mRNA turnover seems related to the rate of protein synthesis (Norris and Koch, 1972; Roth and Dampier, 1972) and is much more intensive than that of tRNA and rRNA in growing bacteria (Norris and Koch, 1972) and in growing Lemna minor (Table 1). Cost of RNA turnover in growing cells is small compared to the cost of amino acid polymerization (e.g. Stouthamer, 1973). Maintenance of RNA and enzymes for biosynthetic processes was estimated to consume the energy of about 1 ATP molecule per peptide bond (Penning de Vries, Brunsting and Van Laar, 1974). In analogy,it is assumed that the cost of maintaining proteases and their mRNA do not exceed 1 ATP molecule per peptide bond of the degraded proteins. Polymerization cost is 3 or 4 ATP molecules per peptide bond (Lucas-Lenard and Lipmann, 1971), so that the lowest turnover

Table 1. Some enzyme and RNA turnover rates in various plant tissues under normal conditions.

enzyme and tissue	turnover rate (day^{-1})	reference
NO_3^--reductase, maize seedlings	3.6	Glasziou, 1969
NO_3^--reductase, maize roots, degradation rate in a NO_3^--free medium	4.1	Oaks et al., 1972
hexose uptake system, Chlorella sp.	4.1	Tanner et al., 1970
phenylalanine ammonia lyase, mustard seedlings, degradation rate in darkness	2.9	Glasziou, 1969
isocitrate lyase, Chlorella sp., degradation rate in darkness	2.2	John et al., 1970
invertase, sugar cane	4.8	Glasziou, 1969
invertase, artichoke and carrot	1.5	Trewavas, 1972
invertase, sugar beet and red beet	0.7	Trewavas, 1972
cellulase, pea epicotyl	0.6	Glasziou, 1969
RuDPCase, expanded barley leaves,		
degradation rate in darkness	0.06-0.38	Peterson et al., 1973
synthesis rate in light	0.12-0.55	Peterson et al., 1973
isocitrate lyase, melon	0.36	Glasziou, 1969
malate synthetase, melon	0.36	"
RNA-ase	0.	"
peroxidase	0.	"
NO_3^--reductase mRNA, maize roots	48.	Oaks et al., 1972
mRNA, potato tuber	7.	Glasziou, 1969
peroxidase,mRNA, sugar cane	7.	"
cytoplasma rRNA, Lemna minor	0.17	Trewavas, 1970
chloroplast rRNA, Lemna minor	0.05	"

cost per amino acid is about 5 or 6 ATP molecules per bond, and this corresponds to 0.22 - 0.26 g glucose per g protein.

Some amino acids, however, are not recycled but combusted and new amino acids are formed. This process causes $^{14}CO_2$ release from previously labelled proteins. Synthesis of 1. g of proteins requires 1.655 g glucose plus NH_3, while its degradation yields NH_3 plus as much energy as 1.22 g glucose does, when it is completely oxidized. In this case protein and amino turnover require 0.43 g glucose per g protein. The latter value should be used when loss of $^{14}CO_2$ from labelled protein is determined, and one between these two values if loss of enzyme activity is measured. The magnitude of the fraction recycled and that combusted is almost unknown (Hellebust and Bidwell, 1964a; Trewavas, 1972). From the different turnover rates of various amino acids in Trewavas experiments it may be inferred that an important fraction was recycled in that particular case; Lehninger (1970) mentions that in man over 75 % of the amino acids are recycled. No other indications about this important parameter were found.

In a detailed description of many experiments Shlyk (1970) reported an average rate of about 0.10 day^{-1} for breakdown and de novo synthesis of chlorophyll in growing and mature leaves of higher plants. From the biochemical pathways leading to chlorophyll it can be calculated that synthesis of 1. g chlorophyll requires 2.29 g glucose plus some NH_3 and Mg, while its breakdown yields about 1.5 g glucose per g chlorophyll. Leaves contain only 15-35 mg chlorophyll per g nitrogenous compounds, and it follows from these figures that cost of chlorophyll turnover is much smaller than that of leaf proteins.

To calculate the total cost of maintenance of the leaf nitrogenous compounds roughly some assumptions are made. Lexander et al. (1970) found that the non-protein nitrogen fraction in the leaves of 22 species was about 10 % of the total nitrogen fraction, with only two exceptions: Helianthus annuus (2.5 %) and Brassica napus (31 %). It is assumed therefore that 10 % of the leaf nitrogenous compounds are stable. Furthermore it is assumed that 44 %

of the leaf organic nitrogen is in RuDPCase, and that this enzyme is degraded for 10 hours per day at a rate of 0.144 day^{-1}, that the resulting amino acids are stored in protein and that the enzyme is resynthesized for 6 hours per day at a rate of 0.24 day^{-1}. Three percent of the organic nitrogen is supposed to be in chlorophyll, and the rest in proteins with a constant turnover rate of 0.15 day^{-1}. From these rates it follows that the maintenance cost of nitrogenous compounds is between 28 and 53 mg glucose per gram per day.

Because the rate of protein synthesis is not constant, has has been demonstrated in vivo by Steer (1973), the cost of protein turnover shows a diurnal pattern. A relatively large fraction of these costs are required in the first hours after the onset of light. It is thus likely that induction of enzyme synthesis at a sudden onset of light causes a burst in energy requirements, and thus in respiration. Heichel (1970) reported a stimulation of maize leaf respiration for 2-3 hours following an illumination of 20 minutes in CO_2 free air. These experiments were repeated by the author with mature leaves of Lolium perenne, Phaseolus vulgaris, Zea mays and Helianthus annuus, grown in high light conditions. An illumination period of 60 minutes (300 W m^{-2}) was used, preceded by a period of darkness of 1 - 24 hours, and in an atmosphere of 300 ppm CO_2. The "post illumination burst", from which is subtracted the cost of translocation of the assimilates formed (Penning de Vries, 1974), was small after 1 and 3 hours darkness, and about twice as large as that observed by Heichel after 6 - 24 hours, except in Lolium where stimulation remained small. Heichel found that the CO_2 produced in this "post illumination burst" depends on the irradiance. It corresponds with the amount of CO_2 formed, when 1 - 4 % of the leaf proteins are broken down and others resynthesized. Zucker (1972) reported that after induction 5 - 70 % of the proteins synthesized may be a single species, the amount of which may well exceed 1 % of the total protein (Mandelstam, 1960; Tanner, Grimes and Kandler, 1970). Amino acids for these enzymes may come from chymotrypsin inhibitor 1, which serves as a reserve protein (Ryan and Huisman, 1970). It is suggested

that the physiological phenomenon of "post illumination respiration" and the biochemical phenomenon of enzyme induction are different aspects of the same process.

Breakdown and resynthesis of lipids. Bieleski (1972) observed that ^{32}P released from phospholipids in growing Spirodela was less than 0.2 day^{-1}. Kawaga et al. (1973) measured disappearance of ^{14}C from membranes in germinating castor bean endosperm, and observed rates of overall membrane turnover of 0.4 - 1.7 day^{-1}, and of 4.8 day^{-1} of a part of the "light membrane fraction". Lecithine molecules and even larger membrane fractions were possibly recycled. In non-growing animal cells membranes turned over at a rate of 0.5 - 1.2 day^{-1} (Pine, 1972). Estimating that 4 % of the dry weight consists of membranes (from estimates of total membrane surface, its thickness and density), and assuming arbitrarely that one tenth of its proteins and lipids are completely degraded and resynthesized from glucose and that the rest is recycled, the cost of membrane maintenance is 1.7 mg glucose per g dry matter per day.

Breakdown and resynthesis of other cell components. Bacterial cell wall components were found to have a turnover rate of 0.3 - 1.0 per generation time (Mauck and Glaser, 1970), but cell walls of higher plants are stable. Holmsen and Koch (1964) found no labelling of polysaccharides after adding labelled glutamate to the leaves. Auxin was shown to be rapidly degraded within the cell (34. day^{-1}, DelaFuente and Leopold, 1970), but this concerns an extremely small amount. Cell organells are not turned over as units, except for very small organalls (Pine, 1972).

