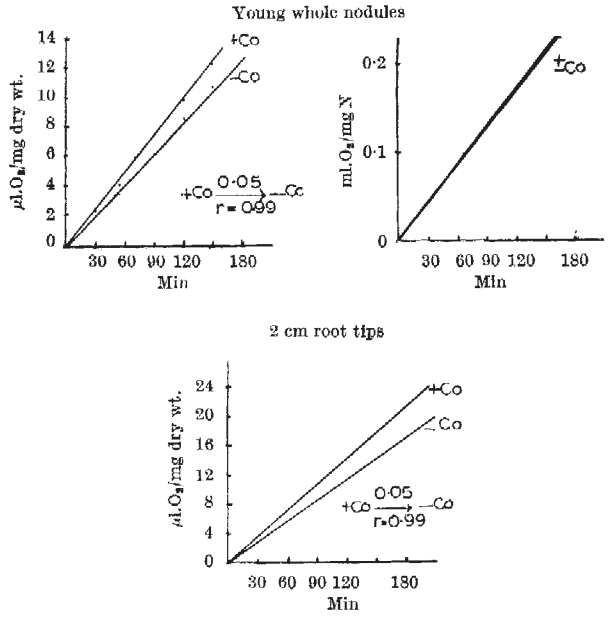

Fig. 1. Respiration of nodules and root tips (50-day plants)

The respirometer curves for the nodules and root tips are given in Fig. 1.

Apart from the general interest in the possible role of cobalt in the metabolism of a plant, this appears to be the first time that a differential requirement for a nutrient has been found, when the leaf tissues of a plant show no response to an element which has a marked and characteristic effect on the metabolism of the roots.

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[1] Ahmed, S., and Evans, H. J., *Biochem. and Biophys. Res. Comm.*, 1, 271 (1959).
[2] Hallsworth, E. G., Wilson, S. B., and Greenwood, E. A. N., *Nature*, 187, 79 (1960).
[3] Reisenauer, H. M., *Nature*, 186, 375 (1960).
[4] Kliewer, M., and Evans, H. J., *Nature*, 194, 108 (1962).
[5] Greenwood, E. A. N., and Hallsworth, E. G., *Plant and Soil*, 12, 49 (1960).
[6] Wilson, S. B., and Hallsworth, E. G., *Plant and Soil*, 20 (in the press).

MICROBIOLOGY

Preparation of ^{14}C-D(—)-β-hydroxybutyric Acid from $^{14}CO_2$ using 'Knallgas' Bacteria (*Hydrogenomonas*)

Organisms which fix carbon dioxide can be used to advantage for the preparation of radioactive labelled compounds. During growth, radioactive carbon from $^{14}CO_2$ can be incorporated into all cellular substances. If growth is inhibited, assimilated carbon is bound primarily in the form of reserve material. Several 'knallgas' bacteria of the *Hydrogenomonas* type incorporate $^{14}CO_2$ into poly-β-hydroxybutyric acid (PHBA) in a hydrogen–oxygen atmosphere in the absence of a source of nitrogen[1,2]. PHBA can be depolymerized to D(−)-β-hydroxybutyric acid (HBA) by using either hydrazine[3] or enzymatic hydrolysis[4,5].

^{14}C-PHBA is prepared using the apparatus shown in Fig. 1. Chemolithotrophically grown cells[6] are gathered, washed, and suspended (0·5 mg dry weight/ml.) in a nitrogen-free mineral medium. From this suspension 100 ml. are pipetted into the 2 l. round flask and stirred at a temperature of 30° C. After opening stopcocks *A*, *B*, and *C*, 5 l. of carbon-dioxide-free 'knallgas' (70 per cent H_2 + 30 per cent O_2) are allowed to flow through the apparatus. After closing stopcock *A*, the suspension is stirred for a further 30 min, stopcock *B* is then closed and the main reaction flask evacuated through stopcock *A*. Perchloric acid, 15 ml. of a 10 per cent solution, is then allowed to flow into flask *D*, which contains barium carbonate-^{14}C (5 mc.). By opening stopcock *B* and *C* the $^{14}CO_2$ which has been generated is carried over into the main reaction flask by the incoming 'knallgas' until the pressure is equilibrated. Stopcock *B* is now closed and the suspension stirred for 3 h. At the end of this time, the cells are killed with formic acid, washed and lyophilized. PHBA is isolated by using a sodium hypochlorite solution[7] or by means of chloroform extraction and is then hydrolysed. From 5 mc. of barium carbonate-^{14}C (spec. act. 20–26 mc./m.mole), a yield of 3–3·5 mc. of HBA (60–70 per cent theoretical) with a specific activity of 12–20 mc./m.mole was obtained in a number of experiments.

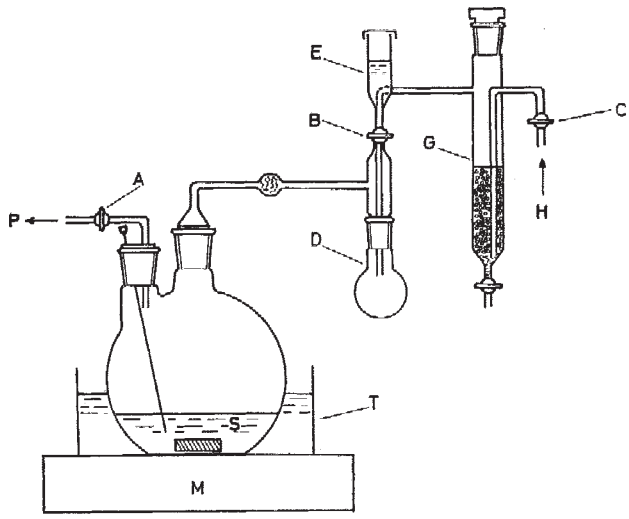

Fig. 1. Apparatus for the preparation of ^{14}C-poly-β-hydroxybutyric acid. *P*, connexion to vacuum pump; *S*, bacterial suspension; *M*, magnetic stirrer; *T*, constant temperature bath; *D*, flask containing barium-carbonate-^{14}C; *E*, 10 per cent perchloric acid; *G*, washing tower containing 20 per cent KOH; *H*, hydrogen–oxygen mixture

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[1] Schlegel, H. G., Gottschalk, G., and Bartha, R. von, *Nature*, 191, 463 (1961).
[2] Gottschalk, G., *Arch. Mikrobiol.*, 47, 236 (1964).
[3] Ottaway, J. H., *Biochem. J.*, 84, 11 (1962).
[4] Schlegel, H. G., and Chowdhury, A. A., *Naturwiss.*, 49, 502 (1962).
[5] Chowdhury, A. A., *Arch. Mikrobiol.*, 47, 167 (1963).
[6] Schlegel, H. G., Kaltwasser, H., and Gottschalk, G., *Arch. Mikrobiol.*, 38, 209 (1961).
[7] Williamson, D. H., and Wilkinson, J. F., *J. Gen. Microbiol.*, 19, 198 (1958).

Growth of 'Knallgas' Bacteria (*Hydrogenomonas*) using Direct Electrolysis of the Culture Medium

In view of the recent discussion pertaining to the use of the 'knallgas' bacteria for the regeneration of exhaled air[1–3], we should like to point out that it is possible to produce the oxygen–hydrogen mixture directly in the culture vessel by electrolysis of the mineral medium. Since the 'knallgas' bacteria of the *Pseudomonas* type (*Hydrogenomonas* strains *H* 16 and *H* 20) grow in chloride-