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Abstract

For the investigation of iron metabolism in the intestinal mucosa in various blood diseases, intestinal biopsy (duodenum) was performed on 10 healthy controls and 35 cases with various blood diseases. The following are the results of the studies on distribution of stainable iron, amounts of non-hemin iron in the biopsied materials, and iron uptake of the intestinal epithelial cells. 1) An evaluation of distribution of stainable iron by Berlin blue reaction showed none or very mild degree, if any, inhealthy controls, an increase in aplastic anemia, pernicious anemia, some of leukemias and in iron deficiency anemia following iron therapy, and a decrease in idiopathic hypochromic anemia, anchylostomiasis anemia, anemia with cancer, myxedema, hemolytic anemia, and in some of leukemias. Some of anemia with cancer, however, showed an increase of a certain degree. In iron absorption tests, no changes were found other than a very mild increase in aplastic anemia. 2) Non-hemin iron was 70-112γ/g in healthy controls, increased in aplastic anemia approximately to 100-200γ/g, ranging 40-130γ/g in leukemia, and decreased in idiopathic hypochromic anemia and in anemia with cancer ranging 30-60γ/g and 30-50γ/g respectively. Amounts of non-hemin iron and serum iron or sideroblasts show a fair correlation. The fractionation of nonhemin iron in aplastic anemia didn't show any difference in relationship of each fraction from healthy controls despite the increased amount in the former. 3) A radioautographic evaluation of iron uptake by intestinal epithelium was performed by our device for evaluation of intestinal absorption capacity. The iron uptake was mild in healthy controls, almost none in aplastic anemia, and marked in iron deficiency anemia where it was decreased approximately to the level of healthy controls following iron therapy. 4) The intestinal tiue iron showed a series of changes similar to those of iron present in the serum or erythroblasts, and the non-hemin iron in the intestinal mucosa is inversely correlated with iron uptake of epithelium and is considered to regulate the absorption according to its amount.

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IRON METABOLISM OF INTESTINAL MUCOSA IN VARIOUS BLOOD DISEASES

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The problem of intestinal absorption of iron in blood diseases is of importance and of interest in association with the causes of anemia. Most investigations, however, have been focused on changes of serum iron, transportation of radioactive iron in the body and amounts of iron secreted in stools, and there are found only few direct evaluations of patients' intestinal mucosa. The theory of "mucosal block"[1] was put forth to explain the mechanism of intestinal absorption of iron but only with difficulty in some points, and at present investigations have been made for better understanding of the mechanism. Detailed observations of mucosal iron absorption have been made possible in various blood diseases, some of which were non-fatal, with the use of intestinal biopsy capsule devised by W. H. CROSBY[2] and then the investigation of the biopsied materials by our device for evaluation of intestinal absorption capacity. The followings are the results of our systematic investigations on findings of stainable tissue iron, non-hemin iron contents, and iron absorption of intestinal epithelium with Fe^{55} in patients with blood diseases.

MATERIALS AND METHODS

Materials consisted of 10 healthy controls, 35 patients with various blood diseases (5 of aplastic anemia, 1 of pernicious anemia, 6 of leukemia, 11 of idiopathic hypochromic anemia, 8 of anemia associated with cancer, 1 of anemia with anchylostomiasis, 1 of hemolytic anemia, 1 of myxedema, 1 of simple purpura), totalling 45 cases.

As for experimental methods, intestinal biopsy (duodenum) was performed with Crosby's intestinal biopsy capsule[2] to yield tissues 4—7 mm in diameter histologically consisting of lamina propria mucosae, lamina muscularis mucosae, submucosa, which were studied with Hematoxylin-Eosin staining, iron staining, and by measurement and fractionation of non-hemin iron (Fig. 1). Simultaneously, comparisons with serum iron and sideroblast and evaluations of change in iron absorption tests were performed. Next, an autoradiographic evaluation of Fe^{55}