Use of turnover. The use of turnover processes in plants may be shortly discussed here because of its important implications for plant productivity. Siekevitz (1972) speculated about protein denaturation by heavy metals, incorrectly constructed

proteins and optimalization of RNA and DNA use as possible reasons leading to or justifying protein turnover. Mandelstam's suggestion (1960), however, that protein breakdown and synthesis mainly provides a means of formation of other enzymes when net cell growth is stopped is now widely accepted for bacteria and animals (Lehninger, 1970; Pine, 1972), and explains the mode of adjustment of the biochemical machinery to environmental changes and possibly anticipation thereof. Only rapid and constant turnover of some proteins in growing bacteria is not explained by this hypothesis. Adaptation to a changed temperature can occur by formation of (iso-)enzymes in mature cells. Such a process has been observed for the enzym NADP-isocitrate dehydrogenase in rainbow trouts (Moon and Hochachka, 1971) and is a form of adaptation for which breakdown and synthesis of proteins are required. Adaptation supports competition vigor and thus survival, but its burden is cost of protein turnover.

Plant cells function in a variable physical environment, but their chemical environment is much more stable than that of bacteria. The time needed for adaptation of enzyme activity in leaf cells to a changed level of light intensity or temperature is considerably larger than the time needed by bacteria to respond to a modified medium composition. This allows the cost of protein turnover in plant cells to be correspondingly lower than in bacteria.

The degradation and resynthesis rates of RuDPCase, NO_3^--reductase and other enzymes (Table 1) enable much more rapid adaptation than is required in modern agricultural systems, where much of the care for interspecific competition has been taken over by the farmer, and where man also breeds for better varieties. For NO_3^--reductase and other rapidly vanishing enzymes in particular, the degradation rate seems more rapid than is required for adaptation processes under all circumstances (cf. Schimke, 1969). It is suggested that an artificial reduction of the rate of protein turnover may increase net crop growth rates, because the crop maintenance cost is lowered more than the reduction of assimilation caused by a decreased rate of adaptation to changing conditions. Complete removal of protein turnover reduces maintenance cost by

about 10 mg glucose per g dry weight per day, or 10 - 40 kg carbohydrates per hectare per day. It is suggested that partial or complete inhibition of protein turnover in full-grown leaves can be obtained for a part or the whole growing season by use of chemicals, phytohormones or by plant breeding. Various substances have been shown to affect the rate of protein synthesis and degradation at different stages. Chemicals and phytohormones inhibiting protein turnover should be applied in such a way that biosynthesis in growing parts is not reduced and reallocation of nitrogenous compounds in the reproductive phase is not hampered. Manipulating the maintenance respiration cost may also be applicable in many different fields of plant production. The role of protein turnover in plant resistance against diseases, however, should be studied carefully.

Maintenance of ion concentrations

The presence of an indiffusible ion species in the cell, such as negatively charged proteins, causes concentration gradients of the diffusible ions across the cell membrane according to a "Donnan-equilibrium". Except for the possible cost of maintaining the indiffusible ion, maintenance of these concentrations does not require energy because the electrochemical potential is the same on both sides of the membrane. Diffusion of ions through the membrane causes a passive flux from and into the cell. The actual intracellular ion concentrations would be strongly influenced by the ion composition of the medium if controlled only by the "Donnan equilibrium". By means of active transport systems, that move ions across the cell membrane at the expense of metabolic energy, cells maintain certain ion concentrations in their compartments. Thus the distribution of ions between cytoplasm and environment usually does not correspond with a "Donnan-equilibrium". Across the tonoplast of algal cells ion gradients are reported, and this holds probably also in higher plants (Hope, 1971; Anderson, 1972).

Compared with studies on active transport in nerve and muscle cells little research has been carried out on plant cells (Anderson, 1972). No reports have been found on ion fluxes across cell membranes in tissues of higher plants in vivo. Active ion fluxes have been studied in algal cells and in dark grown tissues of higher plants bathing in a nutrient solution. Data of active fluxes in steady state conditions were collected in Table 2. In actual plants the ion concentration of xylemsap was found to be 0.1 to 0.7 times that of a normal nutrient solution (cf. Milthorpe and Moorby, 1969), and it is likely that ion concentrations are higher in intercellular spaces of leaves, from where water evaporates. The ion composition of the liquid in these spaces is different from that of a nutrient solution; nevertheless it is assumed that fluxes observed in nutrient solution give a fair impression of the order of magnitude of fluxes in vivo.

In autotrophic cells in- and efflux can be enhanced 2 to 5 fold by light (e.g. Hope, 1971), but only fluxes in darkness will be considered, because this study was made to obtain an estimate of maintenance cost of plant cells in darkness. Possible extra expenses in the light are neglected because the method employed to compute the crop daily gross assimilation (cf. De Wit, Brouwer and Penning de Vries, 1970) accounts for this directly (Penning de Vries, 1974).

More is known about energy expenditure for active transport processes. In animal cells and mitochondria ion extrusion or accumulation and ATP consumption are clearly related (Stein, 1967; Lehninger, 1970). Extrusion of Na^+ from the cell requires about 1 ATP molecule per translocated ion. This transport occurs by two mechanisms, in one of which uptake of K^+ is coupled to export of Na^+ in a 1:1 relation, and in the other uptake of K^+ for other ions, amino acids or glucose is loosely coupled to Na^+ transport. Animal mitochondria do not accumulate K^+ or Na^+, but Ca^{2+} and inorganic P are taken up at a cost equivalent to 0.6 and 1.0 ATP molecules respectively (Lehninger, 1970). Higher plants seem to use different transport mechanisms: active Na^+

Table 2. Some active fluxes across plasmalemma (p) or tonoplast (t) in darkness in steady state conditions.

flux	membrane	rate 10^{-12} mole/cm^2/sec	tissue and condition	reference
K^+ influx	p	1.4	Avena sativa, coleoptyle, in nutrient solution	Pierce and Higinbotham, 1970
Na^+ efflux	p	> 0.3	"	"
Cl^- influx	p	0.5	"	"
K^+ influx	t	1.7	"	"
Na^+ influx	t	0.3	"	"
Cl^- influx	t	0.1	"	"
K^+ influx	p	0.7	Pisum sativum, epicotyl, in nutrient solution	Macklon and Higinbotham, 1970
K^+ influx	t	1.	"	"
all ions, efflux	p	1.3-2.8	Nitella sp.	Vredenberg, 1972
K^+ influx	p	15.-30.	Acetabularia sp.	Saddler, 1970
Na^+ efflux	p	3.-10.	"	"

efflux has been observed, but also active K^+ and Cl^- influx independent of Na^+ extrusion. Formation of these transport systems is often inducible (Anderson, 1972). Information about the energy cost of moving ions across plasmolemma or tonoplast is mainly qualitative (Anderson, 1972), but it is unlikely that these processes are much less efficient in plants than in animals. Fisher and Hodges (1969) and Kirk and Hanson (1973) reported values of 0.6 to K^+ ion per ATP molecule in maize mitochondria, and such values were also found for erytrocytes (Lehninger, 1970). There is still discussion whether ATP is used as an intermediate to provide energy for transport (Anderson, 1972), but the net cost of such mechanisms can always be expressed in ATP units, since finally energy consumption is always competitive with ATP production.

