SCIENCE

VOL. 96 FRIDAY, JULY 10, 1942 No. 2480

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

Lancaster, Pennsylvania

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary in the Smithsonian Institution Building, Washington, D. C.

CARBON DIOXIDE UTILIZATION IN ANIMAL TISSUES[1, 2]

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IF we consider living organisms in terms of their nutritional demands upon the environment in which they live, we can place the plant with its ability to synthesize all the complex components of its structure from light energy and simple inorganic substances such as carbon dioxide, water and ammonia at one extreme and the animal with its fastidious demands for preformed dietary constituents such as vitamins, certain amino acids and certain fatty acids at the other. The carbon requirements of the plant can be satisfied completely by carbon dioxide. For animals the sources of carbon are the energy-rich organic molecules of the diet, and carbon dioxide is regarded traditionally as a metabolic end product. Experimentally, this is justified in that one can demonstrate a photosynthetic uptake of carbon dioxide in plants, while with animal tissues a continuous metabolic production of carbon dioxide is observed.

The photosynthetic process can be generally formulated:[3]

(1) $CO_2 + 2H_2A + \text{energy} \longrightarrow (CH_2O) + 2A + H_2O$

[HA is any oxidizable substance: A the oxidation product of HA]

[1] Read before the American Chemical Society at Memphis, Tennessee, on April 22, on the occasion of the conferring of the Eli Lilly and Company Award in biological chemistry for 1942.

[2] The original work reported in this paper was aided in part by grants from the John and Mary R. Markle Foundation and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[3] C. B. Van Niel, *Cold Spring Harbor Symp. Quant. Biol.*, 3: 138, 1935.

In the chlorophyl-containing plant-cell this becomes:

(2) $CO_2 + 4H_2O + \text{light} \longrightarrow (CH_2O) + O_2 + 2H_2O$

It would be well to reserve the term photosynthesis for this reaction, which is uniquely characterized by (1) the production of free O_2 as an end product and (2) by being the only process involving the reduction of CO_2 which results in a gain in energy by the assimilating system, since the energy for reduction has not been furnished by cellular metabolism but by activated chlorophyl.[4] The process of CO_2 reduction or more precisely of CO_2 fixation and reduction is, therefore, not the most characteristic feature, although an integral part, of the photosynthetic process.

There exist other and numerous examples of carbon dioxide utilization apart from photosynthesis. In certain non-photosynthesizing, so-called autotrophic, unicellular organisms the ability to use CO_2 as a sole source of carbon has been recognized for many years. The energy required for the reduction of CO_2 in these organisms is derived from a coupled chemical reaction and the process is termed "chemosynthetic." In 1935 the utilization of CO_2 by typical heterotrophic bacteria was demonstrated by Wood and Werkman at Iowa State.[5] While the carbon requirement of the heterotrophic bacteria can not be met by CO_2 alone, they utilize CO_2 in the metabolic reactions by which they maintain their existence.

Within the last year and a half evidence has been obtained that a non-photosynthetic utilization of CO_2 occurs also in animal cells.[6] CO_2 utilization, therefore, is a process which is of general biological occurrence.

It should be emphasized that two types of reactions are embraced by the phrase "carbon dioxide utilization." In the "dark reaction" of photosynthesis, a *fixation* of CO_2 as carboxyl groups apparently occurs,[7] *i.e.*,

$$R.H + CO_2 \rightleftharpoons R.COOH$$

A similar carboxylation or fixation of CO_2 in the carboxyl groups of the dicarboxylic acid seems to occur in animal cells. This type of reaction is to be differentiated from the true *reduction* of CO_2 that occurs in the photosynthetic conversion of CO_2 to carbohydrate, in the production of methane from CO_2 by *Methanobacterium omelianskii*[8] and in other systems. It follows, therefore, that the utilization of carbon dioxide by a given system can not be termed a reduction of CO_2 unless specific information as to the fate of the inorganic carbon is available. Both types of CO_2 utilization, fixation and reduction are of biological importance.

Recognition that CO_2 utilization occurs in animal tissues came as the result of investigations of carbohydrate metabolism. Since current attempts to interpret the importance of the process reflect this fact, some consideration of carbohydrate metabolism, particularly of the mechanism of oxidation of pyruvic acid, is pertinent to the discussion.

