Function of Carbon Dioxide in the Metabolism of Heterotrophic Cells

IN recent years the indispensability of small quantities of carbon dioxide for the growth of very diverse heterotrophic cells has been amply demonstrated. For a fairly recent survey of literature, reference may be made to the paper of Gladstone, Fildes and Richardson[1]. Until now, however, no explanation has been given for this unexpected phenomenon.

Two possibilities present themselves. In the first place, the fact that very common types of bacteria, yeasts and fungi proliferate in a given medium if traces of carbon dioxide are present, but do not do so in its absence, may mean that carbon dioxide is the starting point for the synthesis of some essential cell constituent. This implies, of course, that carbon dioxide will be chemically converted, or in other words reduced, by the heterotrophic cells of the inoculum. Although it seems improbable that this power is a universal property of the numerous heterotrophic organisms for which the indispensability of carbon dioxide has already been demonstrated, the possibility cannot be at once rejected in view of the results obtained in the recent studies of Woods[2], Wood and Werkman[3], Barker[4] and others.

It may, however, suffice to state here that all attempts made so far to obtain experimental evidence in favour of this assumption have been unsuccessful.

A second possible explanation for the necessity of carbon dioxide for the growth of heterotrophic cells might be sought in a demonstration that this compound is essential for the functioning of the energy-yielding reactions normally occurring in the cells of the inoculum. Since only aerobic organisms have been studied, this would mean that the ultimate removal of carbon dioxide from the cells interferes with the respiration process. This would imply that carbon dioxide is not only essential for the proliferation of heterotrophic cells, but also for the respiration of a suspension of preformed 'resting cells'.

After experiments to test this hypothesis had already been started, I found that a similar suggestion had been made by Krebs[5], who, however, did not offer experimental support for his assumption that carbon dioxide might act as a hydrogen carrier in the respiration of *B. coli*.

I have now succeeded in proving that carbon dioxide is, indeed, essential in the normal functioning of the oxidation-reduction catalysts in various heterotrophic cells, in so far at least that complete removal of carbon dioxide leads to an inhibition of methylene blue reduction. It was found that this reduction under the usual conditions is fully prevented if the medium is rigorously freed from the last traces of carbon dioxide previous to the test. For this purpose, some solid caustic potash was placed in the side bulb of a Thunberg-Keilin tube, and the tubes were shaken, keeping them at a temperature of 0° C. to prevent reduction before the removal of the carbon dioxide was completed. After some hours the tubes were incubated in a water bath at 37° C. in order to test whether or not reduction of the dye would occur. Using *B. coli* or *B. prodigiosum* as test organisms, no reduction took place although the experiment was continued for 24 hours; control tubes submitted to a similar pre-treatment, with calcium chloride instead of caustic potash in the side bulb, readily reduced the methylene blue.

With organisms like *Saccharomyces ellipsoideus* (Chambertin yeast), *Aspergillus niger* or *Prototheca* species which ordinarily contain large quantities of reserve carbohydrates—easily leading to carbon dioxide production in the cells—no absolute inhibition of methylene blue reduction could be obtained, but the time necessary for the reduction was considerably prolonged, showing that here the same condition prevails.

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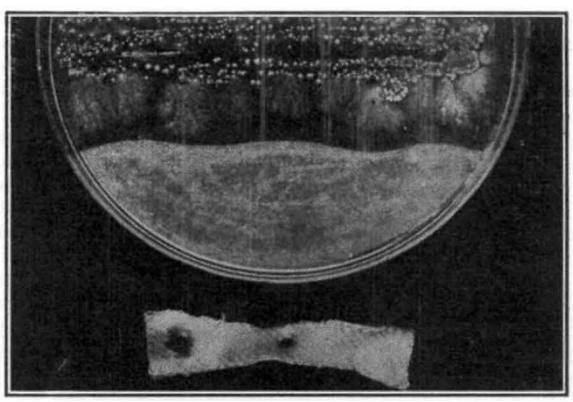
[1] *Brit. J. Exp. Path.*, **16**, 335 (1935).
[2] *Biochem. J.*, **30**, 515 (1936).
[3] *Biochem. J.*, **30**, 48 (1936).
[4] *Archiv f. Mikrobiol.*, **7**, 404 (1936).
[5] *Biochem. J.*, **31**, 2095 (1937).

Dissolving Action of Micro-Organisms on Milk-Wool

MILK-WOOL is now becoming of universal interest, and samples are being shown now and then of textile products containing 50 or even 100 per cent of this material. It appears to us, however, that due consideration should be given to the question, whether these products can withstand the biological agencies provided by Nature in the form of organisms able to assimilate and mineralize protein substances of the class to which casein belongs.



ABOVE: MILK-WOOL PLATE. DISSOLVING ACTION OF MICRO-ORGANISMS.
BELOW: PIECE OF 100 PER CENT MILK-WOOL TEXTILE. LEFT: TWO HOLES, CAUSED BY STERILE PIECES OF CLARIFIED MILK-WOOL AGAR. RIGHT: PIECE OF SAME AGAR, NON-INOCULATED, AS CONTROL (NO CHANGE).

These considerations have induced us to investigate the possibility of isolating casein-splitting organisms, which are also capable of attacking the formol-hardened casein, milk-wool.

It was found that nearly all casein-splitting micro-organisms, isolated from soil or manure, or gathered by infection from the ordinary atmosphere, are able to attack the wool. They belong to all classes: bacteria, moulds, actinomyces, many of which show a very strong dissolving action when grown on water-agar plates containing the usual