2. The displacement of preëxisting thiamin by injected thiamin demonstrates that a significant amount of the vitamin remains in the tissues even after 36 days of a B_1-free diet; larger quantities are present under normal nutritional conditions. This does not imply that the amount retained after a prolonged B_1-free diet is an adequate protective amount of this vitamin.

3. The metabolism (interchange and destruction) of vitamin B_1 is rapid and thus resembles that of the main metabolites—protein, fat and carbohydrate.

4. The rapid destruction of thiamin yields in the urine neutral sulfur compounds and inorganic sulfate.

5. The losses incurred by excretion and destruction are inevitable in the maintenance of a physiologically adequate concentration of thiamin and cocarboxylase in the blood and tissues.

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† This paper was presented at the meeting of the American Society of Biological Chemists in New Orleans, March 14–16, 1940.

[1] Cf. E. R. Buchman, *Jour. Am. Chem. Soc.*, **58**, 1803 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *Ibid.*, **59**, 1052 (1937).

[2] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[3] R. A. Cooley, Don M. Yost and Edwin McMillan, *Jour. Am. Chem. Soc.*, **61**, 2970 (1939).

[4] Robert Goodhart and H. M. Sinclair, *Jour. Biol. Chem.*, **132**, 11 (1940).

[5] Henry Borsook, Geoffrey Keighley, Don M. Yost and Edwin McMillan, *Science*, **86**, 525 (1937).

RADIOACTIVE CARBON IN THE STUDY OF RESPIRATION IN HETEROTROPHIC SYSTEMS

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It is now well established[1] that the presence of small amounts of CO_2 are indispensable for the growth of many types of heterotrophic organisms. Also it has been reported[2] that the reduction of methylene blue by certain bacteria is dependent upon traces of CO_2. It appears that some of the experiments we have been doing with radioactive carbon have direct bearing on these interesting results.

Several heterotrophic (non-photosynthetic) systems, namely, yeast (bakers'), *B. coli*,[3] ground plant (barley) roots, ground liver (rat) tissue, etc., have now been found to assimilate small quantities of radioactive carbon as CO_2. This assimilation is inhibited by the presence of HCN. Since in the respiratory processes of these heterotrophic cells there is a net production of CO_2 it is clear that a CO_2 assimilation can best be studied directly by isotopic tracer methods using either stable (C^{13}) or radioactive (C^{11} and C^{14}) carbon.

It is also apparent that the presence of radiocarbon in oxidation states lower than +4 does not necessarily mean a net reduction of CO_2 has occurred. It may be due to the existence of a reversible reaction involving CO_2 as an end-product. At the present time no such reversible reactions in the respiratory process are known. It seems reasonable in view of the work of Hes and others that the formation of reduced radiocarbon by a respiring cell is due not to a simple interchange but rather that CO_2 plays the rôle of a highly specific oxidizing agent.

It would seem that the entrance of a CO_2 molecule into the system is necessary for the production of an additional large number of CO_2 molecules. In a sense, then, respiration is auto-catalytic.

It has been most convenient to work with yeast and the results described below have been obtained with fresh yeast cells suspended in distilled water. The suspensions were exposed to a C^*O_2 (0.5 to 2 cm.)-air mixture at approximately atmospheric pressure. Since short lived C^{11} (21-minute half-life) was used the exposures were of short duration (5 to 100 minutes). At various intervals $NaHCO_3$ was added to the suspensions, which were then boiled vigorously with strong acid to remove dissolved C^*O_2. The method of measuring the activity has been described elsewhere.[4] The uptake of C^*O_2 by aqueous suspensions of yeast cells as a function of length of time of exposure to C^*O_2 is shown in figure 1.