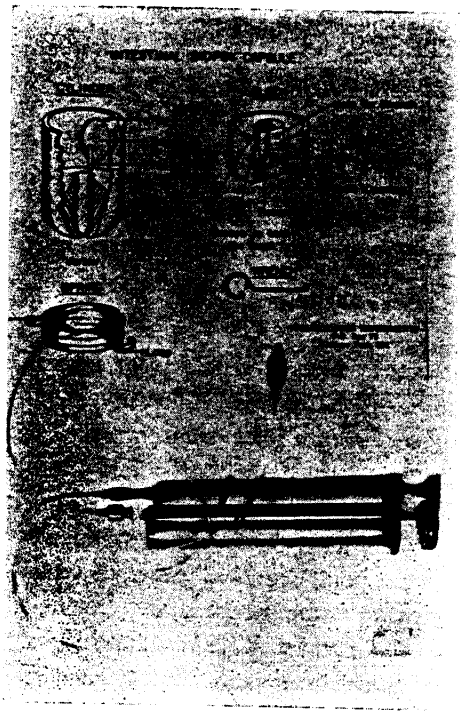

Fig. 1 Intestinal Biopsy Capsule
(by W. H. Crosby)

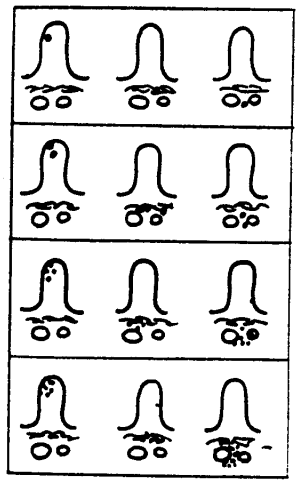

Fig. 2 Iron distribution of intestinal tissue

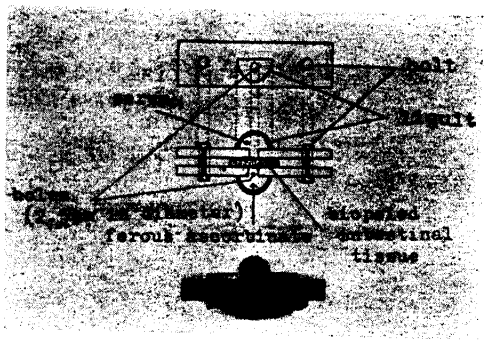

Fig. 3 Device for evaluation of intestinal absorption capacity

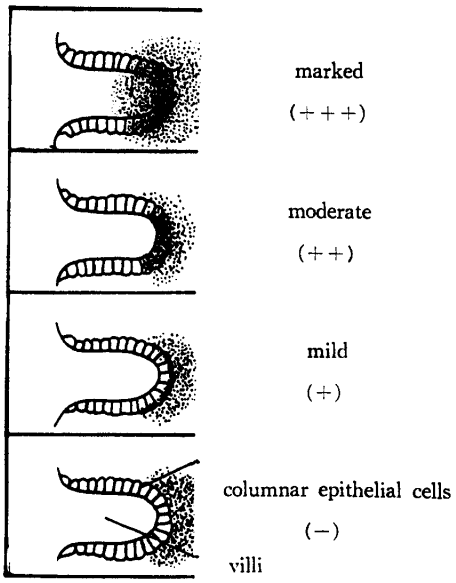

Fig. 4 Uptake of radioactive iron in columnar epithelial cells

uptake by intestinal epithelium was done by our device for evaluation of intestinal absorption capacity (Fig. 3).

Berlin blue reaction was used for iron staining and as shown in Fig. 2 iron stained preparations were graded as very mild, mild, moderate, and marked respectively when either lamina propria mucosae, lamina muscularis mucosae, or submucosa, contains 1 stainable iron grain in a few fields magnified by 400, 2—3 grains, 4—10 grains, and more than 10 to numerous grains. In this way, the distribution of stainable irons was evaluated. Intestinal non-hemin iron contents were measured by BRÜCKMANN-ZONDEK's method[3], and the fractionation was done by YONEYAMA-KONNO's method[4]. BARKAN's method[5] was used for serum iron and KAPLAN's method[6] for sideroblasts.

