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Abstract

The serum iron contents and the number of sideroblasts from various patients and the radioactivity of erythroblasts from the same patients incubated with Fe55 have been observed. The results have proved that in the case with accelerated erythropoietic function like polycythemia vera and in the iron deficient state like idiopathic hypochromic anemia, the serum iron level and the number of sideroblast are lower than those in normal persons and higher in radioactivity in erythroblasts, whereas in the case with low erythropoietic function like hypoplastic anemia the former values are higher and lower in radioactivity of erythroblasts. There is an inverse correlation between the average number of stainable iron granules and the average rate of radioactive iron appearance in erythroblasts, and the observation on these factors will give an important clue for judging the utilization process of iron in each disease. The limitation of the iron uptake correlating with the hemoglobin synthesis have been discussed.

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STUDIES ON THE IRON METABOLISM OF ERYTHROBLASTS IN VARIOUS BLOOD DISEASES

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Owing to the application of radioisotopes with the advanced technique of autoradiography, the metabolic pathway of iron, the absorption, storage, excretion and the incorporation into heme, have been greatly revealed in detail (1, 2, 3, 4). In the meanwhile, the studies on sideroblasts, the erythroblasts containing the iron granules which give positive reaction to blue Prussian, have brought forth a new cytological problem on the non-hemin iron in the cell.

Our previous investigations on these sideroblasts suggested that the iron detectable in erythroblasts by Prussian blue reaction indicated the iron being taken up from serum into the cytoplasm and destined to be incorporated into heme, and we noted that in the course of the hemoglobin metabolism iron passed through essentially three steps, the uptake by the cell, retention in the cytoplasm and the incorporation into protoporphyrine (6), though there are some limitations on the transfer of iron from serum into erythroblasts. The data from which the just mentioned conclusion has been deduced are those obtained from the chemical and morphological observations by using non-radioactive iron as the iron source.

In the present paper the authors present the evidences to prove that the observation on autoradiographic examination and sideroblasts will give an important clue for judging the mechanism of the disturbances in iron metabolism of erythroblasts in various diseases.

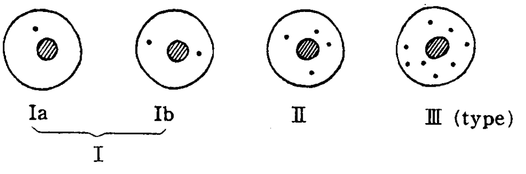
MATERIALS AND METHODS

Experimental materials used are the sera and bone marrow of 21 cases including three normal persons, three idiopathic hypochromic anemia, three Banti's disease, three gastric cancer, one hepatic cancer, one Hodgkin's disease, one multiple myeloma and one polycythemia vera, three leukemia and two hypoplastic anemia.

As for the experimental method, the serum iron contents are measured by Barkan's method (7), and the detection of sideroblasts are conducted with smears of the bone marrow aspirated from patients. At the same time, 4 μc (1γ) Fe^{55} is added to 1 ml of the serum from the patient, and the mixture is left standing for one hour. Then 0.3 ml aspirated bone marrow of the same patient is added to the mixture. After keeping the latter mixture in a roller tube at the constant temperature of 37°C for 24 hours, smears are prepared and autoradiographs of erythroblasts are taken. Since this experiment takes a relatively long time, the radioisotope Fe^{55}, possessing a longer half-life, has been used. Finally, the sideroblast appearance of the patient and the results of the autoradiography from the same patient are compared in the manner as to be described later.

For the detection of sideroblasts we have used a modified method of KAPLAN (5), and observing 100 erythroblasts the percentage of sideroblasts has been calculated, classifying into three types as shown in Fig. 1, according to the number of iron granules found in the cytoplasm; then placing the percentage of each type at ordinate and type of sideroblast at abscissa, a sideroblastogram has been made (6). Also the average number of iron granules in individual erythroblasts has been calculated.

Fig. 1. Diagrammatical Drawing of Sideroblasts and their Classification.