To obtain an order of magnitude of the energy consumption for active ion transport in plants, it is assumed, on basis of the information of Table 2, that active fluxes amount to $1\text{-}2.10^{-12}$ mole per cm^2 plasmalemma per sec. Furthermore it is assumed that a considerable fraction of this flux represents coupled active transport, and that per active ion movement the energy of 1 ATP molecule is required. Thus an energy flux may be consumed equivalent to 1.10^{-12} mole ATP per cm^2 plasmalemma per sec. Cells with dimensions of 40x40x40 μ have about $1.2\ 10^4\ cm^2$ plasmalemma per g dry weight, thus maintenance of these fluxes consumes about 4 mg glucose per g dry weight per day. In young and small cells this value may be larger, while in large parenchyma cells it will be lower. Active fluxes across the tonoplast may be roughly similar to that across the plasmalemma (Table 2). Pitman (1969) on basis of simulation, concluded to considerable activity of the tonoplast in barley root cells, and Lüttge, Cram and Laties (1971) concluded that salt stimulated respiration above a salt concentration of 0.5 mMol is related to transport across the tonoplast. Without much evidence it is assumed that other membranes do not require much energy to maintain ion gradients, and that the cost of re-uptake of glucose and amino acids leaked from the cell are

negligible. The total energy requirement for maintenance of ion gradients across the cell membranes is thus estimated to be 6-10 mg glucose per g dry weight per day. This figure depends on the concentration and concentration gradient maintained and on membrane permeability. Probably mainly the latter varies with tissue and species, and the second with environmental conditions.

When the total maintenance requirement for plant tissue is about 40 mg glucose per g dry weight per day, the cost of maintaining ion gradients corresponds to about 20 %. This value is about similar to that estimated by Keynes and Maisel (1954) for the relative cost of this process in resting frog muscle cells. The respiratory energy in erytrocytes and active kidney cells, however, is required mainly for maintenance of the ion gradient (Netter, 1969, pg. 763) and ion uptake (Lehninger, 1970, pg. 616), respectively.

The high active fluxes in Acetabularia (Table 2) can be maintained only as a result of the small surface volume ratio of the large cells. The surface volume ratio of bacteria is very much larger, and it can be inferred that either their membrane permeability or the gradient maintained is much smaller. Stouthamer and Bettenhausen (1973) observed that maintenance respiration in Azotobacter aerogenes is enhanced by increasing NH_4Cl concentration in the medium, but this did not occur in Azotobacter vinelandii because of highly impermeable membranes (Knowles and Smith, 1971). This difference demonstrates the variability that exists among species in this respect.

The use of a constant active transport of ions across cell membranes, other than to maintain certain ion concentrations, is unknown. It has been suggested that part of the uptake of amino acids and glucose into animal cells is coupled to passive Na^+ influx (cf. Lehninger, 1970), and also that ion uptake may be related to protein synthesis at membranes (Sutcliffe, 1973). It is also possible that plants have not yet been able to develop membranes with mechanisms by which exclusively essential substances are transported.

Other maintenance processes and wasteful respiration. There is no indication that a noticeable amount of respiratory energy is required in plants to provide heat (except in a few, particular cases), or for displacements other than of ions. Active leaf movements require very small amounts of energy. There is some indication (Pickard, 1972) that protoplasmastreaming is not a separate energy requiring process, but results from other processes.

The existence of wasteful respiration processes in plants ("uncoupled respiration" or "idling respiration") has been suggested to explain unexpected high respiration rates (Beevers, 1970) or low yields (Tanaka, 1972), but no conclusion evidence has as yet been found. Beevers (1970) suggested that a considerable fraction of glucose consumed in mature leaves may be degraded by the pentose phosphate pathway, as an alternative to the Krebs cycle. The $NADPH_2$ generated by this pathway is mainly oxidized by cytoplasmic oxidases and does not yield ATP. Also a high concentration of fatty acids in plant cells decreases the P:O ratio (Baddely and Hanson, 1967). However, whether these examples indicate a useless decrease of efficiency of substrate utilization is difficult to assess since we do not have a criterium for usefulness of biochemical processes. It was suggested (pg. 10) that a part of protein turnover may represent a process of little use, e.g. the rapid degradation and resynthesis of NO_3^--reductase. Measured as respiration rates, useful and useless processes cannot be distinguished. The presence of wasteful processes related to biosynthesis in rapidly growing maize and sunflower plants was ruled out earlier (Penning de Vries, 1972, 1974).

MEASURED MAINTENANCE RESPIRATION RATES

The maintenance respiration rate of organs that do not grow or transport substances can be determined directly, because maintenance processes are then the only processes causing CO_2 production. Care should be taken in preparing the samples since cutting or slicing can affect the internal structure and

(thereby) the metabolic rate considerably (Eberhardt, 1960; MacDonald, 1968). Some measurements obtained in this way (method a) are presented in Table 3; their values are always very low.

Measuring the rate of maintenance respiration of metabolically active organs is principally more complicated: maintenance processes are seldom not accompanied by biosynthesis of structural dry matter or by active translocation of sugars and amino acids. If the latter processes are stopped, the rate of maintenance processes will adjust itself. Moreover, the rate of protein turnover in mature leaves is probably maximal in the morning, after the onset of light (pg. 8), and is sensitive to changes in the environment. Fortunately protein metabolism in darkness does not seem much altered until nearly all soluble carbohydrates are consumed (James, 1953; Hellebust and Bidwell, 1964b). This may last 6-24 hours, or even a few days in leaves of some species (James, 1953). The respiratory quotient in this period is about unity.

One method to determine the rate of sugar consumption for maintenance processes is by extrapolating the relation between growth rate (increase in structural dry weight) or rate of export of assimilates and respiration rate to zero. It requires that growth and degradation do not occur simultaneously in the subject considered (cf. Penning de Vries, 1974), while independence of the rate of maintenance processes of that of other metabolic processes is presupposed. To avoid changes in the system to be maintained, measurements may not be taken with long time intervals. This can be demonstrated by plotting shoot respiration rates (at 10°C) of Helianthus annuus (data from Kidd, West and Briggs, 1921) versus growth rates obtained during the growing season: this yields a very low rate of 4 mg glucose per g dry matter per day. An easy way to obtain a range of growth rates within one day without changing conditions for maintenance processes is still lacking: although the CO_2 assimilation rate can be changed instantaneously, the growth rate responds slowly due to the buffering capacity of the pool of reserve carbohydrates (De Wit et al., 1970; Penning de Vries, 1974). Some data obtained by this

Table 3. Maintenance respiration rates obtained with various methods. P stands for the tissue protein content (in %) and T for temperature (in degree C); the assimilation rate in the days prior to measurement is indicated by H (high, in full sunlight), M (moderate) or L (low, at 100 W m^{-2} or less). The respiration rate is expressed in mg glucose per g dry matter per day; values originally expressed in other units were converted, assuming a respiratory quotient of 1.

References:
1 Huber and Ziegler, 1960
2 derived from Yoda et al., 1965
3 Tamiya and Yamagutchi, 1933
4 McCree, 1970
5 Thornley and Hesketh, 1972
6 Penning de Vries, 1974
7 Penning de Vries and Van Laar, unpublished
8 derived from Heichel, 1970
9 Alberda, unpublished
10 Louwerse, unpublished
11 Prinz zur Lippe, 1956
12 James, 1953

Table 3, continued

species and organ	conditions	maintenance respiration rate	method	reference
Avena sativa and Hordeum vulgare, seeds	water content 9-11 %	0.0002-0.0010	a	1
Pisum sativum, seeds	air dried	0.0039	a	1
various conifers, stem wood	core, T=15	0.02-0.13	a	2
"	bark, T=15	1.3	a	2
Aspergillus niger, mycelium	T=30, $[O_2]$ =80%, non growing	337.	a	3
Trifolium repens, plants	T=20, L	15.	b	4
Gossipium sp., bolls	field conditions	6 ± 10	b	5
Helianthus annuus, plants	P=24, T=18, M	28.	b	6
"	P=24, T=25, M	47.	b	6
"	P=15, T=25, M	41.	b	6
Zea mays, plants	P=23, T=18, M	7.	b	6
"	P=23, T=25, M	15.	b	6
"	P=23, T=33, M	44.	b	6
Helianthus annuus, leaves	T=25, H	60.	c	7
Zea mays, leaves	T=25, H	40-60.	c	7
"	T=25, H	57.	c	8
"	T=25, L	39.	c	8
"	T=25, L	8-10.	c	9
Lolium perenne, leaves	T=25, H	40.	c	7
Phaseolus vulgaris, leaves	T=25, H	80.	c	7
"	T=25, L	12.	c	10
Phaseolus multiflorus, leaves, 14 days old	T=18-25, L	55.	c	11
" 28 " "	T=18-25, L	25.	c	11
" 48 " "	T=18-25, L	18.	c	11
" 24 " "	T=20,L,daylength=6 hrs	18.	c	11
" 24 " "	T=20,L,daylength=12 hrs	18.	c	11
" 24 " "	T=20,L,daylength=18 hrs	30.	c	11
Hordium sp. and Triticum sp., leaves	T=20	50-150	d	12
Prunus lauracerasus	T=20	10-20	d	12
Zea mays, leaves	T=20, M	27 ± 10	d	7
" "	T=25, M	26 ± 10	d	7
" "	T=30, M	46 ± 10	d	7
Phaseolus vulgaris, leaves	T=25, M	27 ± 10	d	7