For the evaluation of iron uptake by intestinal epithelium with Fe^{55}, our device for evaluation of the intestinal absorption capacity shown in figure enabled us to place Fe^{55} (25μc) on the surface of biopsied mucosa as ferrous ascorbinate solution in iron concentration of 1 cc 100[7] which was washed with physiological saline solution 5 minutes later to yield tissue preparations for an autographic evaluation. According to stripping method, Fuji radioautograph films ET-2E were exposed for 20 days and following the development and fixing, Aluminium Kern Echt Rot or Hematoxylin-Eosin staining was performed. As shown in Fig. 4, observed findings were graded as marked, moderate, and mild, when radioactive iron appeared in lamina propria mucosae beyond columnar epithelium of villi, in the epithelial cells diffusely, and in a part of epithelial cells respectively.

RESULTS

1. Findings of stainable iron(Figs. 5, 6 ; Photo 1)

In healthy controls, no or little, if any, distribution of iron was found. Many cases of aplastic anemia showed a marked and pernicious anemia a mild iron distribution. Some cases of leukemia showed increased distribution, but many others did not. None was found in all the cases of idiopathic hypochromic anemia. Some cases of anemia with cancer revealed a marked distribution, but most cases did not. None was noted in any of anchylostomiasis anemia, myxedema, simple purpura, hemolytic anemia. Increased iron distribution was found in idiopathic hypochromic anemia and pernicious anemia following one month of iron therapy.

When found, iron was distributed mainly in the capillary vessel wall of lamina propria mucosae, reticulohistiocytic cells in its neighbors, and in the columnar epithelial cells. In the iron absorption tests of biopsy materials taken 3 hours after 1g of ferrous gluconate intake, the results proved healthy controls,