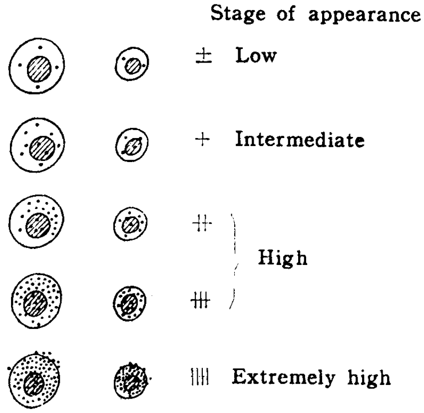
Type I ······ possessing 1—2 iron granules
Ia······possessing 1 granule
Ib······possessing 2 granules
Type II ······ possessing 3—5 granules
Type III ······ possessing over 6 granules

The stripping emulsion method is used for antoradiography. Namely, the bone marrow smears are dried and fixed with methanol. These are first treated with gelatin and then these smears are covered with stripping film in a dark room. This covering is conducted in 1% glycerine distilled water solution added with KBr at the concentration of 50 mg/1. After desiccating the smears, these are placed in a box containing silicagel, sealed tightly and shielded from light, and the exposure is conducted for 15 days in a refrigerator. They are developed on Fuji FD-111 for three minutes, and then are immersed in the fixing solution of 1 mol. sodium thiosulfate.

The films are washed in running water for 20 minutes, and after drying they are stained with Giemsa, and the appearance of darkened silver granules on erythroblasts has been observed with microscope.

The erythroblasts are classified into five types, as demonstrated in Fig. 2, according to the rate of appearance of darkened silver granules with radioactivity. The average rate of radioactive iron appearance in individual cells is calculated from the distribution density curve drawn on the 200 cells observed.

Fig. 2. Diagrammatical Drawing of Erythroblasts and the Classification according to the Rate of Radioactive Iron Appearance in Autoradiography.



RESULTS

Sideroblasts observed by Prussian blue reaction :

The investigations of sideroblasts conducted on the bone marrow smear from the patients in iron deficient state (idiopathic hypochromic anemia, Banti's disease and malignant tumor), and polycythemia vera, in which the accelerated erythropoietic function is supposed, proved the low rate of sideroblast appearance as shown in Figs. 3-A, 4-A, and the sideroblastogram showed a tendency to left shift as can be seen in Figs. 3-B, 4-B.

On the contrary, in the cases of hypoplastic anemia and leukemia, in which the decreased erythropoietic function is supposed, the sideroblast appearance is high, as indicated in Fig. 6-A and also a tendency to right shift in sideroblastogram can be recognized as seen in Fig. 6-B.

In the normal persons the values are found to be intermediate between the two groups, the activated and suppressed ones in hematopoiesis (Fig. 5).

Acta Medica Okayama, Vol. 14 [1960], Iss. 2, Art. 2

Fig. 3. A. Myelogram concerning Sideroblasts and Radioactivity in One Case of Idiopathic Hypochromic Anemia (Iron Deficient State)

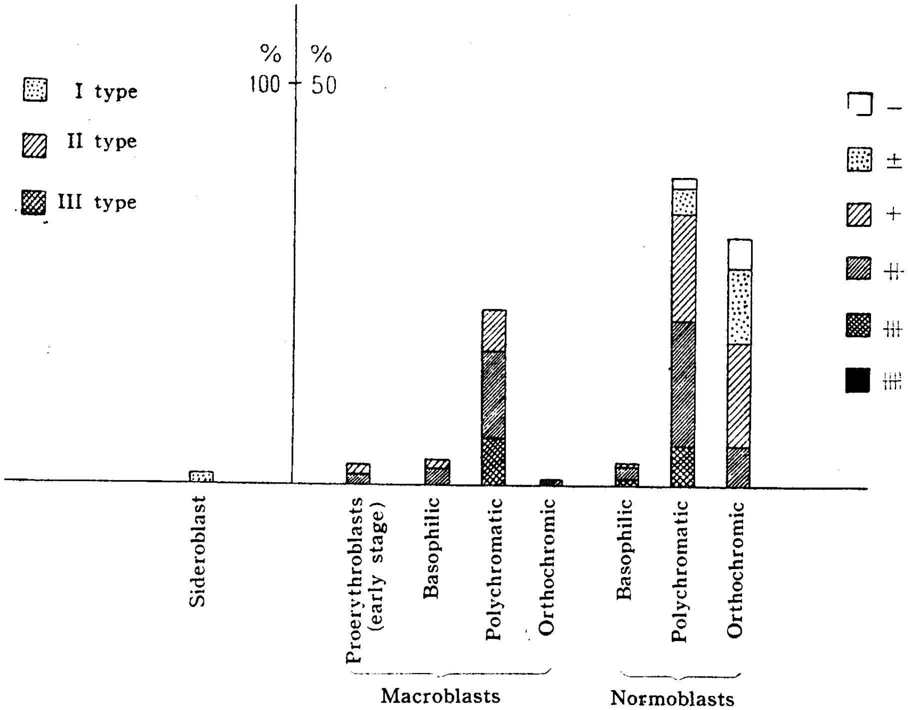


Fig. 3. B. Sideroblastogram and Fe^{55} Uptake in each maturing Stage of Erythroblasts