The work of a number of investigators has shown that the oxidative reactions by which carbohydrate is metabolized in many tissues are centered around the 4-, 5- and 6-carbon dicarboxylic acids. In pigeon breast muscle[9,10,11,12] and in pig heart muscle,[13] pyruvic acid is metabolized by the reactions of the so-called citric acid cycle. If we add malonic acid to this system the cyclic reaction stops, due to the poisoning of the enzymes responsible for the conversion of succinic acid to fumaric acid; pyruvic acid is no longer utilized and the oxygen uptake of the tissue is reduced to about 10 per cent. of its original value. These facts apply to muscle tissue alone; in liver the picture is quite different. While we can demonstrate the existence of the necessary enzymes to bring about the conversions of the citric acid cycle, the addition of sufficient malonic acid to inhibit the succinic dehydrogenase enzyme is not followed by any decrease in the utilization of pyruvic acid or oxygen as it is in muscle. At the same time citric, α-ketoglutaric and succinic acids accumulate. From this and from other facts it appears that there exists in liver a reaction by which pyruvic acid is converted into these dicarboxylic acids —a reaction that is not poisoned by malonic acid.[14] Although we are unaware of the mechanism of this reaction, we know that it involves the direct participation of carbon dioxide.

When pyruvic acid is oxidized by an animal tissue such as pigeon liver, a continuous production of carbon dioxide occurs. The fact that a portion of the carbon dioxide is being incorporated into organic molecules can only be demonstrated by the use of radioactive carbon dioxide or of the stable isotope of carbon. It was, perhaps, inevitable that the preparation of carbon isotopes suitable for use by the biologist should result in recognition of the widespread occurrence of CO_2 utilization in living tissues by various independent groups of workers. Investiga-

[4] H. Gaffron, *Biochem. Zeits.*, 292: 269, 1937.
[5] H. G. Wood and C. H. Werkman, *Jour. Bact.*, 30: 332, 1935.
[6] E. A. Evans, Jr., and L. Slotin, *J. B. C.*, 136: 301, 1940.
[7] S. Ruben, M. D. Kamen, W. Z. Hassid and D. C. Devault, SCIENCE, 90: 570, 1939; *Jour. Am. Chem. Soc.*, 62: 3443; 3450; 3451, 1940.
[8] H. A. Barker, S. Ruben and M. D. Kamen, *Proc. Nat. Acad. Sci.*, 26: 426, 1940.
[9] H. A. Krebs and W. A. Johnson, *Enzymologia*, 4: 148, 1937.
[10] H. A. Krebs and L. V. Eggleston, *B. J.*, 34: 442, 1940.
[11] H. A. Krebs, *B. J.*, 34: 460, 1940.
[12] H. A. Krebs, *B. J.*, 34: 775, 1940.
[13] D. H. Smyth, *Biochem. Jour.*, 34: 1046, 1940.
[14] E. A. Evans, Jr., *B. J.*, 34: 829, 1940.

tions from Berkeley, from Iowa State and Minnesota, from Harvard and from Chicago have contributed to the rapid development of this field.

In the propionic acid bacteria, Wood and Werkman have postulated that the utilization of CO_2 involves a condensation of CO_2 with a 3-carbon particle such as pyruvic acid to form oxaloacetic acid. This substance is then reduced to succinic acid. In experiments with radioactive carbon and the stable carbon isotope, C_{13},[15,16] the incorporation of CO_2 into succinic acid could be shown. However, the intermediate formation of oxaloacetic acid was not directly demonstrated.

If one assumes that the same reaction occurs in pigeon liver, *i.e.*, that carbon dioxide and pyruvic acid condense to form oxaloacetic acid, the mechanism of the dicarboxylic acid synthesis from pyruvate in liver could be explained by a series of reactions resembling those described for muscle: *i.e.*, the condensation of oxaloacetic and pyruvic acids to form citrate and the oxidation of this to α-ketoglutaric acid.

In experiments with a suspension of minced pigeon liver in a bicarbonate buffer containing radioactive carbon dioxide, Slotin and I[6] were able to demonstrate the utilization of carbon dioxide to form α-ketoglutaric acid. About 5 per cent. of the total radioactivity of the system, originally present as inorganic carbon dioxide, could be accounted for as this substance. All the radioactivity present in the α-ketoglutaric acid was located in the carboxyl group α to the carbonyl oxygen. On oxidation of the radioactive α-ketoglutarate with permanganate the succinic acid formed was entirely inactive[17] and the entire original radioactivity of the α-ketoglutarate was found in the carbon dioxide evolved during the oxidation. Likewise, succinic acid formed enzymically during the oxidation of pyruvate by malonate-poisoned liver was devoid of radioactivity.[17] These facts can not be reconciled with the intermediate formation of citric acid, and it is impossible that a symmetrical molecule of the type of citric acid could be intermediate in the process.