method (b) are given in Table 3. Due to scatter in the measurement and while the maintenance respiration rate probably has a diurnal pattern this method is not accurate. This extrapolation method has been used also to determine the maintenance requirement of animals (Kleiber, 1961) and of growing bacteria. Since growth and maintenance processes are not independent in bacteria (pg. 3) the "maintenance rate" obtained in this way is only valid for growing bacteria (cf. Pirt, 1965). In higher plants this complication is absent, since at all growth rates most cells do not increase in structural dry weight (total dry weight minus reserve substances); only seedlings may be an exception.

Temperature changes may be useful in modifying rapidly the rate of conversion of reserves into structural material, and so to extrapolate to growth rate zero. Both the response of the rate of biosynthetic and maintenance processes to temperature should then be known.

Another method (c) measures the rate of CO_2 production of attached organs under conditions where no growth or translocation is expected to occur, but maintenance is still unaffected. This method has been used for mature leaves after 6-24 hours darkness. Values obtained by this method are somewhat larger than those obtained by (b), but a few very low values were found as well (Table 3). This method is inaccurate because it is difficult to establish whether all processes except maintenance have stopped.

A fourth method (d) measures the rate of respiration or the rate of dry weight decrease of full grown, detached organs. If these are not exhausted from carbohydrates the respiration rate is not much changed during the first few days (James, 1953). Wound respiration from a small part of the leaf or petiole is unlikely to influence the respiration rate noticeably, so that measurements of leaves of well illuminated plants for the first day after detachment probably approximate normal rates. Such rates are presented in Table 3, and are about similar to those obtained by other methods; the high values for primary bean leaves (80 and 55) probably reflect some remained

biosynthetic activity. The very high rates reported by James (up to 150) must be erroneous since not enough carbohydrates are present to sustain such high rates for 7 days, as was reported.

Table 3 shows a range of maintenance respiration rates of 8-60 mg glucose per g dry weight per day at 25°C. The information is still too limited to decide whether different species have different rates of maintenance respiration under similar conditions, but that of H. annuus seems always higher than that of Zea mays. A comparison of the values for one species at one temperature (Table 3) shows that leaves with high assimilation rates in the days preceding the measurement have higher respiration rates than those with low assimilation rates. Thus also measurements suggest a relation between metabolic activity and maintenance cost. The possibility might be investigated that active ion fluxes across cell membranes require per g dry weight an amount of energy independent of the assimilation rate (but dependent on temperature and salinity, see below), while protein turnover consumes a variable amount, which equals 2-7 % of the daily gross assimilation. Tamiya and Yamagutchi (1933) described a component of maintenance respiration related to the growth rate in Aspergillus niger of 12 % of the total substrate consumption at 30°C and an oxygen concentration of 80 %.

The rate of maintenance respiration predicted from basic data equals 15-25 mg glucose per g dry matter per day, and is about correct for some plants grown under moderate and low ligh intensities, but is too low in other cases. It is suggested that this should be attributed to the fact that protein turnover rates used for prediction were not obtained from plants grown at high light intensities, but from less active ones. It remains to be established, however, that wasteful respiration does not occur in such leaves.

In a simulation of growth of a maize crop De Wit et al. (1970) assumed that maintenance processes consume 15 mg glucose per g dry matter with 4 % nitrogen per day. The simulated growth rate agreed well with observed rates,

whereas a two times higher value underestimated the growth rate considerably. The assimilation rate simulated with this model, however, is probably too low, and the relation of metabolic activity to maintenance cost is presumably more pronounced than was simulated. Ryle, Brockington, Powell and Cross (1973) used a value of 30 mg glucose per g dry matter per day to simulate growth of uniculm barley and obtained an encouraging agreement between experimental and simulated results.

It is obvious that measuring the rate of maintenance respiration is a difficult task, and it is not surprizing that in spite of the enormous amount of work done on plant respiration only a few values can be interpreted as maintenance respiration with reasonable certainty. For a better understanding of the various processes many observations are still to be made, where especially metabolic activity may be an important reference value. Because the rates of individual maintenance processes are variable and because the maintenance respiration rate is so easily exceeded by that of biosynthetic processes, it seems that the methods described previously are not suitable for accurate determinations of maintenance respiration in plants, and that this process should be approached on a biochemical level instead.

EFFECT OF ENVIRONMENTAL FACTORS ON MAINTENANCE PROCESSES

To limit this study effects of growth retardants will not be covered, but it is well known that these may influence protein synthesis and degradation and decrease the efficiency of oxidative phosphorylation. Also effects of plant disease will not be considered.

Numerous measurements have been made of the effects of environmental factors on plant and leaf respiration rates, many of which are presented in the Encyclopedia of Plant Physiology, volume 12,2. However, frequently the contribution of CO_2 from biosynthetic processes to the total CO_2 production is not known, nor the effect of the changed factor on the rate of these

biosynthetic processes. As a result, still little is known about the effects of environmental factors on the rate of maintenance processes. Measurements in which the change of an environmental condition persists should be distinguished from short term changes, because adaptation processes may modify the response.

Temperature. In spite of the general knowledge that temperature regulates the rate of many processes, such as respiration, it effects on maintenance processes have hardly been studied.

Lyons and Raison (1970) demonstrated that temperature did not alter the P:O ratio in isolated mitochondria of several species between 1.5°C and 25°C. Probably because the procedure followed in preparing mitochondria was not sufficiently subtle (cf. Romani and Ozelkok, 1973) absolute values of the P:O ratio's were fairly low (1.5). Chilling of cold resistant cucumber varieties did not affect the P:O ratio, while it decreased from 1.5 to 0.5 in non-resistant varieties (Kushnirenko et al.,1969). The P:O ratio may thus be about 3 in the range of temperatures normal to a plant, but reduced at relatively high or low temperatures.

The rate of thermal protein turnover increases exponentially with temperature, but it is small at temperatures normal to the species considered (0.013 and 0.044 day^{-1} in mammals at 37° and 40°C, respectively, Morowitz, 1968). The only descrition found of experiments about effects of temperature on the rate of active protein turnover concerns E.coli, where the rate of protein degradation increased exponentially from about 0.2 day^{-1} at 25°C to about 2.0 day^{-1} at 45°C; up to 42°C the rate of degradation was probably equal to that of resynthesis (Pine, 1973). In thermophlic bacteria the rate of protein turnover at 70°C is not higher than that of mesophylic bactera at 35°C (Pine, 1972). Similar experiments in higher plants have not yet been performed.

The increase in the diffusion coefficient of ions with temperature is small (about 1.3 per 10°C), but the response of membrane permeability to temperature

is considerable in animal cells (Stein, 1967) and in algae (e.g. Thorhaug, 1971). Hope (1971) reports that active fluxes are enhanced 3 to 4 fold per $10^{o}C$ temperature increase in nerve cells, and Waisel (1972) presents some indirect evidence for a slightly smaller response in plants. A large increase of cost of maintenance of ion concentrations is in agreement with observations that high temperatures amplify the damaging effect of saline media (Strogonov, 1964).

The basic information thus suggests that temperature increase raises the cost of maintenance by a considerable stimulation of protein turnover and of active ion fluxes.