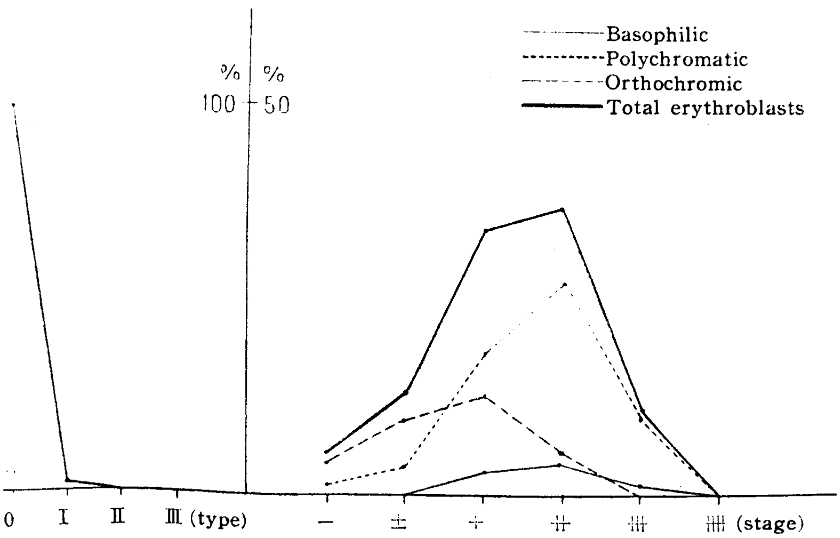


Fig. 4. A. Myelogram concerning Sideroblasts and Radioactivity in One Case of Polycythemia Vera (Accelerated Erythropoietic Function)

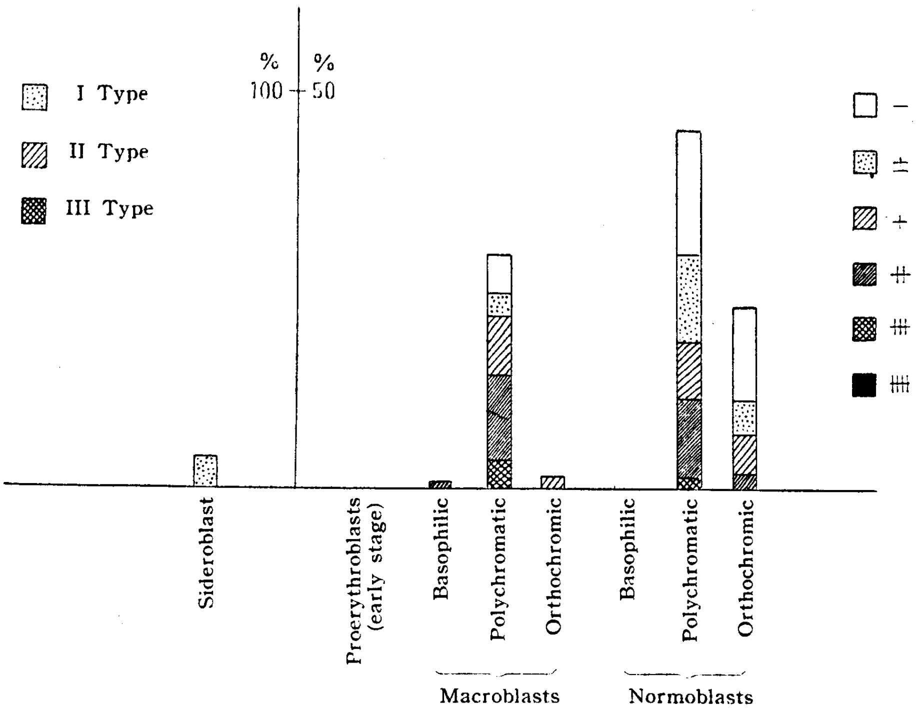


Fig. 4. B. Sideroblastogram and Fe^{55} Uptake in Each Maturing Stage of Erythroblasts