The measurements are, of course, corrected for radioactive decay, and are therefore comparable. 10^7 counts/min. corresponds to 0.01 cc. C^*O_2 (S. T. P.). The rate of CO_2 production by each suspension was 0.01 cc. (S. T. P.) CO_2 per minute. Thus under these conditions one C^*O_2 molecule was reduced for every 50 molecules produced in respiration. These figures are to be considered merely semi-quantitative. A constant rate of C^*O_2 reduction is obtained only when the amount of C^*O_2 present at the start of the experiment is large compared to the CO_2 evolved during the exposure. If this is not the case then the C^*O_2 is diluted, and in addition lack of rapid equilibration between the freshly produced CO_2 within the cell and the C^*O_2 in the rest of the vessel becomes important. Thus at high respiratory rates the accumulation of reduced radioactive carbon may be less.

Attempts to chemically identify the active molecules have thus far been unsuccessful. When the cells are boiled with dilute acid for approximately

one minute the cell-free aqueous extract contains more than 90% of the reduced C*. If the boiling acid treatment is omitted the cell-free medium has only ~1% of the activity. Osazones prepared with phenyl hydrazine as well as hydrazones of 2,4 dinitro phenylhydrazine are inactive (<1% of the activity). In the chemical analyses a mixture of aldehydes, sugars, acids, etc., was added to furnish carriers. Special attention was centered on pyruvic acid since the decarboxylation of pyruvic acid ($CH_3C(=O)C(=O)OH = CO_2 + CH_3CHO$) is known to play an important part in catabolic reactions. That the pyruvic acid fraction was inactive (<0.5%) indicates this reaction is irreversible. Salts of Ba^{++} precipitated in 80% ethanol were very active and even after several reprecipitations contained ~50% of the radioactivity. Decarboxylation of the active Ba salts was attempted at 250°C. for one hour. Only ~3% of the C* was converted to $BaCO_3$ by this treatment.

It is of interest at this point to mention that a non-photochemical[5] reduction of CO_2 is carried out byg reen plants (barley, wheat, sunflower, chlorella, etc.). This seems to be definitely a part of the photosynthetic mechanism.[6] The dark pick-up of CO_2 by plants has also been observed and measured by other investigators using different methods.[7, 8]

The evidence accumulated thus far indicates differences exist between the dark C^*O_2 reduction by a photosynthetic (chlorella) system on one hand and a non-photosynthetic (yeast) on the other. The time course of the C^*O_2 dark reduction by chlorella is shown in figure 2 and is to be contrasted with figure 1.

Decarboxylation experiments (dry distillation of the Ba salt at 250° for 1 hour) on the active material from chlorella suggest the major part of the C* is present in a —COOH group. Furthermore the radioactive molecules formed in yeast were found in diffusion experiments to have a higher diffusion coefficient (and are very likely of lower molecular weight) than the active compounds formed in chlorella. The dark reduction of C^*O_2 by chlorella is reversible. It is not certain whether the C^*O_2 uptake by yeast is reversible. If the yeast cells are allowed to react with C^*O_2 for 60 minutes and then flushed with a continuous stream of N_2 for 30 minutes, there is no decrease in the reduced C* present in the cells. If a stream of (inactive) CO_2-N_2 (50–50 mixture) is used, then ~15% of the reduced carbon is removed. Whether this loss occurs via the same path as the C^*O_2 reduction or by means of other reactions is still uncertain. In any case it is now an accepted fact that CO_2 reduction is no longer an exclusive characteristic of photosynthetic and chemosynthetic autotrophic organisms.[9]

It is conceivable, as Professor G. N. Lewis and Professor W. C. Bray

have suggested to us, that the primary reactions resulting in CO_2 reduction may be similar in many respects for photosynthetic and non-photosynthetic systems. The secondary reactions may differ enormously in the autotrophic as compared to the heterotrophic organisms, since in the former the net reaction is an accumulation whereas in the latter it is combustion of organic matter. It may prove easier to investigate the photosynthetic primary step because the secondary reactions take place only in the light. This is not the case in the heterotrophic systems since one must deal with a steady state rather than an equilibrium condition.

