identical with rhizocarpic acid (mixed m.p., IR).

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Isolation of Intrinsic Factors from Human Gastric Juice

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Many attempts have been made to isolate intrinsic factor (IF), mostly utilizing hog stomach as the starting material. However, the most active preparations have been dissimilar,[1,2] for instance in respect to the sedimentation constants (5.4—4.0). In 1962, we published a method [3] for purifying IF from human gastric juice. The method is based on a series of chromatographic and gel filtration steps and utilizes the well established fact [4,5] that vitamin B_{12} is bound to and stabilizes IF. From gastric juice, neutralized outside the stomach and to which radioactive cyanocobalamin had been added, three vitamin B_{12} complexes were isolated, the IF-active complexes S and I and the non-IF-active complex R. Complexes S and I have later been found to be antigenically identical.[6] R has been shown to be ubiquitous in the body fluids [6] and to be produced by leucocytes.[7] Complex S possessed IF activity in the Schilling test in a dose of about 40 μg of protein bound to 1 μg of vitamin B_{12} [3] (protein determined by the Lowry method using Clinton standard bovine serum as the reference).

Owing to the low content of IF in the starting material, very large quantities of the latter are needed to prepare sufficient amounts of the active substance for molecular characterization. For this purpose we now collected a pool of 40 litres of neutralized gastric juice derived from 376 persons subjected to Histalog® stimulation. To the pool was added an excess of cyanocobalamin containing a trace of ^{57}Co-labelled vitamin. Two-litre batches of the pool were subjected to treatment with CM-cellulose and DEAE-cellulose chromatography, whereupon the pools were combined and rechromatographed on DEAE-cellulose, followed by DEAE-Sephadex and CM-Sephadex. Complex S was thereafter run through Sephadex G-200, whereas complex I was rechromatographed on DEAE-Sephadex before being filtered through Sephadex. The yields, as dry weight of protein, were 9.4 mg of complex S and 4.8 mg of complex I.

Complex S was found to be homogeneous in the ultracentrifuge and to have a concentration-dependent sedimentation constant, the extrapolated S_{20w} being 5.75 ± 0.08. The diffusion constant of the complex was determined in the ultracentrifuge by the synthetic boundary method and found to be $D_{20} = 4.4 \pm 0.2$ (uncorrected for concentration). It was also homogeneous in polyacrylamide disc electrophoresis [8] over a wide concentration range. Its nitrogen content was 12.1 %. Depending on the partial specific volume, the molecular weight can be assumed to be in the range of 100 000—120 000. The vitamin B_{12} content of complex S was 25 μg per mg, indicating that 2 moles of cyanocobalamin were bound per mole of IF. Compared with the vitamin B_{12}

preparation which had been added to the pool and which gave the normal vitamin B_{12} absorption spectrum, the complex exhibited some differences. The ratios of the absorbancies at 361/550 nm and 361/520 nm were 4.1 and 4.2, respectively, compared with 3.3 and 3.75 for the cyanocobalamin. In addition, there was the usual protein absorbance at lower wavelengths.

In the ultracentrifuge, complex I appeared to contain a trace of a slower-sedimenting impurity, also seen in disc electrophoresis, and amounting to 5 % at the most. Gel filtration of the complex through polyacrylamide (Biogel P-60) did not remove the impurity. The extrapolated sedimentation constant of complex I was 5.5 ± 0.1.

In our previous report[3] the isolated complexes S and I were shown to be the sole carriers of IF activity in gastric juice. The IF activity of complex S was again confirmed in this study.

The slightly lower sedimentation constant of complex I compared with that of complex S, and its absence in stomach mucosa[9] and in gastric juice neutralized in the stomach[6] appears to indicate that it is a breakdown product of the original intrinsic factor molecule.

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The Positional Distribution of Fatty Acids in Triglycerides and Lecithins of Human Chylomicrons

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Analyses of the positional relations of the fatty acids in human lymph lecithins[1-3] have demonstrated that the distribution of fatty acids on the α- and β-position is non-random. Saturated fatty acids are usually found esterified predominantly on the α-positions of the lecithins and polyunsaturated fatty acids on the β-position. A similar distribution of the fatty acids is also found in human bile and liver lecithins.[4] The existing data in the literature do not give any information about the structure of the various triglycerides in man. Such information is necessary in order to be able to elucidate the metabolic interrelations between phospholipids and triglycerides in man.

Experimental studies on the stereospecific mechanisms involved in fat absorption in animals have shown that some asymmetry is usually present in lymph triglycerides during chylomicron formation,[5-7] with a tendency to more unsaturated fatty acids at the β-position and saturated fatty acids at the α-position.

Studies on the specificity of fatty acid esterification during fat absorption in man have recently been reported from this laboratory.[8,9] In these studies, mixtures of several ^{14}C-labelled fatty acids were fed to patients with thoracic duct fistula, and the distribution of labelled and unlabelled fatty acids was measured for several different lymph lipids. Chylomicron triglyceride formation showed no specificity