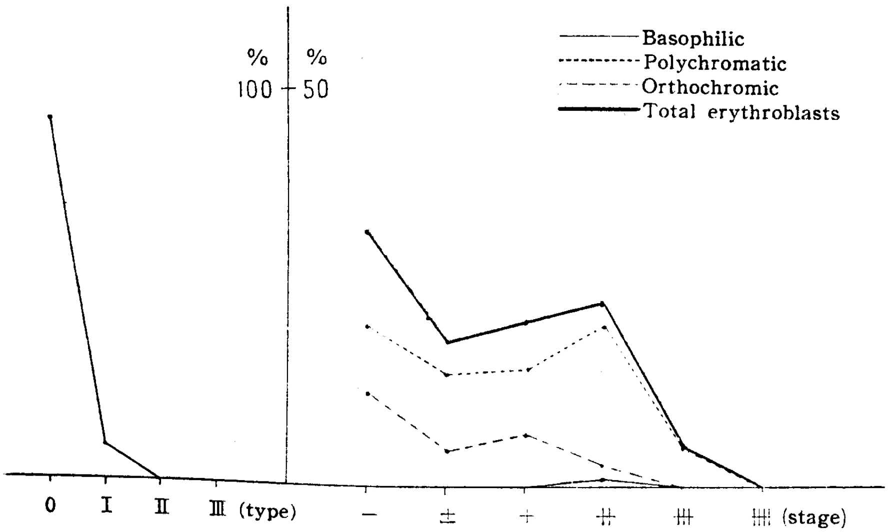


Fig. 5. A. Myelogram concerning Sideroblasts and Radioactivity in One Case of Normal Person

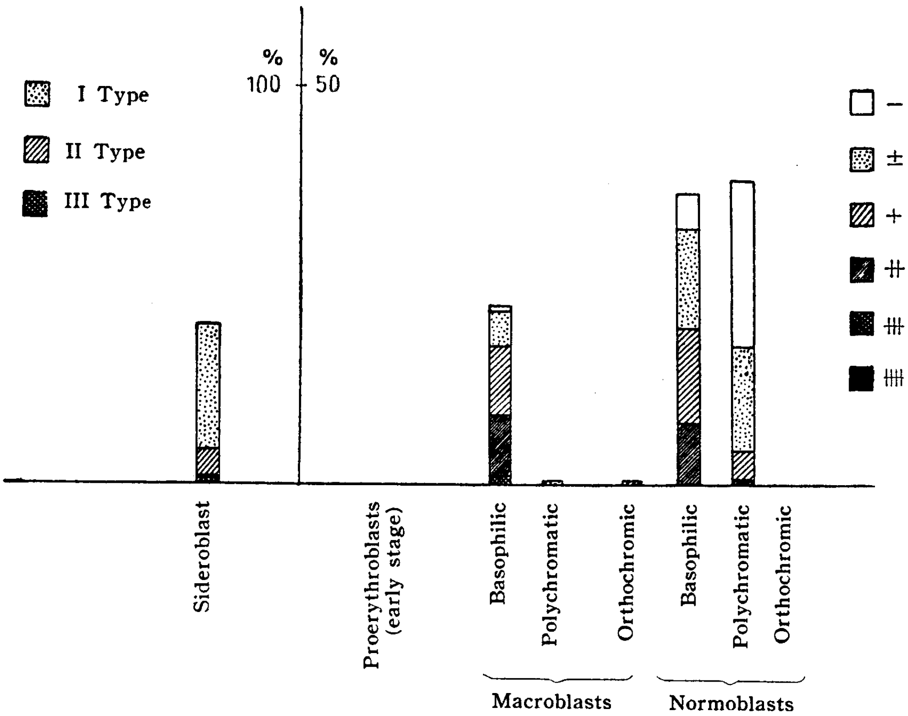


Fig. 5. B. Sideroblastogram and Fe^{55} Uptake in Each Maturing Stage of Erythroblasts