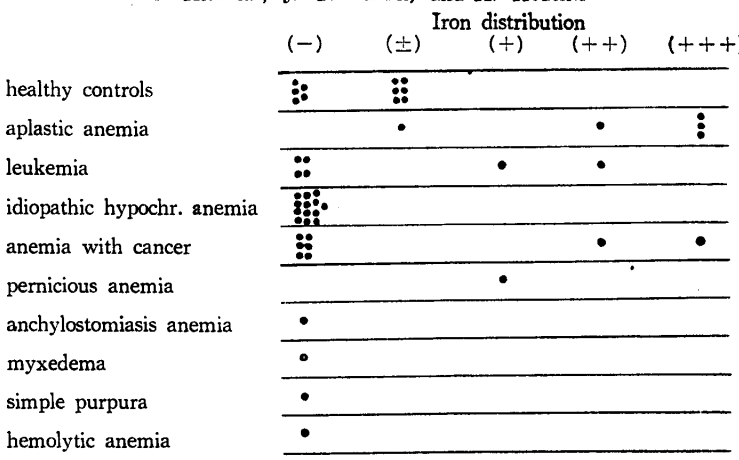

Fig. 5 Iron distribution in intestinal tissue according to diseases

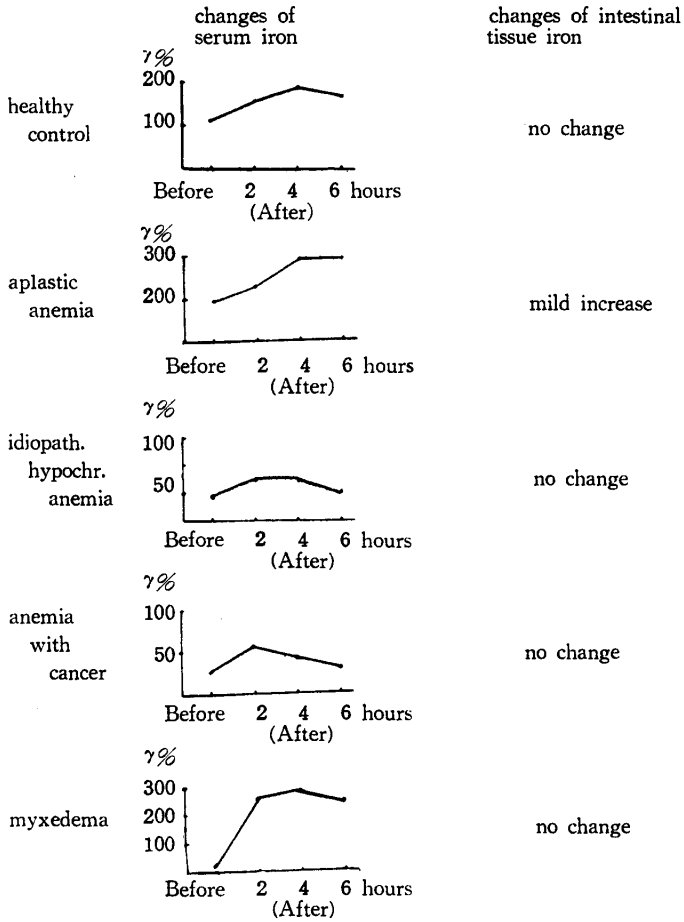

Fig. 6 Changes of intestinal tissue iron by iron absorption test

idiopathic hypochromic anemia, anemia with cancer, myxedema, to show no change of intestinal tissue iron despite increased serum iron, and aplastic anemia to show a tendency for a mild increase.

2. Non-hemin iron (Figs. 7—10)

Intestinal tissue non-hemin iron amounted to 70—112 γ/g in healthy controls. Aplastic anemia showed an increase in non-hemin iron as in stainable iron, ranging 100-200 γ/g. A mild increase or decrease was noted in leukemia as compared with healthy controls, the values ranging approximately 40-130γ/g. The value was lower in all cases of idiopathic hypochromic anemia than in healthy controls, ranging 30-60 γ/g, giving more evident difference than findings with iron staining. Similar low values were noted in anemia with cancer, ranging 30-50 γ/g.

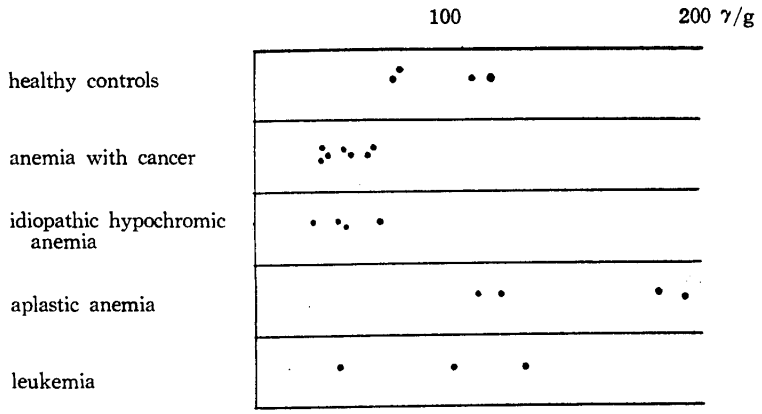

Fig. 7 Intestinal tissue iron content

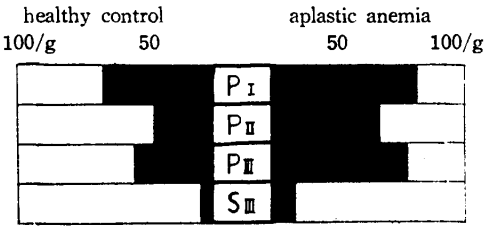

Fig. 8 Fractionation of intestinal tissue iron
(by Yoneyama-Konno's method)