Although at the present stage of knowledge a prediction of how the maintenance respiration rate in higher plants respons quantitatively to a change in temperature is of little value, the above conclusion seems confirmed by direct respiration measurements, in which often a stimulation of CO_2 production has been reported of about 3 fold per $10^{o}C$ temperature increase at low temperature to 2 fold at higher temperatures from below $0^{o}C$ in some species up to $45^{o}C$ in others (Kidd et al., 1921; Yamamoto, 1933; Forward, 1960, Table 3). Unfortunately, many of these measurements were made in short term experiments, and thus may not always be representative for long term changes (cf. Forward, 1960). So not only the relations between temperature and the individual maintenance processes are poorly understood, but also the overall effect of temperature on maintenance respiration in not yet well established.

Respiration rates of plants of different species at their optimum growth temperatures, which may be $20^{o}C$ apart, are about similar (Forward, 1960), which agrees with the supposition (pg. 18) that a large fraction of the maintenauce cost is related to metabolic activity.

Water stress and salinity. Water stress and salinity are considered together because these processes have an increased ion concentration in cells in common.

In spite of the agricultural importance of these factors, there is as yet little insight into their effects on a physiological or cellular level. The influences of salinity on plant growth have been reviewed recently by Waisel (1972).

In media with a high salt concentration the P:O ratio of isolated mitochondria remains unaffected (e.g. Greenway and West, 1973). Morozovski and Kabanov (1970) found that the P:O ratio of a salinity sensitive species did not decrease up to a soil NaCl concentration of 0.4 %, and that of a salt resistant species up to 2 %. Also in these experiments absolute values of the P:O ratio were fairly low. A direct effect of water stress on the efficiency of oxidative phosphorylation has not been found. It is therefore expected that water stress and salinity at a level normal to the species considered do not uncouple oxidative phosphorylation.

The activity of many enzymes decreases when water stress persists, while that of other enzymes remains unaffected (e.g. Bardzik et al., 1972). Similar responses were observed in plants at media salinized with various salts, but naturally the reactions of glycophytes at low salt levels differed from that in halophytes (Waisel, 1972).

No report has been found about the effect of water stress or salinity on the active fluxes across cell membranes. Osmotic shrinkage of the cell size did not affect the respiration rate of Azotobacter vinelandii (Knowles and Smith, 1971). An increase of the plant salt concentration generally stimulates processes for maintenance of ion concentrations (Waisel, 1972), but how soil salinity affects ion gradients across cell membranes is still undescribed. It is most likely that also the type of salt causing salinity affects the degree of stimulation of these maintenance processes. In case of water stress plant cells obtain most of their increase in osmotic potential by accumulation of inorganic ions (Waisel, 1972). Since then also the salt concentration in the intercellular spaces rises the gradients to be

maintained increase if the ion species accumulated in cells is different from that in intercellular spaces.

It is therefore expected that continuous water stress reduces the rate of maintenance respiration mainly via a decreased plant metablic activity (although the turnover rate of some enzymes may be increased), and that a short period of water stress modifies it only slightly. Salinity, however, can increase the cost of maintaining intracellular ion concentrations markedly. Thus roots with a high ion selection capacity and salt excreting leaf cells may protect the majority of cells against the development of unnecessary gradients, and maintenance cost. Consequently both are a facet of plant salt resistance (cf. Waisel, 1972). To what extend salinity increases the total maintenance cost cannot yet be estimated. Rain may decrease ion concentrations in the intercellular spaces in leaves.

Again these ill-described expectations are supported by direct respiration measurements. An increasing water shortage reduces the rate of leaf maintenance respiration to less than 50 % of its initial value (Huber and Ziegler, 1960; Boyer, 1970; Gordon and Bichurina, 1970); that of oat seedlings, however, doubled (Huber and Ziegler, 1960). Rehydration increases respiration rates temporarily 2 to 6 fold (Huber and Ziegler, 1960), which probably results from enzyme induction and other biosynthetic processes. The energy requirement for maintenance in Saccharomyces cerevisiae in a 1. M NaCl medium is 4 times larger than in a NaCl free medium (Watson, 1970). In halophytes metabolic activity is not lowered at low salinity levels, while respiration seems stimulated (Waisel, 1972), which provides indirect evidence that active processes to maintain ion concentrations are intensified.

Starvation from nutrients and carbohydrates. Zaitseva et al. (1970) reported that short term P-deficiency did not decrease "mitochondrial functioning", although it reinforced a growth rate reduction in water stressed and in

flooded plants (Samuilov et al., 1970). When growth of E. coli and of Torulopsis utilis is limited by iron the P:O ratio falls from 3 to about 1 (Rainnie and Bragg, 1973).

Trewavas (1972) found that absence of NO_3^-, PO_4^{3-}, SO_4^{2}, Mg^{2+} or Ca^{2+} increased the rate of protein turnover in Lemna minor 2 to 3 fold. In E. coli the rate of proteolysis is about doubled in amino acid starved cells (Pine, 1973). The turnover rate of phospholipids in phosphorus deficient Spirodela is reduced (Bieleski, 1972). Syrett (1960) showed that deficiency of K^+, Mg^{2+}, and Ca^{2+} slightly increased the plant respiration rate, while severe deficiency of these and of N and of P decreased plant metabolic activity and respiration.

The main effect of slight nutrient deficiencies may be a change of the chemical composition of biomass synthesized, a decrease in metabolic activity, and an increased rate of protein turnover and of nitrogen redistribution among plant organs, When growth is limited by nutrients the rate of protein turnover is probably increased and the P:O ratio decreased.

Starvation of carbohydrates induced by prolonged darkness, generally forces the cell to degrade protein because lipids are often present in very small amounts only. The term "maintenance" is confusing in this situation since the plant does not maintain its structures, and, sooner or later, its assimilation capacity decreases.

It has often been observed that plant productivity can be depressed at high night temperatures (e.g. Went, 1957). At relatively high temperatures the maintenance cost is large and the rate of biosynthetic processes is stimulated, so that the pool of reserve carbohydrates may be depleted before the end of the night (Challa, pers. comm.). The resulting damaging effect of a high night temperature is avoided when the night period is sufficiently short to prevent exhaustion, or when sufficient reserves are formed in daytime. Translocation of sugar from leaves of tomato plants stops at high night temperatures (Went, 1957), so that carbohydrate starvation in

heterotrophic parts results, in spite of the presence of reserves in the plant.

Oxygen, carbon dioxide, ozon and UV radiation. The effects of a wide range of O_2 and CO_2 concentrations on dark respiratory processes are small, in contrast to their effects on photorespiration. The rate of O_2 diffusion into thick tissues is usually sufficient to avoid anaerobic conditions (MacDonald, 1968). At a high O_2 concentration (80 %) the growth rate of yeast is reduced (e.g. Tamiya and Yamagutchi, 1933), possibly due to stimulation of protein turnover by its oxidation.

Ozon and UV radiation destroy cell structures, and thus stimulate repair processes (e.g. Das et al., 1972), but under normal conditions their effects are negligible for respiration studies. Even in polluted areas the ozon concentration remains generally below a concentration that stimulates maintenance respiration noticeably (cf. Pell and Brennan, 1973).

Mechanical stress. A 2-5 fold stimulation of respiration has been observed in manipulated leaves and branches, in fluttering leaves and even in leaves in an airstream but fixed on a frame (Went, 1957; Eberhardt, 1960; Todd, Chadwick and Tsai, 1972). There is as yet no way to explain these results, Eberhardt (1960) suggested that it is essentially the same phenomenon as "wound respiration", which largely consists of stimulated biosynthesis. A comparison of experimental and simulated results of maize crop growth, where mechanical stimulation of maintenance was neglected (De Wit et al., 1970), suggests that this phenomenon hardly contributes to respiration in field conditions, and it is therefore expected that the effect of wind outdoors is much smaller than measured in short term, climate room experiments.