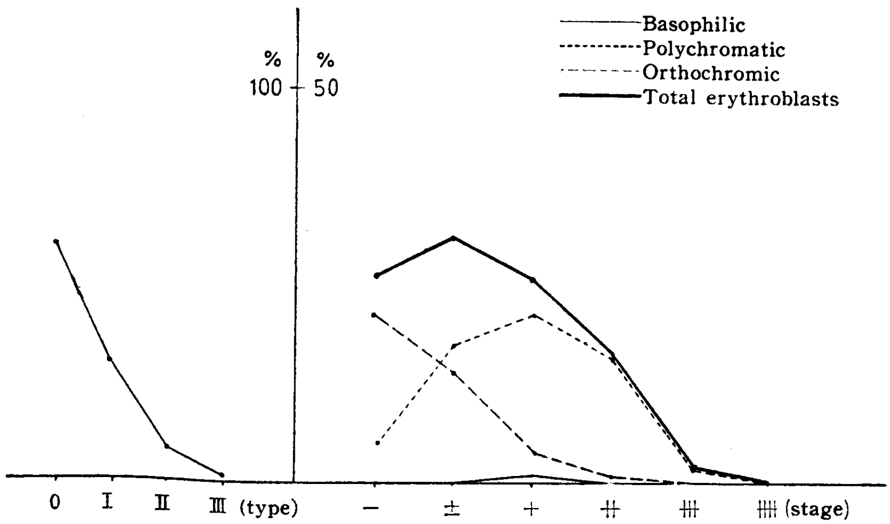


Fig. 6. A. Myelogram concerning Sideroblasts and Radioactivity in One Case of Hypoplastic Anemia (Low State of Erythropoietic Function)

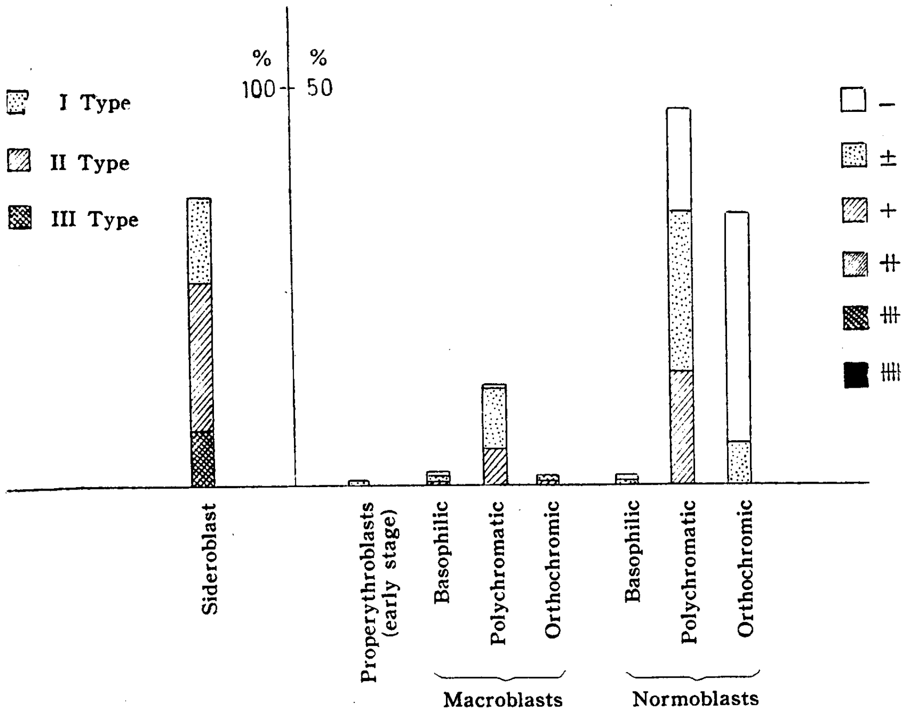
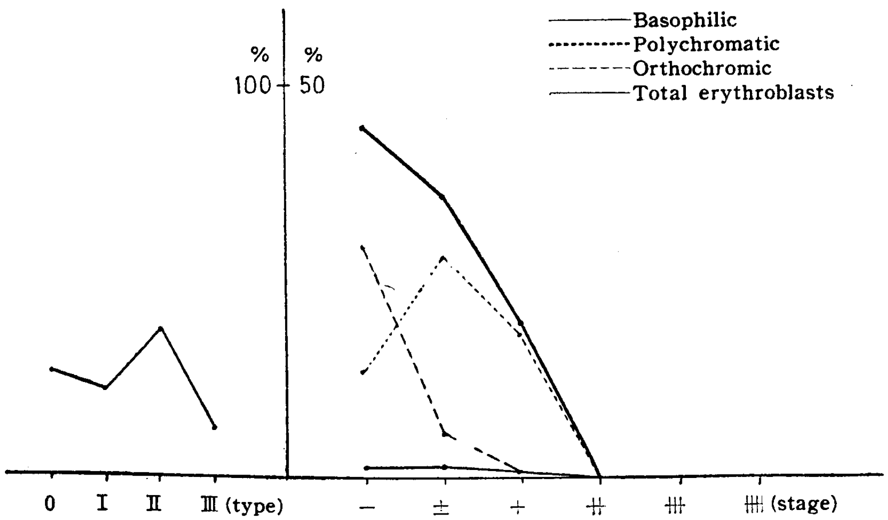


