The Study on Uptakes of ^{131}I Labelled-Linoleic Acid by Intestinal Absorption Method and Lipid Fractions Method

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In 20 patients which consisted of 3 of control group, 10 of postgastrectomy group, 4 of pancreatic disease group and 3 of intestinal disease group, determination of the intestinal absorption and the uptake to the lipid fractions used ^{131}I labelled-linoleic acid was performed. Radioactivity in blood showed the most highest levels in the control group. On the ther hand, the excretion ratio in feces showed 5.4% in control group, 16.4% postgastrectomy group, 29.8% in pancreatic disease group and 21.0% in intestinal disease group.

Statistical significance was observed between in control group and in the other groups in the excretion ratio in feces.

The next problem was to investigate how changed the fatty acids in serum by the hour and how they exsisted in any types were. The serum was incubated as same as body temperature. The serum lipids were devided into 5 fractions by silic acid columns, modified as Fillerup method in 2 cases. The serum in 2 cases as normal control was tested by this method. In this result, radioactivity of each fractions was shown the same patterns. The first fraction showed the peaks. The third and fourth fractions showed the constant value untill 24 hours after incubation in 2 cases indicated the highest value at 24 hours.

The above indications suggested that the linoleic acid was well combined with cholesterol ester during 2 to 6 hours after incubation. After all, linoleic acid was combined with phospholipid easily.

In our results, we understood that the incubation method was entirely appropriate by gass-chromatographic method.

Intestinal Absorption of ^{35}S Labelled Thiamine and Its Derivative and a Basic Study of Measurement of ^{35}S Containing Materials

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Absorption of ^{35}S-thiamine and ^{35}S-dicarbethoxythiamine with specific activities of 95 μCi/mg and 20 μCi/mg, respectively, was investigated in rats dogs and man. The materials included feces, intestinal contents, urine, blood and tissue homogenate, and the instrument was a liquid-scintillation counter of Shimadzu LSG-3.

The solvent system of dioxane-nephthalen was found to be superior to others particularly in its capacity to hold aqueous solution. 15 ml of the solvent containing 3% Car-O-Sil gel was used throughout. The counting efficiency was 54.7% as determined with standard ^{35}S-lithium sulfate, at 10-1000V pulse height. Hydrogen ion gave significant quenching but the effect of alkali in the presence of proteins was negligible. Plasma and tissue homogenate could be added to the solvent with only 10-30% quenching. Feces were digested with NHO_3 and H_2O_2 and then diluted and neutralized for measurement. Precipitation procedure gave poor recovery.

B_{12} and the derivative were given in the dose of 25 mg per so and urinary excretion and blood levels were determined, as well as net absorption from 4 day fecal excretion. It was found that DCET was absorbed much

faster and to a much greater extent than thiamine in man and in dogs. In rats, it was disclosed also that absorption of B_1 is rather limited to the upper intestine while that of DCET was efficient even in the ileum and colon.

Study on Human Gastric Intrinsic Factor Using Radioactive Vitamin B_{12}

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Purification of human intrinsic factor (IF) is of prime importance in the study of vitamin B_{12} (B_{12}) metabolism. It is generally accepted that the cause of pernicious anemia (PA) is the decreased secretion or lack of IF in the gastric juice. Pepsin-inactivated and neutralized normal human gastric juice (NHGJ) was dialyzed, lyophilized, added ^{57}Co or ^{60}Co B_{12} at room temperature for 30 minutes. This mixture was filtered through Sephadex G-100 gel. Bound B_{12} peak was named Fraction I, pooled, concentrated with Carbowax-6000 and chromatographed through DEAE-cellulose. A single peak of radioactivity was obtained and named Fraction II. Fraction II was reconcentrated with Carbowax-6000 or by lyophilization and finally filtered through Sephadex G-50, producing Fraction III in similar meanings. Fraction II was found active at 1.5 mg in a total gastrectomy patient by Schilling test. The ultracentrifugation of Fraction III revealed that it consisted of a single protein peak and its sedimantation coefficient was 10.1 S, and the molecular weight was about 155,000. In order to study if Fraction III and PA gastric juice (PAGJ) have IF activity or not, in vitro assay was performed using everted sacs of guinea pig intestine. The incubation media consisted of either 1000 pg B_{12} alone, 1000 pg B_{12} + NHGJ, 1000 pg B_{12} + Fraction III or 1000 pg B_{12} + PAGJ. Average B_{12}—uptake by the sac was 59.4 pg, 202.7 pg, 211.5 pg or 13.6 pg, respectively. This shows Fraction III is sufficiently active as IF. On the other hand, the B_{12} uptake by the sac from PAGJ and B_{12} containing medium was, on the average, 13.6 pg, rather lower than when B_{12} alone was added to the medium. This decrease in B_{12} uptake by the sac from JAGJ may be accounted for by the possible presence of non-IF B_{12} binding substances, although PAGJ contains no more proteins than NHGJ. Non-IF B_{12} binding substances may inhibit the uptake of B_{12} by the sac. Taking this fact into consideration, we proceeded to the subsequent experiment. After being chromatographed through Sephadex G-100 and DEAE-cellulose, PAGJ was separately pooled for radioactive B_{12} peak and for protein peak which appeared after the B_{12} peak. Each pool was studied for IF activity by the everted sacs of guinea pig intestine. Inhibitory effect of each pool was shown by depressed B_{12} uptake by the sac, but the protein pool was more inhibitory as compared with radioactive B_{12} pool. This suggests the possible presence in PAGJ of B_{12} utilization-inhibitory substances. We were not convinced of the presence in PA serum of what is called antibody against IF as long as immunologically obtained data are concerned, however.