to the behaviour of the bound vitamin B_{12} in liver extracts.

Wijmenga *et al.*[5] have pointed out that no method of isolating cyanocobalamin from liver extracts has been described that does not involve a proteolytic step, and that when Smith[9,10] originally described the isolation of vitamin B_{12} he reported that two pink bands were formed on partition chromatography, one of which appeared to be transformed into the other on treatment with a proteolytic enzyme. Cooley *et al.*[6] have suggested that vitamin B_{12} may be present in liver in the form of a cobalichrome in which the cyano group is replaced by a protein or polypeptide moiety. That this may be partially true is suggested by the fact that we have invariably found less cyanocobalamin in liver extracts by the chemical method of Boxer and Rickards[11] than by microbiological assay, unless the liver extract has first been subjected to cyanide treatment, when the agreement between the two sets of results is fairly good. This confirms Boxer and Rickards's conclusion that "in liver concentrates, cyanocobalamin usually accounts for only a small fraction of total cobalamins".

By contrast, the cyano group is apparently unaffected when intrinsic factor is added to cyanocobalamin, since the results obtained with such a mixture by the method of Boxer and Rickards was in good agreement with the amount of cyanocobalamin present. This supports the observation of Wijmenga *et al.*[12], based on a study of absorption spectra, that the cyano group is still present in the cyanocobalamin-intrinsic factor complex.

F. A. Robinson
M. E. H. Fitzgerald
K. Fehr
J. J. Grimshaw

Research Division,
Allen and Hanburys, Ltd.,
Ware, Herts.
June 15.

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A Riboflavin-producing Bacterium

Riboflavin synthesis has been demonstrated in a wide variety of micro-organisms by means of growth studies; these have been adequately reviewed from time to time[1]. Among the bacteria, members of various genera have been reported to produce riboflavin in very small amounts[2], and none has been reported which exceeds the capacity of *Cl. acetobutylicum* to produce this vitamin.

During the course of routine work, an air-contaminant was found to produce an intense greenish-yellow pigment in a medium consisting of casein hydrolysate and beef-extract. This pigment appears to be riboflavin; the compound, like the vitamin, has the following properties: (*a*) it exhibits an intense greenish-yellow fluorescence in ultra-violet light; (*b*) the fluorescence is instantaneously discharged on the addition of sodium hydrosulphite; (*c*) it has absorption maxima at 225, 269, 372 and 445 mμ, as measured in the Beckman spectrophotometer.

The air contaminant was found to be a bacterium. It was isolated in the pure state by the usual methods. On microscopic examination it was found to be coccoid, arranged in irregular groups of sizes 0·8–1·0 μ in diameter. It is Gram-negative (observed after 6 hr., 12 hr., 24 hr. and 48 hr.).

The amount of riboflavin produced by this organism was determined by carrying out static and agitated fermentations, using a medium containing casein hydrolysate and beef-extract. Riboflavin production was assayed by the fluorimetric method using a pure riboflavin solution as the standard and found to be of the order of 150–250 μgm./ml., the maximum amount being produced in the reciprocating shaker at 26° C., the yield obtained being 250 μgm./ml. This high yield of riboflavin by a bacterium is very interesting and further studies on the identification of the organism are in progress.

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Subrata Ganguly

Department of Applied Chemistry,
University College of Science and Technology,
92 Upper Circular Road,
Calcutta 9.
May 13.

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Inhibition of D-Amino-acid Oxidase

Peptides containing D-amino-acids are not attacked by D-amino-acid oxidase[1,2]. We have found that the action of pig kidney D-amino-acid oxidase on DL-leucine was inhibited by DL-leucinamide, DL-leucylglycine, DL-leucylglycylglycine, and by glycyl-DL-leucine. These compounds also inhibited the enzymic oxidation of DL-valine and DL-phenylalanine. Thus, DL-leucinamide when added to DL-leucine, DL-valine or DL-phenylalanine in a molar ratio 5 : 1 inhibited the oxidation by 35–45 per cent. DL-Leucylglycine inhibited the oxidation of DL-leucine by 40–45 per cent, of DL-phenylalanine by 50 per cent, and of DL-valine by 60 per cent. DL-Leucylglycylglycine inhibited the oxidation of DL-phenylalanine by 25 per cent, of DL-leucine by 30 per cent, and of DL-valine by 65 per cent. Glycyl-DL-leucine gave similar results. With glycylglycine no inhibition was obtained.

It was also found that derivatives of DL-valine, DL-leucine and DL-phenylalanine in which one of the amino-hydrogens was substituted by ethyl, hydroxyethyl or cyclohexyl radicals also acted as inhibitors of D-amino-acid oxidase. Thus, DL-N-ethylphenylalanine inhibited the oxidation of DL-phenylalanine

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