Fig. 6. B. Sideroblastogram and Fe^{55} Uptake in Each Maturing Stage of Erythroblasts



Concerning the average number of stainable iron granules of erythroblasts, the number is high in hypoplastic anemia and in leukemia, followed by normal persons, and decreases in the order of Banti's disease, cancers, polycythemia vera, idiopathic hypochromic anemia.

·Quantitative analysis of serum iron conducted simultaneously on these patients showed some correlation of the serum iron contents to the appearance of sideroblasts, i. e. low values in the cases of accelerated erythropoietic function as one patients in iron deficient state, and high in the case of low erythropoietic function.

Radioactivity of erythroblasts :

The autoradiographic observations revealed that the most marked radioactivity are found in the polychromatic erythroblasts, followed by orthochromatic erythroblasts, though some of basophilic ones show a high radioactivity. Most of orthochromic erythroblasts showed radioactivity but low in grade and ones showing high radioactivity can hardly be recognized.

In normal persons, a representative case of which is shown in Fig. 5, the erythroblasts having radioactive iron can be recognized, but they occupy about 70 per cent of total erythroblasts and the majority of them show a moderate to low activity and those having a high radioactivity are a few in number.

Viewing the radioactivity of erythroblasts in each disease, in those diseases that show iron deficiency and hypochromic anemia, such as idiopathic hypochromic anemia, Banti's disease, malignant tumors, generally almost all erythroblasts possess radioactivity as in the representative case of idiopathic hypochromic anemia shown in Fig. 3, and their radioactivities are high. For example, in idiopathic hypochromic anemia the radioactive iron appears in 94.7-97.1 per cent of erythroblasts, and about 70-80 per cent of them show a moderate to high radioactivity. In Banti's disease the radioactivity can be recognized in 85.9-93.9 per cent of erythroblasts, and the majority of them present a moderate radioactivity, and some of them show a low activity and others a high radioactivity, In Hodgkin's disease, radioactive iron appears in 94 per cent of erythroblasts. Most of them, about 75 per cent, show a moderate to a high radioactivity presenting fairly similar values to those of idiopathic hypochromic anemia. In cancers (gastric and hepatic cancers) the radioactive iron appears in 70-94.3 per cent of erythroblasts, and most of them show either a moderate or high radioactivity in two cases, while the other two cases show a moderate or low activity showing a fairly wide range in distribution, though even in the former the degree of appearance of radioactive iron is lower than what can be observed in idiopathic hypochromic anemia. In these four cases mentioned above, the appearance of radioactive iron is higher than in the cases of normal persons. In multiple myeloma radioactive iron appears in 90.1 per cent of erythroblasts, and many of them, about two thirds in number, show moderate or high degree of radioactivity and a few of them presented an extremely high radioactivity.

In the case of polycythemia vera which will mean an accelerated erythropoiesis, erythroblasts without radioactivity occupy as high as 32.4 per cent, and most of radioactive erythroblasts show a high radioactivity (Fig. 4)

In the cases of hypoplastic anemia and leukemia which will be in the state of low erythropoietic function radioactive iron appears only in about 50-70 per cent of erythroblasts, and moreover, the majority of them, as shown by the representative case of hypoplastic anemia in Fig. 6, show only an extremely low degree of radioactivity. In the cases of hypoplastic anemia one case shows a low or intermediate degree of radioactivity in 55.6 per cent of erythroblasts but no erythroblasts with high radioactivity can be recognized. In another case, however, 73.2 per cent of erythroblasts showed the radioactivity, and of them about 80 per cent possess low or moderate activity, and about 20 per cent possess the high activity, showing the conditions rather similar to those of normal persons. In the case of leukemia, radioactivity can be recognized in 57.6 per cent of the erythroblasts in one case and these, like in hypoplastic anemia, show only a low to moderate redioactivity. While in another case of leukemia 99.1 per cent of erythroblasts show radioactivity and about 90 per cent of them showed a moderate to high radioactivity.