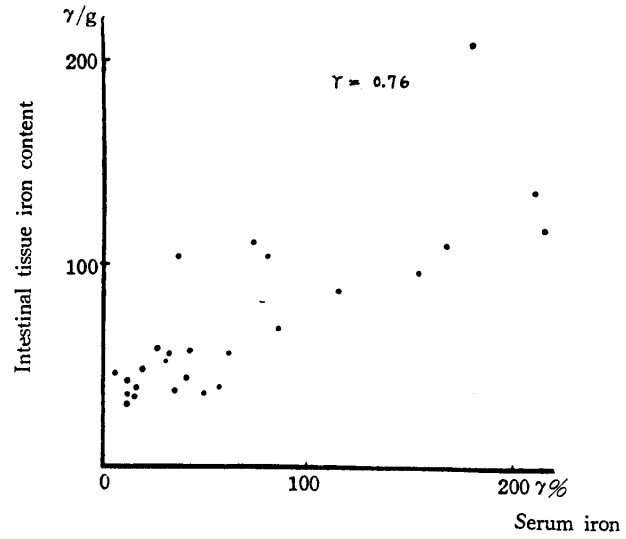

Fig. 9 Relationship between intestinal tissue iron and serum iron

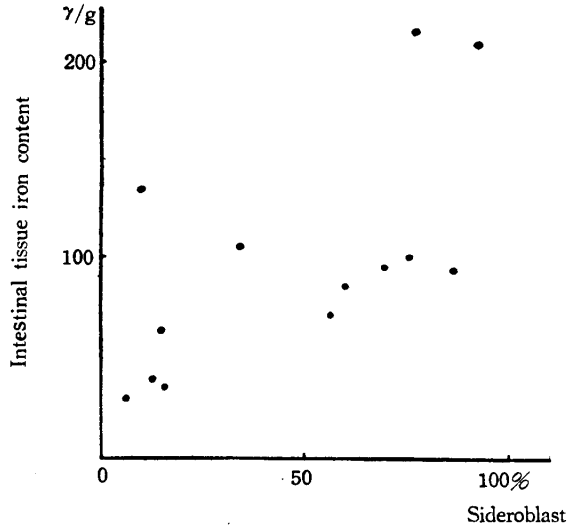

Fig. 10 Relationship between intestinal tissue iron and sideroblast

In addition, the contents of non-hemin iron of intestinal tissue were in a comparatively close correlation with those of serum iron in the same individual.

The appearance rate of sideroblasts, the iron containing erythroblasts in the bone marrow, also showed a close correlation with the iron contents of the small bowels suggesting a parallel linkage between the iron metabolism in the bone marrow and intestine. Fractionation of intestinal tissue non-hemin iron was performed in healthy controls and aplastic anemia. An increase in each fraction (PI, PII, PIII, SIII) such as hemosiderin fraction, ferritin fraction etc. was noted in aplastic anemia which showed a high value of non-hemin iron, but no apparent differences from controls in relationship among these each fractions.

3. Iron uptake of mucosal epithelium (Photos. 2—5)

As described previously, an evaluation of radioactive ferrous ascorbinate absorption by biopsied intestinal mucosa was done with our device for evaluation of the intestinal absorption capacity. In healthy controls, who showed very mild degree of stainable iron in biopsy, radioactive iron made its appearance in a part of columnar epithelial cells, indicating mild iron uptake. Aplastic anemia showed no radioactive iron in contrast to a remarkable increase of intestinal tissue iron in biopsy. In idiopathic hypochromic anemia, a marked iron uptake was indicated by the appearance of radioactive iron in lamina propria mucosae beyond the epithelial cells, despite the absence of stainable iron in biopsy. Findings after one month of iron therapy showed a tendency for a decrease in radioactive iron appearance approximating the value of healthy controls, despite moderate increase of stainable iron in biopsy.

COMMENTS

Many investigations on intestinal iron absorption have been focused on iron absorption tests based on serum iron, ferrokinetic studies with radioactive iron. There have been few, however, based on an observation of the intestinal mucosa itself in various blood diseases. The use of intestinal biopsy materials enabled us to investigate the iron metabolism in the intestinal mucosa in various blood diseases, and our device for evaluation of the intestinal absorption capacity led to a dynamic evaluation, yielding noteworthy findings as the result.