ACKNOWLEDGEMENTS

Mr. B. Boerboom has made a compilation of relevant literature at an early stage of this report. Discussions with dr. A.H. Stouthamer and

dr.ir.C.T. de Wit were most valuable and their help is greatly appreciated. Miss A.H. van Rossem kindly corrected the English text.

LITERATURE CITED

ANDERSON,W.P., ION TRANSPORT IN THE CELLS OF HIGHER PLANT TISSUES. ANN.REV.PLANT PHYSIOL.,23,51-72,1972

BADDELEY, M.S. AND J.B.HANSON, UNCOUPLING OF ENERGY-LINKED FUNCTIONS OF CORN MITOCHONDRIA BY LINOLEIC ACID AND MONOMETHYLDECENYLSUCCINIC ACID. PLANT PHYSIOL.,42,1702-1710,1967

BARDZIK,J.M.,H.V.MARSH AND J.R.HAVIS. EFFECTS OF WATER STRESS ON THE ACTIVITIES OF THREE ENZYMES IN MAIZE SEEDLINGS. PLANT PHYSIOL. 47,828-831,1971

BEEVERS,H. RESPIRATORY METABOLISM IN PLANTS. HARPER AND ROW,NEW YORK, 1961

BEEVERS,H. RESPIRATION IN PLANTS AND ITS REGULATION. IN PREDICTION AND MEASUREMENT OF PHOTOSYNTHETIC PRODUCTIVITY,209-214,1970. PUDOC,WAGENINGEN

BEEVERS,L.AND R.H.HAGEMAN. NITRATE REDUCTION IN HIGHER PLANTS. ANN.REV. PLANT PHYSIOL.,20,495-522,1969

BIELESKI,R.L. TURN OVER OF PHOSPHOLIPIDS IN NORMAL AND PHOSPHORUS DEFICIENT SPIRODELA. PLANT PHYSIOL.,49,470-475,1972

BOYER,J.S. LEAF ENLARGEMENT AND METABOLIC RATES IN CORN,SOYBEAN AND SUNFLOWER AT VARIOUS LEAF WATER POTENTIALS. PLANT PHYSIOL.,46,233-235,1970

DAS,J., J.MALINOFF AND S.B.BHATTACHARJEE, DARK AND LIGHT REPAIR IN ULTRAVIOLET-IRRADIATED ACHOLEPLASMA LAIDLAWII. BIOCH.BIOPHYS.ACTA,159,189-197,1972

DELA FUENTE,R.K. AND A.C.LEOPOLD. TURN OVER IN THE TRANSPORTABLE POOL OF AUXIN. PLANT PHYSIOL.,45,642-643,1970

DYER,T.A. AND D.J. OSBORNE,LEAF NUCLEIC ACIDS.J.EXP.BOT.,22,552-560,1971

EBERHARDT,F., DER EINFLUESZ VON MECHANISCHER BEANSPRUCHUNG, VERLETZUNG,

UND INFEKTION AUF DIE ATMUNG.PG 388-412, 12.2.
IN HANDBUCH DER PFLANZENPHYSIOLOGIE, ED. W.RUHLAND, SPRINGER VERLAG, HEIDELBERG. 1960

FISHER,J. AND T.K.HODGES, MONOVALENT ION STIMULATED ADENOSINE TRIPHOSPHATE FROM OATS ROOTS. PLANT PHYSIOL.,44.385-395.1969

FORWARD, DOROTHY F., EINFLUESZE AEUSZERER FAKTOREN AUF DIE ATMUNG. 3A. EFFECT OF TEMPERATURE ON RESPIRATION. IN HANDBUCH DER PFLANZENPHYSIOLOGIE,12,2,PG 234-255, ED.W.RUHLAND.SPRINGER VERLAG, HEIDELBERG, 1960

GLASZIOU, K.T., CONTROL OF ENZYME FORMATION AND INACTIVATION IN PLANTS. ANN.REV.PLANT PHYSIOL.,20,63-88,1969

GORDON, L.K. AND A.A.BICHURINA. EFFECT OF INCREASING WATER DEFICIT ON RESPIRATORY METABOLISM IN PLANTS. DOKLADY BOTANICAL SCIENCES, 192,36-38,1970 (TRANSLATION FROM RUSSIAN)

GREENWAY,H. AND K.R.WEST. RESPIRATION AND MITOCHONDRIAL ACTIVITY IN ZEA MAYS ROOTS AS AFFECTED BY OSMOTIC STRESS. ANN.BOT., 37,21-35,1973

HEICHEL,G.H. PRIOR ILLUMINATION AND THE RESPIRATION OF MAIZE LEAVES IN THE DARK. PLANT PHYSIOL.,46,359-362,1970

HELLEBUST,J.A. AND R.G.S. BIDWELL, PROTEIN TURN OVER IN ATTACHED WHEAT AND TOBACCO LEAVES. CAN.J.BOT.,42,1-12,1964

HELLEBUST, J.A. AND R.G.S. BIDWELL, PROTEIN METABOLISM AND RESPIRATION IN ATTACHED AND DETACHED PRIMARY WHEAT LEAVES. CAN.J.BOT. 42,357-366,1964

HOLMSEN,T.W. AND A.L.KOCH, AN ESTIMATE OF PROTEIN TURN OVER IN GROWING TOBACCO PLANTS. PHYTOCHEMISTRY,3.,163-172,1964

HOPE,A.B. ION TRANSPORT AND MEMBRANES.(121 PP) BUTTERWORTH AND CO PUBLISHERS (LTD), LONDON, 1971

HUBER,B. AND H.ZIEGLER, EINFLUESSE AUSZERER FAKTOREN AUF DIE ATMUNG. 2. WASSER UND MINERALSALZE. 12,2, PG 150-167,

IN HANDBUCH DER PFLANZENPHYSIOLOGIE, ED. W.RUHLAND, SPRINGER VERLAG, HEIDELBERG. 1960

JAMES,W.O. PLANT RESPIRATION. OXFORD CLARENDON PRESS.1953

JOHN, P.C.L., C.F.THURSTON AND P.J.SYRETT, DISAPPEARANCE OF ISOCITRATE LYASE ENZYME FROM CELLS OF CHLORELLA PYRENOIDOSA. BIOCH.J., 119,913-919,1970

KAWAGA,T.,J.M.LORD AND H.BEEVERS. THE ORIGIN AND TURN OVER OF ORGANELLE MEMBRANES IN CASTOR BEAN ENDOSPERM. PLANT PHYSIOL. 51,61-65,1973

KEYNES,R.D. AND G.W.MAISEL, THE ENERGY REQUIREMENT FOR SODIUM EXTRUSION FROM A FROG MUSCLE. PROC.ROY.SOC.SER.B., 142,383-392,1954

KIDD,F., C.WEST AND G.E.BRIGGS. QUANTITATIVE ANALYSIS OF GROWTH OF HELIANTHUS ANNUUS.PART 1. PROC.ROY.SOC.SER.B.,92,368-384,1921

KIRK, B.I. AND J.B.HANSON, THE STOICHIOMETRY OF RESPIRATION-DRIVEN POTASSIUM TRANSPORT IN CORN MITOCHONDRIA. PLANT PHYSIOL., 51,357-362,1973

KLEIBER, M., THE FIRE OF LIFE, WILEY AND SONS. NEW YORK. 1961

KNOWLES,CH.J. AND L.SMITH, EFFECT OF OSMOTIC PRESSURE OF THE MEDIUM ON THE VOLUME OF INTACT CELLS OF AZOTOBACTER VINELANDII AND ON AND ON THE RATE OF RESPIRATION. BIOCH.BIOPHYS.ACTA. 234,144-152,1971

KUSHNIRENKO,S.V., E.B.KURKOVA, A.YU.ROGACHEVA AND V.N.ZHOLKEVICH. INFLUENCE OF LOW POSITIVE TEMPERATURES ON OXIDATIVE PHOSPHORYLATION AND ULTRASTRUCTURE OF THE MITOCHONDRIA IN CUCUMIS SATIVUS L. SOVIET PLANT PHYSIOL.,16,398-403,1969