It is possible to estimate roughly from the ratio of radioactivity per milligram carbon of the medium to that of the α-ketoglutarate that about 1 carbon atom in 10 of the synthesized α-ketoglutarate is derived from the medium. Since the radioactivity is confined entirely to the carboxyl group of the α-ketoglutaric acid, the number of carbon atoms of the α-ketoglutaric acid derived from the medium can not exceed 1 in 5. Calculation of the 1 in 10 value is approximate and indicates only the order of magnitude of the synthesis. It seems probable that one mole of carbon dioxide is derived from the medium for every mole of α-ketoglutarate synthesized and that we are concerned with a stoichiometric utilization of CO_2.

Implicit in what has been said is the assumption that the appearance of radioactivity in α-ketoglutaric acid reflects the direct synthetic utilization of inorganic carbon. It is possible, however, that the radioactivity could result by a simple process of exchange between an intermediate in the reaction and carbon dioxide of the medium. That is, reactions of the type

$$CH_3C(=O)\cdot COOH \rightleftharpoons CH_3CHO + CO_2$$

$$COOH\cdot CH_2\cdot C(=O)\cdot COOH \rightleftharpoons CO_2 + CH_3C(=O)\cdot COOH$$

could, by virtue of their reversibility, introduce radioactivity into the cyclic chain of chemical reactions which are occurring in the metabolizing tissue. However, estimates of the extent of such reactions are too low to account for the observed utilization of CO_2.[18,19]

Under our experimental conditions, the uptake of radioactive CO_2 by pigeon liver suspensions may involve 25 to 30 per cent. of the total radioactivity of the system. Only about 25 per cent. of the radioactivity which has been converted into organic combination can be accounted for as α-ketoglutarate. Of the non-α-ketoglutarate activity, about 25 per cent. can be released as carbon dioxide by treatment with ninhydrin or chloramine-T, suggesting the presence of isotopic carbon in the carboxyl groups of α-amino acids. Wood and Werkman,[20] using the stable carbon isotope, have shown that dicarboxylic acids such as fumaric, malic and succinic acids, and also lactic acid, contain isotopic carboxyl carbon, and these substances must account for another portion of the non-α-ketoglutarate radioactivity.

In the earlier stages of the work, a picture of the fate in pigeon liver of that portion of carbon dioxide which is concerned with pyruvate metabolism was drawn by assuming that a preliminary condensation of carbon dioxide and pyruvic acid to oxaloacetic acid occurs. By the further assumption of the reactions of the citric acid cycle it would be possible to account for the appearance of isotopic carbon in the carboxyl groups of the dicarboxylic acids. The formation of isotope-containing lactic acid could be accounted for by a reversible enolization and hydration of oxaloacetate and its decarboxylation to pyruvic acid. In substance then this would imply that the so-called citric acid cycle of muscle exists also in liver with the addition of the CO_2 utilizing reaction.

There are, however, strong objections to this scheme.

[15] S. F. Carson and S. Ruben, *Proc. Nat. Acad. Sci.*, 26: 422, 1940.

[16] H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, *J. B. C.*, 135: 789, 1940; 139: 365, 1941.

[17] E. A. Evans, Jr.. *Bull. Johns Hopkins Hospital*, 69: 225, 1941.

[18] E. A. Evans, Jr., and L. Slotin, *J. B. C.*, 41: 439, 1941.

[19] E. A. Evans, Jr., in "A Symposium on Respiratory Enzymes," University of Wisconsin Press, 1941.

[20] H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, *J. B. C.*, 142: 31, 1942.

First, there is a complete lack of experimental evidence that oxaloacetic acid is the initial product of carbon dioxide fixation. However, this substance is extremely unstable and rapidly metabolized and a final decision as to its role in carbon dioxide utilization will come in all probability only after purification of the enzyme systems involved. The second objection involves the intermediate formation of citric acid itself. I have already pointed out that the available data eliminate this substance as a possible intermediate in the formation of α-ketoglutaric acid. Wood and Werkman[20] have attempted to answer this by assuming that the reaction goes through isocitric rather than through citric acid. However, Johnson[21] has shown that in pigeon liver as well as other tissues an enzymic equilibrium is maintained between citric and isocitric acids. Until supporting data are forthcoming there seems little reason to accept this modification of the original citrate-intermediate theory.