On comparing the appearance of radioactive iron in erythroblasts, it is the highest in idiopathic hypochromic anemia, followed by polycythemia vera, Banti's disease, malignant tumors, and normal persons, leukemia, hypoplastic anemia, in the descending order mentioned, as shown in Fig. 7. However, in some of malignant tumors and leukemia, the appearance rate is found to be in the range equal to or more than that of idiopathic hypochromic anemia, and even in the hypoplastic anemia, the exceptional case in which the rate of appearance is close to the normal level can also be recognized.

As can be understood from the above mentioned data, between the average rate of radioactive iron appearance in erythroblasts and the average number of stainable iron granules of erythroblasts there can be observed an inverse correlation which is clearly shown in Fig. 8, i. e. there occurs a decrease in the appearance of radioactive iron when stainable iron granules in erythroblasts are on the increase, while the radioactive iron increases when the stainable iron granules are on the decrease.

Fig. 7. Average Number of Stainable Iron Granules and Average Rate of Radioactive Iron Appearance in Erythroblasts of Various Diseases

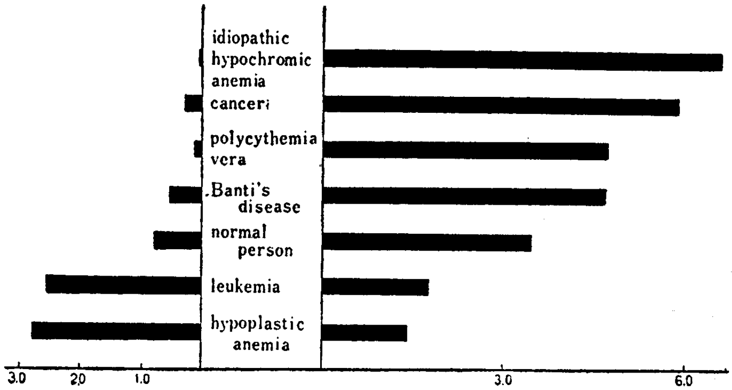
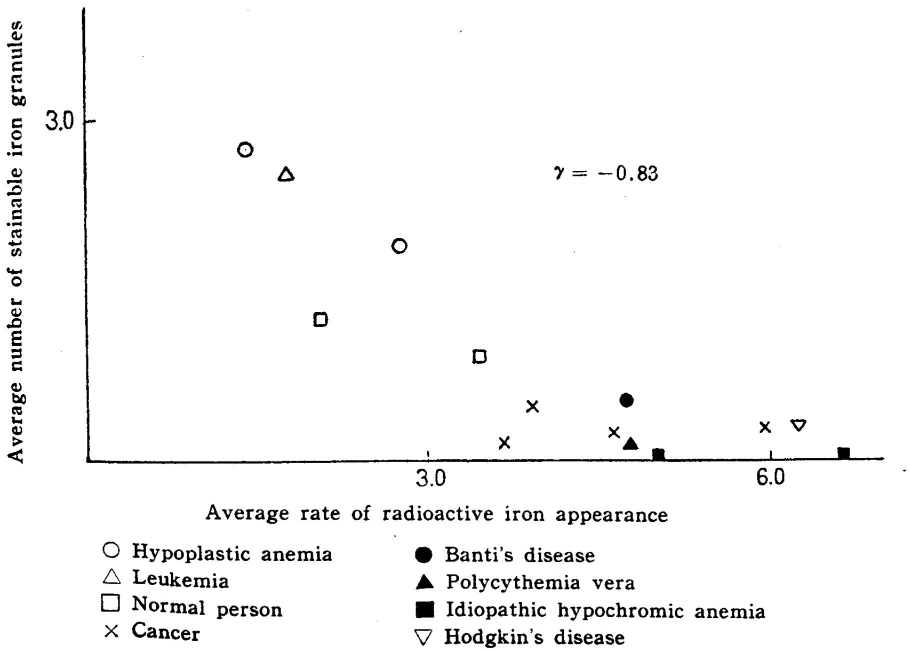


Fig. 8. Correlation Between the Average Number of Stainable Iron Granules and Average Rate of Radioactive Iron Appearance



DISCUSSION

The autoradiography of erythroblasts *in vitro* reveals the Fe^{55} taken into erythroblasts and it is quite a convenient methnd for tracing the introduction of iron into erythroblasts, but not possible in distinguishing non-hemin iron from heme iron.

