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SPECIAL ARTICLES

PHOTOSYNTHESIS WITH RADIO-CARBON

ANTICIPATING the appearance of a complete report now in preparation, we wish to summarize very briefly the results obtained in the past year using unicellular green algae (*Chlorella pyrenoidosa*)[1] and radioactive carbon dioxide ($\overset{*}{C}O_2$). In some respects the $\overset{*}{C}O_2$ reduction is similar to that found in barley and sunflower plants previously reported.[2]

The rate of photosynthesis was measured simultaneously by the Warburg manometer method and the radioactive technique. The two methods agreed within the experimental error. The $\overset{*}{C}O_2$ reduction was found to be markedly affected by such poisons as hydrogen cyanide and phenyl urethane. The light and dark reduction was enormously decreased by 10^{-2}–10^{-3} M. HCN. It was observed, manometrically,[3] that at these concentrations of HCN photosynthesis was strongly inhibited, whereas respiration remained unchanged. We feel certain, therefore, that the radioactive indicator method as applied to *Chlorella* is not complicated by possible reversible steps in the respiratory process.

As in the case of the higher plants,[4] *Chlorella* cells reduce $\overset{*}{C}O_2$ in the dark. We have found this to be a reversible reaction; 85 to 95 per cent. of the *dark* reduced radioactive carbon could be extracted by boiling approximately one minute with water; 70 to 80 per cent. of the C* in this extract was precipitated[5] by barium or lead ion in 80 per cent. ethanol. The barium precipitate from the *light* reduction extract contained a smaller fraction of the C*. Prolonged boiling with 1 N. HCl did not decrease the barium-insoluble radioactivity. These and other experiments indicate the presence of a carboxyl group in the radioactive molecule(s). Decarboxylation (dry distillation of barium salts) carried out on the dark water soluble material showed that most, if not all, of the labeled carbon was in the carboxyl group. To a far lesser extent this was also true of the light extract. A considerable number of naturally occurring carboxylic acids (*i.e.*, formic, acetic, propionic, oxalic, tartaric, malic, maleic, fumaric, citric, succinic, ascorbic) have been found inactive. Water-soluble proteins as well as a large number of molecules (aldehydes, ketones, ketoacids, carbohydrates, hydrolyzable polysaccharides) containing a free carbonyl group were also inactive. Benzoylation experiments indicated the presence of at least one alcoholic hydroxyl group in the radioactive molecule(s).

Preliminary experiments with glass diffusion discs (method of Northrop and Anson[6]) yield values for the diffusion coefficient of the radioactive substance in the water-soluble extract from *Chlorella* exposed to $\overset{*}{C}O_2$ for short times in light or dark which suggest the molecular weight to be large. A lower limit may be set at 200 although all indications are that the molecular weight is considerably greater.[7] Precautions were taken to ensure the diffusion measurements were made with a

[1] Sterile cultures were used. We are indebted to Dr. W. Arnold and Dr. H. A. Barker for much help and advice in this connection.

[2] S. Ruben, W. Z. Hassid, M. D. Kamen, *Jour. Am. Chem. Soc.*, 61: 661, 1939.

[3] O. Warburg, *Biochem. Zeits.*, 100: 230, 1919.

[4] E. D. McAlister, *Jour. Gen. Physiol.*, 22: 613, 1939, using a different method, has also found a dark uptake of CO_2 by wheat.

[5] A mixture of different carrier substances was added in all the chemical analyses.

[6] J. H. Northrop and M. L. Anson, *Jour. Gen. Physiol.*, 12: 543, 1929.

[7] Lack of information about the shape of the molecule(s) prevents a reliable estimation of the molecular weight. Experiments with Professor McBain using the ultracentrifuge are in progress and it is hoped these will make an accurate molecular weight evaluation possible.

true, non-colloidal solution. Within the experimental accuracy, the molecular weight was independent of the extraction method employed as well as time of exposure to C^*O_2.