In any event we are unquestionably concerned with a synthetic reaction in pigeon liver utilizing CO_2 and leading to the formation of the dicarboxylic acids. In so far as the general importance of the reaction is concerned, we have found that a utilization of CO_2 comparable in magnitude to that in pigeon liver occurs in rat liver and beef liver. Presumably, the reaction is of general occurrence in the liver of higher animals. In muscle, CO_2 utilization does not take place to any appreciable extent. Other tissues have not been studied.

In the intact rat, Solomon, Vennesland, Klemperer, Buchanan and Hastings[22] have shown that when radioactive $NaHCO_3$ is injected into fasting rats and lactic acid is fed at the same time, a considerable part of the radioactive carbon appears as glycogen in the liver. There is little precise information as to the manner in which lactic acid is synthesized to glycogen but it is reasonable to assume that the reaction is the reverse of the glycolytic process, *i.e.*, the breakdown of glycogen to lactic acid. This involves (Fig. 1) a series

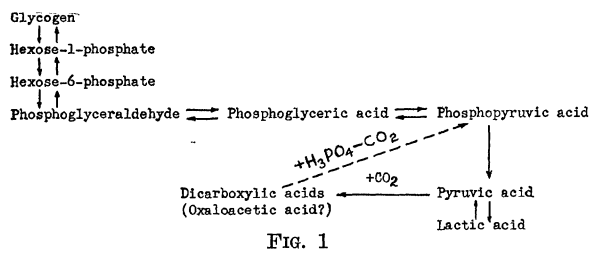

FIG. 1

of reversible reactions excluding the single step by which phosphopyruvic acid is converted into pyruvate. Under these circumstances the appearance of radioactivity in the liver glycogen being formed from lactic acid (and this happens only when there is a demonstrable synthesis of glycogen) argues for a reaction or series of reactions involving CO_2 and serving as a short circuiting mechanism around the irreversible phosphopyruvate-pyruvate step. Hastings and his co-workers have pointed out that by assuming a phosphorylation of the dicarboxylic acid formed from CO_2 and pyruvate and the subsequent breakdown of this product to phosphopyruvic acid, it would be possible to explain the incorporation of carbon dioxide in glycogen by a reversal of the known intermediate stages between glycogen and phosphopyruvic acid.

From another view-point we can regard carbon dioxide fixation as a device for the synthesis of the dicarboxylic acids which apparently play so important a catalytic role in the carbohydrate metabolism of muscle and other tissues. The reaction may serve as a source of the dicarboxylic acids (possibly transported in some other form) for those tissues incapable of their direct formation. We can see the advantage of a reaction which would result in the formation, from pyruvic and CO_2, of the catalytic agents concerned in the metabolic disposal of pyruvic acid, especially under circumstances in which large quantities of pyruvic acid were present.

However, such speculations as these may be entirely overthrown by further study. As I have pointed out previously, a considerable portion of the CO_2 utilized by pigeon liver can not be accounted for in terms of carbohydrate intermediates, although these may be the initial products. It is probable that such cellular-constituents as the purines also arise in the course of the fixation of carbon dioxide. With rat liver slices, a true synthetic utilization of carbon dioxide in the formation of urea has been known for a long time and although this reaction does not occur in pigeon tissue, the formation of other carbon-nitrogen bonds may be involved in the utilization of carbon dioxide.

The problem would be very much simplified, of course, if the carbon utilizing system could be obtained in a cell-free form. Dr. Slotin, Dr. Vennesland and I have accordingly directed our recent efforts to that end. While both minced beef liver and rat liver utilize CO_2, attempts to prepare cell-free extracts from these sources gave preparations of small and variable activity. With pigeon liver, however, we have succeeded in preparing very active cell-free carbon dioxide utilizing systems.[23] We have employed two types of preparations in our studies. The first of these was the cloudy cell-free supernatant obtained after centrifuging a suspension prepared by grinding minced pigeon liver with sand and an equal volume of ice cold phosphate buffer, pH 7.4. In these extracts there is

[21] W. A. Johnson, *B. J.*, 33: 1046, 1939.