LEHNINGER,A.L. BIOCHEMISTRY. WORTH PUBLISHERS,NEW YORK,1970

LEXANDER,K., R.CARLSSON, V.SCHALEN, A.SIMONSSON AND T.LUNDBORG. QUANTITIES AND QUALITIES OF LEAF PROTEIN CONCENTRATES FROM WILD SPECIES AND CROP SPECIES GROWN UNDER CONTROLLED CONDITIONS. ANN.APPL.BIOL.,66,193-216,1970

LYONS,J.M. AND J.K.RAISON, OXIDATIVE ACTIVITY OF MITOCHONDRIA ISOLATED FROM PLANT TISSUES SENSITIVE AND RESISTANT TO CHILLING INJURY. PLANT PHYSIOL.,45,386-389,1970

LUCAS-LENARD,J. AND F.LIPMANN. PROTEIN BIOSYNTHESIS. ANN.REV.BIOCHEM., 40,409-488,1971

LUETTGE,U. ,W.J.CRAM AND G.G.LATIES. THE RELATIONSHIP OF SALT STIMULATED RESPIRATION TO LOCALIZED TRANSPORT IN CARROT TISSUE. Z.PFLANZENPHYSIOLOGIE,64,418-426,1971

MACDONALD,I.R., FURTHER EVIDENCE OF OXYGEN DIFFUSION AS THE DETERMINING FACTOR IN THE RELATION BETWEEN DISK THICHNESS AND RESPIRATION OF POTATO TISSUE. PLANT PHYSIOL.,43,274-280,1968

MACKLON,A.E.S. AND N.HIGINBOTHAM, ACTIVE AND PASSIVE TRANSPORT OF POTASSIUM IN CELLS OF EXCISED PEA EPICOTYLS. PLANT PHYSIOL.,45, 133-138,1970

MANDELSTAM,J. THE INTRACELLULAR TURN OVER OF PROTEIN AND NUCLEIC ACIDS AND ITS ROLE IN BIOCHEMCIAL DIFFERENTIATION. BACT.REV., 24,289-308,1960

MAUCK, J. AND L.GLASER. TURNOVER OF THE CELL WALL OF BACILLUS SUBTILIS W-23 DURING LOGARITHMIC GROWTH. BIOCH.BIOPH.RES.COMM., 39,699-706,1970

MCCREE,K.J. AN EQUATION FOR THE RATE OF RESPIRATION OF WHITE CLOVER PLANTS GROWN UNDER CONTROLLED CONDITIONS. IN PREDICTION AND MEASUREMENT OF PHOTOSYNTHETIC PRODUCTIVITY,221-229,1970, PUDOC,WAGENINGEN

MILTHORPE, T.L., AND J.MOORBY, VASCULAR TRANSPORT AND ITS SIGNIFICANCE IN PLANT GROWTH, ANN.REV.PLANT.PHYSIOL.,20,117-138,1969

MOON, T.W. AND P.W.HOCHACHKA, TEMPERATURE AND ENZYME ACTIVITY IN POIKILOTHERMS. BIOCH.J., 123,695-705,1971

MOROWITZ, H.J., ENERGY FLOW IN BIOLOGY. ACADEMIC PRESS. LONDON, 1968

MOROZOVSKII,V.V. AND V.V.KABANOV, EFFICIENCY OF RESPIRATION IN PEA AND

GLASSWORT UNDER NACL SALINIZATION OF THE SUBSTRATE. SOVIET PLANT PHYSIOL.,17.482-486.1970

NETTER,H. THEORETICAL BIOCHEMISTRY. OLIVER AND BOYD. EDINBURGH. 1969

NORRIS,T.E. AND A.L.KOCH. EFFECT OF GROWTH RATE ON THE RELATIVE RATES OF SYNTHESIS OF MESSENGER,RIBOSOMAL AND TRANSFER RNA IN E.COLI. J.MOL.BIOL.,64,633-649.1972

OAKS,A.,W.WALLACE AND D.STEVENS. SYNTHESIS AND TURNOVER OF NITRATE REDUCTASE IN CORN ROOT. PLANT PHYSIOL.,50,649-654,1972

PELL,E.J. AND E.BRENNAN. CHANGES IN RESPIRATION, PHOTOSYNTHESIS ADENOSINE-5-TRIPHOSPHATE AND TOTAL ADENYLATE CONTENT OF OZONATED PINTO BEAN FOLIAGE AS THEY RELATE TO SYMPTOM EXPRESSION. PLANT PHYSIOL.,51,378-381,1973

PENNING DE VRIES, F.W.T., RESPIRATION AND GROWTH. IN CROP PROCESSES IN CONTROLLED ENVIRONMENTS. ED. REES, COCKSHULL, HAND AND HURD. ACADEMIC PRESS, LONDON, 1972, PP 327-347

PENNING DE VRIES, F.W.T., THE USE OF ASSIMILATES IN HIGHER PLANTS. IN PHOTOSYNTHESIS AND PRODUCTIVITY IN DIFFERENT ENVIRONMENTS. ED. J.COOPER, CAMBRIDGE UNIVERSITY PRESS, 1974, IN PRESS

PENNING DE VRIES, F.W.T., A.M.H. BRUNSTING AND H.H. VAN LAAR, PRODUCTS, REQUIREMENTS AND EFFICIENCY OF BIOSYNTHETIC PROCESSES A QUANTITATIVE APPROACH. J.THEORET.BIOL., IN PRESS 1974

PETERSON,L.W.,G.E.KLEINKOPF AND R.C.HUFFAKER. EVIDENCE FOR LACK OF TURN OVER OF RIBULOSE-DI-PHOSPHATE CARBOXYLASE IN BARLEY LEAVES. PLANT PHYSIOL., 51,1042-1045,1973

PICKARD, W.F., FURTHER OBSERVATIONS ON CYTOPLASMIC STREAMING IN CHARA BRAUNII. CAN.J.BOT., 50,703-711,1972.

PIERCE,W.S. AND N.HIGINBOTHAM, COMPARTMENTS AND FLUXES OF K, NA AND CL IN AVENA COLEOPTYLE CELLS. PLANT PHYSIOL.,46,666-673,1970

PINE,M.J., TURN OVER OF INTRACELLULAR PROTEINS. ANN.REV.MICROBIOL. 26,103-126,1972

PINE, M.J., REGULATION OF INTRACELLULAR PROTEOLYSIS IN ESCHERICHIA COLI. J.BACT.,115,107-116,1973

PIRT,S.J. THE MAINTENANCE ENERGY OF BACTERIA IN GROWING CULTURES. PROC.ROY.SOC.B.,163,224-231,1965

PITMAN,M.G., SIMULATION OF CL UPTAKE BY LOW-SALT BARLEY ROOTS AS A TEST OF MODELS OF SALT UPTAKE. PLANT PHYSIOL.,44,1417-1427,1969

PRINZ ZUR LIPPE, A., UEBER DEN EINFLUSS DES VORANGEGANGENEN LICHT-DUNKEL WECHSELS AUF DIE CO2-AUSSCHEIDUNG DER PRIMARBLAETTER VON PHASEOLUS MULTIFLORUS IN ANSCHLIESSENDER DUNKELHEIT. Z.BOT., 44,297-318,1956

RACUSEN, D. AND FOOTE,M., PROTEIN TURNOVER RATE IN BEAN LEAF DISCS. PLANT PHYSIOL.,37,640-642,1962

RAINNIE, D.J. AND P.D.BRAGG, THE EFFECT OF IRON DEFICIENCY ON RESPIRATION AND ENERGY COUPLING IN ESCHERICHIA COLI. J.GEN.MICROBIOL., 77,339-349,1973

ROMANI,R.J. AND S.OZELKOK, SURVIVAL OF MITOCHONDRIA IN VITRO. PLANT PHYSIOL.,51,702-707,1973

ROTH,R.M. AND C.DAMPIER, DEPENDENCE OF RIBONUCLEIC ACID SYNTHESIS ON CONTINUOUS PROTEIN SYNTHESIS IN YEASTS. J.BACT.,109,773-771972

