Immunologic Study of Hog Intrinsic Factor

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Using DEAE—followed by CM-cellulose column chromatography, hog intrinsic factor was purified and then the antibody agaiinst the purified hog intrinsic factor (PHIF.) was obtained in rabbit serum 4 weeks after the injection of the mixture of PHIF.-saline solution and complete Freund's Adjuvant to rabbits. Ouchterlony's double diffusion between PHIF.-$Co^{60}B_{12}$ complex and the antibody was observed in 48 hours at room temperature. 2 or 3 precipitin lines appeared on the agar gel plate, and only one of them had radioactivity for Co^{60}-B_{12} near the place of intrinsic factor-well on radioautography. The above-mentioned radioimmunodifusion technique was taken up to study the physicochemical properties of hog intrinsic factor. Namely, changes on heating hog intrinsic factor, storing it for long period, and responses of hog intrinsic factor to treatment with HCl, ethyl alcohol, acetone, urea, and enzymes such as pepsin, trypsin, α-chymotrypsin, nagase, and papain were studied.

The results were as follows: 1) When intrinsic factor was heated to 60°~100°C for 30 minutes, large amounts of it were found denatured, but the small amounts still remained unchanged. Storing in the refrigerator at 4°C of intrinsic factor resulted in a considerable loss of intrinsic factor activity as seen by radioimmunodiffusion. 2) Concerning the effect of HCl on intrinsic factor, it was found that no changes were noted with hog intrinsic factor when it was kept mixed with HCl at the final pH of 1.5 for 15 minutes, in sharp contrast to complete denaturation of hog intrinsic factor in 24 hours at the same pH. 3) Ethylalalcohol brings forth the formation of precipitates when added to hog intrinsic factor. The precipitates were dissolved agaiin in saline and subjected to the radioimmunodiffusiion. As a result, this saline solution was found to possess the ability to denature, to a great extent, hog intrinsic factor, when tested on agar gel plates as antigen against intrinsic factor, whether hog intrinsic factor was bound or not bound to radioactive B_{12}. 4) B_{12}-intrinsic factor complex was resistant to acetone, but when intrinsic factor was not bound to B_{12}, it was considerably denatured. 5) Treatment of intrinsic factor with 8 Mol. urea did not significantly change its activity. 6) Intrinsic factor was not destroyed by pepsin treatment for 24 hours at 37°C, if it was previously bound to B_{12}, but was denatured if it was not bound. 7) When intrinsic factor reacted with trypsin at pH 7.4 under the same condition as pepsin, intrinsic was destroyed with trypsin at relatively high concentration, but was resistant to it at low concentration. Similar findings were found with chymotrypsin. 8) Nagase treatment of intrinsic factor resulted in a weak denaturation. Papain had no effect on intrinsic factor.

Measurement of Iron Loss in Stool and its Comparison with Whole Body Counting

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This paper reports the amount of loss of iron in stool, urine, hair and epithelial cells of skin by stool counting and whole body counting.

No significant difference was observed between the values with and without the hair

and desquamated epithelial cells of skin in countings more than 60 days.

Radioiron loss in urine was 1/20 of radioiron in stool in normal subjects, therefore the most of radioiron was lost into the gastrointestinal tract.

The cumulative curve of radioiron loss was constituted from two slopes.

The first slope represents the exfoliation of intestinal epithelial cells and the second slope means the loss of blood in normal subject. Iron loss by exfoliation of this subject was 0.033 mg/day and that by bleeding was 0.0065%/day (0.32 ml as blood, 0.17 mg as iron). Therefore the ratio of iron loss by bleeding to that by exfoliation was 86:14.

In iron deficiency anemia, iron loss by bleeding was 1.30%/day (65 ml as blood, 16.5 mg as iron).

In hemosiderosis, iron loss by exfoliation was 0.36 mg/day and that by bleeding was 0.030%/day (1.5 ml as blood, 0.43 mg as iron).

In polycythemia vera, iron loss by exfoliation was 0.11 mg/day, and that by bleeding was 0.004%/day (0.10 ml as blood, 0.064 mg as iron). In myelofibrosis, the cumulative radioiron loss curve was straight, implying the virtually complete mixing of radioiron with body iron by hemolysis. In this case, iron loss was 0.37%/day (3.50 mg as iron).

The calculation of loss of blood, iron by exfoliation was done by using cumulative radioiron loss curve in stool, radioiron disappearance rate, blood volume, and plasma iron value.

X. Metabolic Tracer I.

Albumin Metabolism after Experimental Laparotomy (Incorporation of ^{14}C-Algal Protein Hydrolysate)

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In the 4th Anual Meeting of the Japanese Association of Nuclear Medicine, we had reported that ^{32}P-incorporation into the s-RNA and r-RNA of mice liver was increased markedly in responce to surgical stress. To evaluate the role of these accentuated nucleic acid metabolism to the postoperative protein biosynthesis, we have studied the effect of the experimental laparotomy upon the incorporation of ^{14}C-labelled amino acids compound both into the liver and plasma protein.

^{14}C-incorporation in vivo of liver ribosome, during the period from 3 to 12 hours after laparotomy, was investigated as follows: 3 microcuries of ^{14}C-algal protein acid hydrolysate per 20g body weight of mouse was injected intraperitoneally 2 hours after operation. From these mice liver the ribosomal fraction was separated and purified after Takanami's procedure. The ribosomal protein nitrogen was measured by Folin's procedure. After adding the Hyamine-10X-OH to these ribosomal fraction and warming, ^{14}C-radioactivity was measured by the liquid scintillation spectrometer (the composition of scintillator PPO 4g, POPOP 0.1g in 1 litre of toluen). Then the specific activity of the liver ribosome after laparotomy was compared to the corresponding specific activity in the control non-operated group at several stages from 3 to 12 hours after injection. Consequently, the ^{14}C-specific activity of the ribosomal fraction was markedly increased in the liver of the operated mice compared with the control group from 6 until 12 hours after injection. These results seemed to be correlated with the before-mentioned increased ^{32}P-incorporation of r-RNA of the mice liver under surgical stress.

Next, the relation between the increased