[22] A. K. Solomon, B. Vennesland, F. W. Klemperer, J. M. Buchanan and A. B. Hastings, *J. B. C.*, 140: 171, 1941.

[23] E. A. Evans, Jr., L. Slotin and B. Vennesland, *J. B. C.*, 143: 565, 1942.

an initial rapid utilization of CO_2, followed by a slower loss of radioactivity. Apparently at least two enzymic reactions are present, one involving the uptake and the second the release of carbon dioxide.

A further simplification of the CO_2 utilizing system can be effected by using an acetone powder of pigeon liver as the starting material. This source is the most promising we have obtained for study of the initial carbon fixation reaction. Extraction of acetone dried minced pigeon liver with water or with buffered solutions yields upon centrifugation a deep-red, cell-free supernatant which shows active CO_2 uptake. In such extracts, with increasing time there is a steady increase in the amount of CO_2 taken up and it appears that the reactions leading to the release of radioactive CO_2 are either absent or inhibited. Both fumaric and pyruvic acids are necessary for the complete activity of these extracts. There is an increase in the amount of carbon dioxide utilized with increasing quantities of pyruvic acid, and we are undoubtedly concerned with a reaction involving both CO_2 and pyruvate. At intervals during the reaction we have tested for the formation of oxaloacetic acid. The results, however, were entirely negative and if oxaloacetic acid is formed during the process of pyruvate utilization by these extracts, its existence must be extremely transitory and at concentrations below those we can detect by present methods. The utilization of CO_2 proceeds to the same extent in nitrogen and carbon dioxide as in the presence of O_2 and CO_2.

When pyruvate is added to these extracts under anaerobic conditions, a slow production of CO_2 can be observed. Whether this reaction is involved in the fixation of CO_2 remains open to further investigation, but in itself it is of interest since it constitutes an example of anaerobic decarboxylation of pyruvic acid by animal tissues. The preparation of an enzyme from pig heart muscle capable of effecting the anaerobic decarboxylation of pyruvic acid was described in 1941 by Green *et al.*[24] Pigeon liver extract apparently contains a second enzyme of this type, although, unlike the pig-heart-muscle enzyme, acetyl methyl carbinol is not formed as the reaction product.

These cell-free extracts, then, readily utilize CO_2 from pigeon liver. They show increased utilization of CO_2 on the addition of pyruvic acid and of fumaric acid. The reaction proceeds anaerobically and there is no evidence of the accumulation of oxaloacetic acid.

I may be excused for presenting this brief summary of our preliminary experiments with cell-free extracts on the ground that they open the way to a purely chemical approach to the problem. It is to be hoped that the characterization and isolation of the components of such extracts will resolve the mechanism of CO_2 utilization in animal cells. The clarification that this may afford our knowledge of carbohydrate metabolism, and of the mechanism of carbon dioxide reduction in photosynthesis serves as both a stimulus and a goal.

It is necessary to modify our former ideas which contrasted the synthesis of organic molecules from CO_2 in plants to the uni-directional reverse breakdown of these molecules to CO_2 in the animal world. Rather, it is necessary to believe that the fixation and reduction of CO_2 may be as biologically important in the animal cell as it is in the plant.

[24] D. E. Green, W. W. Westerfeld, B. Vennesland and W. E. Knox., *J. B. C.*, 140: 683, 1941.

THE RECENT EXPANSION OF THE ALUMINUM INDUSTRY[1]

By T. D. JOLLY

THE ALUMINUM COMPANY OF AMERICA

UNDER present conditions, most of us have our noses so firmly pressed against our own grindstones that our range of vision doesn't extend much beyond our own jobs.

Because this is true in my own case, this will not be a scientific address on proper methods to be followed in purchasing for construction in war times, but simply a recital of our experience during the past two or three troublesome years—in other words, a case history of expansions in the aluminum industry and, in particular, in Aluminum Company of America.

Two months after the Munich conference of 1938, the company initiated a study of its ability to meet any demand which might come from Britain or France, should war suddenly break out. A six-man committee, representing various departments within the company, was appointed. That committee made frequent reports to the management as conditions changed throughout 1939. Based on these reports, and disregarding both the large stock of aluminum on hand and the apparent small requirements of the armed forces of this country, the company inaugurated an expansion program which, with the additions since made to it, calls for a capital expenditure of

[1] Address delivered before the convention of the National Association of Purchasing Agents, Waldorf-Astoria, New York, May 26, 1942.