RYAN,C.A. AND W.HUISMAN, THE REGULATION OF SYNTHESIS AND STORAGE OF CHYMOTRYPSINE INHIBITOR 1 IN LEAVES OF POTATO AND TOMATO PLANTS PLANT PHYSIOL., 45,484-489,1970

SADDLER, H.D.W., FLUXES OF SODIUM AND POTASSIUM IN ACETABULARIA. J.EXP.BOT., 21,605-616,1970

SAMUILOV,F.D.,L.K.GORDON,V.E.PETROV AND A.A.BICHURINA. INFLUENCE OF PHOSPHORUS NUTRITION ON THE ENERGY EFFECTIVENESS OF RESPIRATION OF PLANTS UNDER CONDITIONS OF INSUFFICIENT AND EXCESS MOISTURE. DOKLADY BOTANICAL SCIENCES, 193,68-71,1970 (TRANSLATION FROM RUSSIAN)

SCHIMKE, R.T., ON THE ROLES OF SYNTHESIS AND DEGRADATION IN REGULATION

OF ENZYME LEVELS IN MAMMALIAN TISSUES. CURRENT TOPICS IN CELLULAR REGULATION. 1,77-124,1969 (ACADEMIC PRESS, LONDON)

SCHIMKE,R.T. AND D.DOYLE. CONTROL OF ENZYME LEVELS IN ANIMAL TISSUE. ANN.REV.BIOCH.,39,929-976,1970

SHLYK,A.A. CHLOROPHYLL METABOLISM IN GREEN PLANTS. 1970 ISRAEL PROGRAM FOR SCIENTIFIC TRANSLATIONS,JERUSALEM.

SIEKEVITZ, P., THE TURNOVER OF PROTEINS AND THE USAGE OF INFORMATION. J.THEORET.BIOL.,37,321-334,1972

STEER,B.T., DIURNAL VARIATIONS IN PHOTOSYNTHETIC PRODUCTS AND NITROGEN METABOLISM IN EXPANDING LEAVES. PLANT PHYSIOL., 51,744-748,1973

STEIN,W.D. THE MOVEMENT OF MOLECULES ACROSS CELL MEMBRANES. ACAD.PRESS, LONDON,1967

STOUTHAMER, A.H., A THEORETICAL STUDY ON ATP REQUIREMENT FOR SYNTHESIS OF MICROBIAL CELL MATERIAL. ANTHONY VAN LEEUWENHOEK, IN PRESS

STOUTHAMER.A.H. AND C. BETTENHAUSSEN.UTILIZATION OF ENERGY FOR GROWTH AND MAINTENANCE IN CONTINUOUS AND BATCH CULTURES. BIOCH.BIOPHYS.ACTA, 301,53-70,1973

STROGONOV, B.P., PHYSIOLOGICAL BASIS OF SALT TOLERANCE OF PLANTS. ISRAEL PROGRAM FOR SCIENTIFIC TRANSLATIONS, JERUSALEM, 1964

SUTCLIFFE, J.F., THE ROLE OF PROTEIN SYNTHESIS IN ION TRANSPORT. IN ION TRANSPORT IN PLANTS. ED. W.P.ANDERSON, ACADEMIC PRESS, LONDON, 1973, PP 399-406

SYRETT,P.J., EINFLUESSE AUSZERER FAKTOREN AUF DIE ATMUNG. IB. THE EFFECT OF MINERALS ON PLANT RESPIRATION. , PG 170-181. IN HANDBUCH DER PFLANZENPHYSIOLOGIE, ED. W.RUHLAND, SPRINGER VERLAG, HEIDELBERG, 1960

TAMIYA,H. UND S.YAMAGUTCHI. UEBER DIE AUFBAU- UND DIE ERHALTUNGSATMUNG. BEITRAGE ZUR ATMUNGSPHYSIOLOGIE DER SCHIMMELPILZE. III. ACTA PHYTOCHIMICA,7,(1),43-64,1933

TANAKA,A. EFFICIENCY OF RESPIRATION. RICE BREEDING.INT.RICE RESEARCH INST. PG.483-498,1972. LOS BANOS,PHILIPPINES

TANNER.W., R.GRUENES AND O.KANDLER, SPEZIFITAET UND TURNOVER DES INDUZIERBAREN HEXOSE AUFNAHMESYSTEMS VON CHLORELLA. Z.PFLANZENPHYSIOLOGIE.62,376-386,1970

THORHAUG,A., TEMPERATURE EFFECTS ON VALONIA BIOELECTRIC POTENTIAL. BIOCH.BIOPHYS.ACTA,225,151-158,1971

THORNLEY,J.H.M. AND J.D.HESKETH, GROWTH AND RESPIRATION OF COTTON BOLLS. J.APPL.ECOL.,9,315-317,1972

TODD, G.W., D.L. CHADWICK AND S.D.TSAI, EFFECT OF WIND ON PLANT RESPIRATION, PHYS.PLANT.,27,342-346,1972

TRAVIS,R.L., W.R.JORDAN AND R.C.HUFFAKER, EVIDENCE FOR AN INACTIVATING SYSTEM OF NITRATE REDUCTASE IN HORDEUM VULGARE L. DURING DARKNESS THAT REQUIRES PROTEIN SYNTHESIS. PLANT PHYSIOL. 44,1150-1156,1969

TREWAVAS,A., THE TURN OVER OF NUCLEIC ACIDS IN LEMNA MINOR. PLANT PHYSIOL. ,45,742-751,1970

TREWAVAS,A., CONTROL OF THE PROTEIN TURNOVER RATES IN LEMNA MINOR. PLANT PHYSIOL.,49,47-51,1972

VREDENBERG,W.J. A METHOD FOR MEASURING THE KINETICS OF ENERGY DEPENDENT CHANGES IN THE ELECTRICAL MEMBRANE RESISTANCE OF METABOLIZING PLANT CELLS. BIOCH.BIOPHYS.ACTA,274,505-514,1972

WAISEL, Y. BIOLOGY OF HALOPHYTES. ACADEMIC PRESS, LONDON, 1972

WATSON,T.G., EFFECTS OF SODIUM CHLORIDE ON STEADY STATE GROWTH AND METABOLISM OF SACCHAROMYCES CEREVISIAE. J.GEN.MICROBIOL., 64,91-99,1970

WENT, F.W., THE EXPERIMENTAL CONTROL OF PLANT GROWTH. CHRONICA BOTANICA, WALTHAM, MASS., USA. 1957 (343 PP.)

WIT, C.T. DE,R.BROUWER AND F.W.T.PENNING DE VRIES, THE SIMULATION OF PHOTOSYNTHETIC SYSTEMS. IN PREDICTION AND MEASUREMENT OF

PHOTOSYNTHETIC PRODUCTIVITY, PG 47-69, PUDOC, WAGENINGEN, 1970

YAMAMOTO, A. UEBER DEN EINFLUSS EINIGER GIFTE UND DER TEMPERATUR AUF DEN AUSNUTZUNGSGRAD DER ATMUNGSENERGIE BEIM WACHSTUM DES SCHIMMEL-PILZES. ACTA PHYTOCHIMICA, 7(1), 65-92, 1933

YODA, K., K. SHINOZAKI, H.OGAWA, K.HOZUMI AND T.KIRA. ESTIMATION OF THE TOTAL AMOUNT OF RESPIRATION IN WOODY ORGANS OF TREES AND FOREST COMMUNITIES. J.BIOL. OSAKA CITY UNIVERSITY, 16, 15-26, 1965

ZAITSEVA, M.G., Z.V.TITOVA AND B.SARSENBAEV, PROPERTIES OF MITOCHONDRIA IN ROOTS OF WHEAT GROWN UNDER DIFFERENT CONDITIONS OF PHOSPHATE NUTRITION. SOVIET PLANT PHYSIOL., 17, 819-826, 1970

ZUCKER, M., LIGHT AND ENZYMES. ANN.REV.PLANT PHYSIOL., 23, 133-156, 1972