A native vitamin B_{12}-polypeptide complex

In a recent paper, H. G. WIJMENGA, J. LENS AND S. J. GEERTS announced the isolation of a partially purified cobalamin polypeptide from bovine liver[1]. The method was not described in detail, but it involved no proteolytic step and was on the whole deemed mild enough to keep the complex in a native form[2].

By courtesy of these workers, a small amount of the substance (100 mg, fraction WBC) was put at my disposal for further purification. The partially purified cobalamin conjugate was a fine red powder, readily soluble in water and salt solutions. No red pigment could be removed from an aqueous solution by extraction with *n*-butanol in the presence of ammonium sulphate. This indicates, according to B. ELLIS *et al.*[3], the absence of vitamin B_{12} and low molecular-weight cobalamins.

Preliminary experiments on paper strips suggested further purification by the use of some electrophoretic procedure. Particularly in slightly acid solutions two or three coloured zones could be observed. For the preparative separation, a column similar to that described by J. PORATH[4] was filled with a suspension of cellulose powder in sodium acetate buffer of pH = 4.0 and $\mu = 0.05$. An amount of 25.12 mg of partially purified cobalamin peptide dissolved in 1 ml of buffer solution was used in the experiment. The substance was first displaced to 10 cm below the surface, then a current of 30 mA was passed through the column for 40 hours. After the electrophoretic run, the column was disconnected from the electrode vessels and the buffer containing cobalamin material was displaced from the supporting medium at a rate of 12 ml per hour and collected in 3 ml portions. The light absorption of the various fractions was determined at 274 and 350 mμ (Fig. 1). Complete recovery of the ultraviolet-absorbing material was obtained as estimated by integration of the curve.

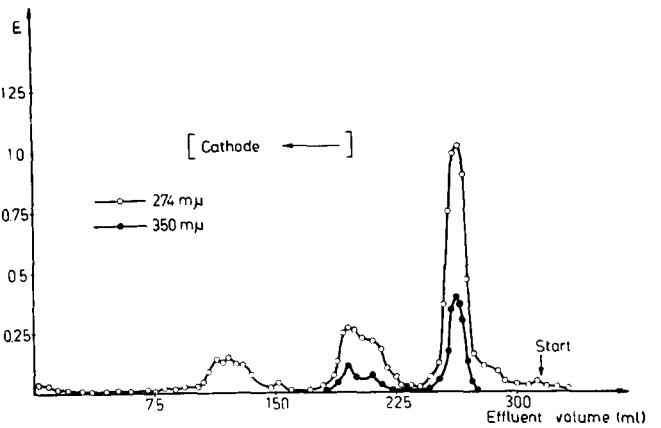

Fig. 1. Zone electrophoresis of a partially purified cobalamin-polypeptide complex from liver. For details, see text.

Under these conditions, two different cobalamin compounds were separated from a protein impurity. The shape of the curve suggested that the main fraction might be homogeneous. The absorption spectra of the 3-ml portions on both sides of the peak gave essentially the same figure with maxima at the wavelengths of 274, 350 and 523 mμ. Paper electrophoresis at pH 7.0 and 8.6 gave only a single spot. The main fraction thus seemed to contain a homogeneous cobalamin peptide. Attempts at getting an approximate measure of the molecular weight by Svedberg's ultracentrifuge gave $s_{20} = 0.5$ S, corresponding to $M < 10{,}000$. The boundary hardly left the meniscus at $260{,}000 \times g$.

After treatment with trace amounts of KCN, the shift in absorption spectrum reported by H. G. WIJMENGA *et al.*[1] was even more pronounced than in the starting material. It was also possible to extract cyano-cobalamine with *n*-butanol[3].

The peptide part remaining in the aqueous phase after splitting the complex with KCN is now the object of further examination. After acid hydrolysis, the following amino acids were preliminary identified by paper chromatography: glycine, alanine, valine, leucine, threonine, aspartic acid, glutamic acid, tyrosine, and histidine. End-group assays and sequence determinations have so far given somewhat inconsistent results, partly due to the minute quantities of material available. Full details of these experiments will be published later.

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