In conclusion, then, experiments with radioactive carbon have shown that a number of heterotrophic systems reduce small amounts of C^*O_2. The chemical identity of the active molecules is thus far unknown. These results offer positive evidence that CO_2 is a specific oxidizing agent in

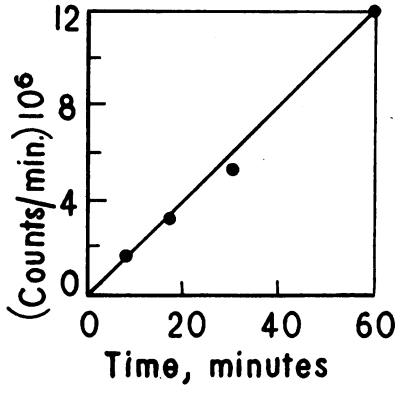

FIGURE 1

C^*O_2 assimilation by yeast.

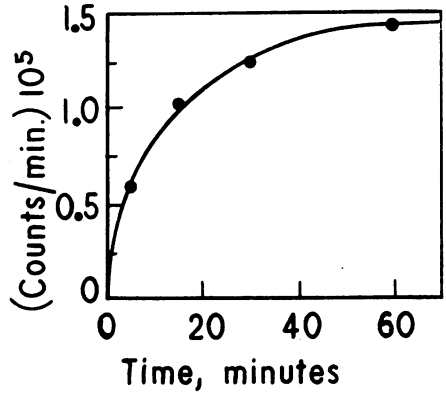

FIGURE 2

Dark C^*O_2 reduction by chlorella.

respiratory processes. This suggestion was first proposed by several investigators as a possible explanation for the fact that small quantities of CO_2 are essential for growth for microörganisms.[1]

It seems certain that further experiments with radiocarbon will yield important information regarding the mechanism.

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[1] For a summary, see Hes, *Ann. Fermentation*, 4, 547 (1938).

[2] Hes, *Nature*, 141, 647 (1938).

[3] We are indebted to Professor C. B. van Niel for the coli and Mr. A. R. Robinson for the preparation of the liver tissue.

[4] Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, **61**, 661 (1939).
[5] Ruben, Kamen, Hassid and DeVault, *Science*, **90**, 570 (1939).
[6] Additional evidence to be published shortly.
[7] McAlister, *Jour. Gen. Physiol.*, **22**, 613 (1939).
[8] Emerson and Lewis, *Am. Jour. Botany*, **26**, 808 (1939).
[9] Cf. van Niel, *Ann. Rev. Biochem.*, **6**, 606 (1937); Gaffron, *Ibid.*, **7**, 986 (1939).

CO_2 ASSIMILATION BY PROPIONIC ACID BACTERIA STUDIED BY THE USE OF RADIOACTIVE CARBON

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The studies of Wood and Werkman[1, 2, 3] as well as the work of Phelps, Johnson and Peterson[4] have shown that propionic acid bacteria can utilize CO_2 during the fermentation of glycerol. In the absence of CO_2 this fermentation can be adequately represented by the equation:[5]

$$\begin{array}{lcl} CH_2OH & & CH_3 \\ | & & | \\ CHOH & = & CH_2 + H_2O \\ | & & | \\ CH_2OH & & COOH \end{array}$$

In the presence of CO_2 the formation of propionic acid is accompanied by the appearance of succinic acid in amounts closely equimolar with the quantity of absorbed CO_2.

This made it seem possible that CO_2 becomes converted into succinic acid by combination with a 3-carbon compound. The formation of succinic acid during the fermentation of pyruvate, dextrose and galactose by *Escher. coli*, particularly its dependence upon the CO_2 partial pressure,[6] supports this view.

It is apparent that important information regarding the mechanism through which CO_2 is utilized can be obtained by the use of radioactive CO_2.[7] We have employed this approach in a study of the fermentation of glycerol by *Propionibacterium pentosaceum*.

The bacteria were grown anaerobically in yeast extract-glycerol media in the presence of CO_2 for 3 to 6 days. For the experiments cells from 250 to 500 ml. of such cultures were centrifuged, washed and suspended in 0.5 per cent phosphate buffer at pH 7.0 with and without added substrates. The suspensions were shaken at 30°C. in the presence of N_2 and C^*O_2[8] for