An evaluation of non-hemin iron in the intestinal mucosa by iron staining or quantitative analysis showed changes of noticeable degree in each of various blood diseases. An increase was noted in aplastic anemia, pernicious anemia, and some cases of leukemia, but a tendency for a decrease in the cases of idiopathic hypochromic anemia, anchylostomiasis anemia and anemia with cancer. Among them, the increase in aplastic anemia was remarkable in many cases,

in contrast to a decrease in idiopathic hypochromic anemia, indicating some clinical diagnostic value, especially when the diagnosis is difficult.

A comparison of the non-hemin iron in the intestinal tissue with serum iron or sideroblasts in the various diseases showed a comparatively close correlation, indicating changes of the former quite similar to those of iron in active use, such as iron found in serum and in sideroblasts. This signifies that non-hemin iron in the intestinal tissue is keeping a certain balance with iron in active use in the iron metabolism of the whole body, being controlled by a series of metabolic mechanisms. It is understandable, however, that as noted in iron absorption tests the intestinal tissue iron shows changes not necessarily similar to those in the serum iron which shows transient changes depending on the situations: an increase of the serum iron in iron deficiency state presumably due to immediate transportation of absorbed iron in an increased rate without increase of the intestinal tissue iron as a result, a mild increase in the case of aplastic anemia presumably due to delayed absorption process.

Furthermore a radioautographic evaluation of iron uptake by epithelial cells with the use of our device for evaluation of the intestinal absorption capacity more clearly showed the above mentioned facts. An increased uptake was noted in non-hemin iron deficiency state, a decrease in excess state. In idiopathic hypochromic anemia, an interesting result was found in that the uptake was decreased to normal value following iron treatment in contrast to a tendency for an increase of the intestinal tissue iron. The above results presumably indicate that iron absorption is influenced by the amounts of non-hemin iron present in the intestinal tissue, i. e. iron present in epithelial cells, reticulohistiocytic cells in lamina propria mucosae. From this, iron absorption is directly regulated by the function of epithelial cells but it is indirectly associated with iron in mucosal reticulohistiocytic cells, in serum and in erythroblasts, and it may be assumed that in a broader sense such a step-wise mechanism is operating for iron absorption.

To explain the iron absorption of intestinal mucosal cells, GRANICK[1] proposed the "mucosal block" theory that the absorption is regulated by the degree of saturation of ferric iron stored in ferritin and ferrous iron present in the cells. Of a more importance in the iron absorption, however, is the state of iron not only just in the epithelial cells but also in the whole body. This is well demostrated by the above-mentioned increase of iron absorption in iron deficiency state and the decrease in excess state. "Mucosal block" theory has difficulty to explain the efficacy of iron stoss therapy, but it is conceivable and supported by our findings that in iron deficiency state, a large amount of iron is rapidly taken up by epithelial cells and immediately transported into the blood low in serum iron without the epithelial cells being saturated by ferrous iron. In iron excess state,

absorption process is delayed in the epithelial cells and is often blocked by saturated ferrous iron. As iron which is concerned with intestinal iron absorption, GRANICK[1] emphasized the importance of ferritin as well as ferrous iron but the importance of hemosiderin in addition is already recognized by SAITO[7]. We also recognized a possibility that both are concerned. In any event, the amount rather than the form of iron present in the intestinal mucosa is presumably important in iron absorption and ferrous iron above all is considered to play a direct important role. Thus we presume the presence in the intestinal mucosa of a comparatively simple mechanism like diffusion by differences in concentration as found in iron uptake of erythroblasts previously described by us[8,9].

CONCLUSIONS

For the investigation of iron metabolism in the intestinal mucosa in various blood diseases, intestinal biopsy (duodenum) was performed on 10 healthy controls and 35 cases with various blood diseases. The following are the results of the studies on distribution of stainable iron, amounts of non-hemin iron in the biopsied materials, and iron uptake of the intestinal epithelial cells.