The reversible uptake of CO_2 in the dark may be written as follows:

$$(I) \qquad CO_2 + RH \rightleftarrows R{-}\overset{\overset{O}{\|}}{C}{-}OH.$$

The formation of carboxyl is a probable reaction energetically and structurally and is suggested by the presence of radioactivity in the carboxyl group. According to thermal data for similar reactions, this process may be exothermic by 1–2 K. cal. The experimental data accumulated thus far indicate $\triangle F$ can be unfavorable by 1–2 K. cal. Free energy values for a reaction such as I, involving large molecules, are not available, however. For small molecules, the free energy change is +5–10 K. cal. Conjugation in the "R" to the carboxyl group may furnish resonance energy to help the reaction. A more favorable free energy balance may be gained by the occurrence simultaneously of processes involving other molecules or taking place within R itself. This reaction is undoubtedly catalyzed by an enzyme system. Evidence bearing on the nature of RCOOH (at least after extraction) is its molecular weight of 3,000 or more, solubility in water, insolubility in ether, absence of free carbonyl groups, presence of alcoholic groups and absence of pigments (chlorophyll, etc.).

At present the dark reversible uptake of CO_2 may reasonably be considered a part of the photosynthetic process,[8] since (1) it is found to be similarly poisoned by HCN, (2) the molecular weight of R is the same as that of the photosynthetic products (whether ultimate or not) formed in the light. That CO_2 does not combine with chlorophyll but with some other substance present in the cell has been suggested by many workers.[9]

Since the net reaction for photosynthesis is

$$nh\nu + H_2O + CO_2 \longrightarrow 1/x(C \cdot H_2O)_x + O_2$$

one may, without specifying the various steps[10] involved, represent the photochemical process by the following schematic equation:

$$(II) \qquad RCOOH + H_2O + nh\nu \longrightarrow RCH_2OH + O_2.$$

The CO–O_2 balance in photosynthesis, together with the absence of carbonyl groups in the observed products, infers reduction to hydroxyl. The similarity between the molecular weights and other properties of the molecules in the light and dark reactions suggests strongly that R in I and II are identical. The energy required for II is about 110 K. cal per mole of CO_2 reduced.

We may add that formaldehyde formation, sometimes postulated, would require about 135 K. cal per mole. In addition the above picture avoids the necessity of polymerization steps. The RCH_2OH may add another CO_2 and repeat the cycle to build up long carbohydrate-like chains. Indeed, R itself may be such a chain. Processes in which carbohydrates are formed by interaction of accumulated reduction products, are of course, not excluded.

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[8] We wish to thank Professor G. Mackinney and Dr. H. A. Barker for friendly criticism.

[9] R. Emerson, *Ergeb. Enzymforsch.*, 5: 305, 1936; H. Gaffron and K. Wohl, *Naturwissenschaften*, 24: 81, 103, 1936; K. V. Thimann, SCIENCE, 88: 50c, 1938.

[10] These may be part of a process involving either a photosynthetic unit (Emerson and Arnold, *Jour. Gen. Phys.*, 16: 191, 1932) or a sequence of reactions as suggested by Franck and Herzfeld, *Jour. Chem. Phys.*, 5: 231, 1937.

THE PREPARATION AND PURIFICATION OF BRUCELLA ANTISERUM[1]

THE constituent of *Brucella* cells responsible for the clinical manifestations of brucellosis in man and animals appears from our studies to be a toxic soluble fraction.[2] Its toxicity may be demonstrated by any route of parental injection.

Preliminary studies have shown that the toxic action of the soluble fraction for experimental animals can be neutralized *in vitro* before injection by the precipitating action of a specific antiserum or by injecting a sufficient amount of antiserum shortly before the toxic antigen.

In order to obtain an antiserum of high precipitin titer or neutralizing power, and as free as possible of non-specific substance for therapeutic application in certain forms of human brucellosis, several procedures were examined.

The serum used was obtained from the cow. The fresh untreated serum precipitated the antigenic frac-

[1] Journal Article No. 396 n.s. from the Michigan Agricultural Experiment Station. This study was supported by a grant from the Horace H. and Mary A. Rackham Fund.

[2] R. B. Pennell and I. F. Huddleson, *Technical Bulletin* 156, Michigan Agricultural Experiment Station, 1937.