1) An evaluation of distribution of stainable iron by Berlin blue reaction showed none or very mild degree, if any, in healthy controls, an increase in aplastic anemia, pernicious anemia, some of leukemias and in iron deficiency anemia following iron therapy, and a decrease in idiopathic hypochromic anemia, anchylostomiasis anemia, anemia with cancer, myxedema, hemolytic anemia, and in some of leukemias. Some of anemia with cancer, however, showed an increase of a certain degree. In iron absorption tests, no changes were found other than a very mild increase in aplastic anemia.

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tinal mucosa is inversely correlated with iron uptake of epithelium and is considered to regulate the absorption according to its amount.

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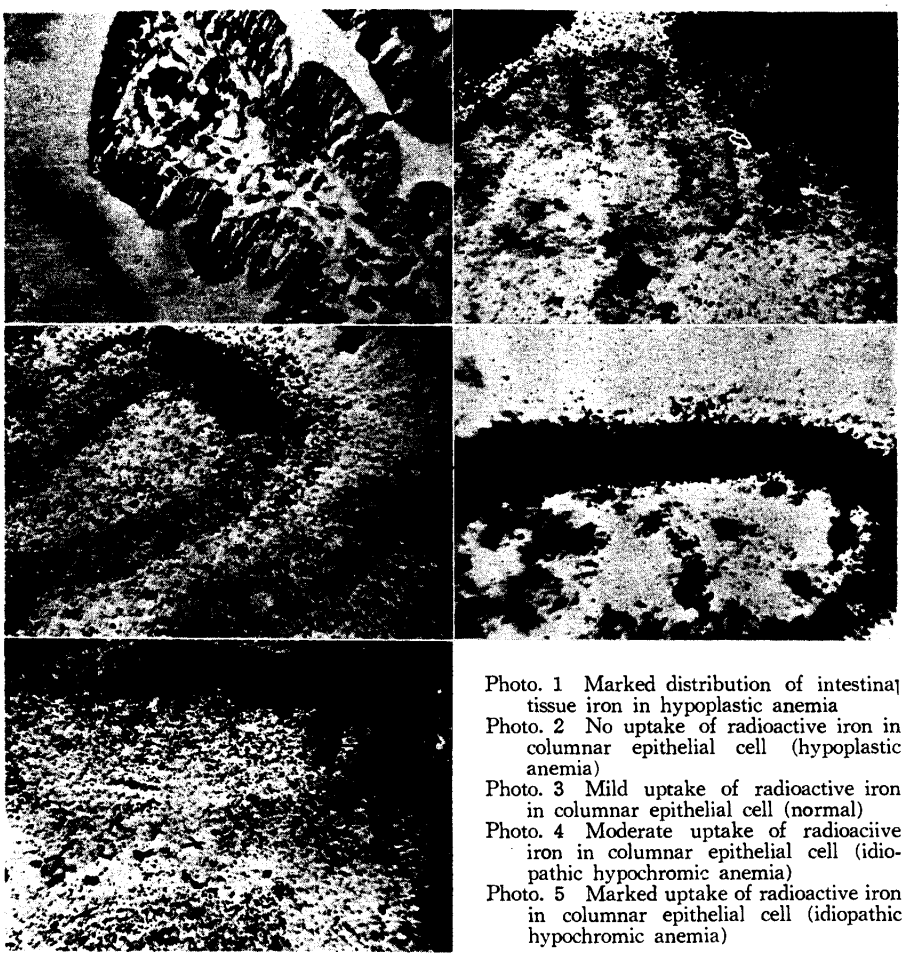

Photo. 1 Marked distribution of intestinal tissue iron in hypoplastic anemia
Photo. 2 No uptake of radioactive iron in columnar epithelial cell (hypoplastic anemia)
Photo. 3 Mild uptake of radioactive iron in columnar epithelial cell (normal)
Photo. 4 Moderate uptake of radioaciive iron in columnar epithelial cell (idiopathic hypochromic anemia)
Photo. 5 Marked uptake of radioactive iron in columnar epithelial cell (idiopathic hypochromic anemia)