In contrast to this, the investigation of the iron granules stainable by Prussian blue reaction in erythroblast, sideroblasts, reveal the non-hemin iron in the cell, which is supposed to be on its way to hemoglobin production after iron is taken up by erythroblast. (6) Consequently it is reasonable to think that more precise knowledge about the iron metabolism in erythroblasts may be obtained by combining the autoradiography method with the potassium ferrocyanide method. Howerver, it has been proven that there are some difficulties technically to investigate the radioactivity on the cells having the Prussian blue reaction on the same specimen. Therefore, we have investigated the iron uptake by applying the autoradiographic method on one specimen and the pathway of the iron taken into cell to the heme synthesis by the potassium ferrocyanide method on another specimen from the same patient. Comparative studies on these two specimen gave a fruitful results revealing the correlation between these as demonstrated in above.

The results have shown that in idiopathic hypochromic anemia, a representative one of iron deficiency cases as already mentioned, both serum iron and sideroblasts are low in their values and a marked iron uptake can be recognized in erythroblasts, clearly indicating the accelerated iron uptake into the erythroblast and an increased incorporation into heme. Similar results have been also obtained in Banti's disease suggesting that in Banti's disease there is hardly any disturbance of the erythropoietic function in the bone marrow, and solely the detention of iron in the reticuloendothelial system mainly in the spleen brings about a poor supply of iron to the bone marrow, which will be the main factor for causing anemia in this disease. Even in the cases of malignant tumors, the similar tendency as in the previous cases can be recognized of serum iron, sideroblasts, and the radioactivity of erythroblasts, indicating that in the patient bearing malignant tumors the iron deficiency anemia will be induced by the fixation of iron in some tissues, probably in cancer tissue or in the reticuloendothelial system.

In polycythemia vera some erythroblasts show a marked uptake of Fe^{55}, but there are a considerable number of erythroblasts that show no radioactive iron, and this is in a marked contrast to idiopathic hypochromic anemia in which the number of sideroblasts is markedly decreased and almost all erythroblasts contain Fe^{55}. In the polycythemia vera there is hardly any iron deficient state in the bone marrow as the erythroblasts contain a considerable amount of hemoglobin, and it is assumed that in polycythemia vera the process of iron uptake into cell and the incorporation of iron into heme is proceeding without any disturbance keeping an equilibilium at a certain level of the increased hematopoiesis. Actually the bone marrow are filled with a number of the relatively matured erythroblasts less in the iron holding capacity (6). In hypoplastic anemia both serum iron and sideroblasts show high values, while, a decrease in the iron uptake of erythroblasts can generally by recognized. This fact substantiates a decrease in the iron utilization owing to the erythropoietic disturbance, though some cases showed the normal iron uptake of erythroblast.

In leukemia, generally the serum iron level and the number of sideroblasts are high and low in the number of those containing radioactive iron, showing the reduction in iron uptake and incorporation into heme, though occationally a marked uptake can be recognized. The irregularity of these data suggests that there are various factors responsible for anemia or the different factors in the different phases in this disease. Namely, there exist the disturbances of the erythropoietic function or the iron deficient state due to the iron detention in the reticuloendothelial system in same cases, and either one of these must have been brought to the surface.

Between the rate of appearance of sideroblasts and the iron uptake as measured by autoradiography in various diseases there can generally be recognized an inverse correlation as already mentioned, and this indicates that the stainable iron in erythroblast can be a criterion for judging the iron uptake capacity of erythroblasts and also, as we have already pointed out, is associated with the inhibition of the iron incorporation into heme. The maximum number of the stainable iron granules seem to be limited in a certain level. This suggests the limitations of the iron holding capacity of erythroblasts in the form of non-hemin iron (6). In other words, even if a quantity of iron is supplied from serum, the iron uptake of erythroblasts is limited by the contents of non-hemin iron in the cell. The maximum capacity may be correlated to the quantity needed for hemoglobin synthesis in erythroblasts in the course of maturation. The mechanism of iron uptake seems to be a relatively simple like a diffusion which can be controlled by the saturation grade of the non-hemin iron in the cell. Therefore, one can know in what process or in what extent the iron metabolism is inhibited in each disease, if he observes the amount of non-hemin iron and the radioactivity in erythroblasts.

SUMMARY

The serum iron contents and the number of sideroblasts from various patients and the radioactivity of erythroblasts from the same patients incubated with Fe^{55} have been observed.

The results have proved that in the case with accelerated erythropoietic function like polycythemia vera and in the iron deficient state like idiopathic hypochromic anemia, the serum iron level and the number of sideroblast are lower than those in normal persons and higher in radioactivity in erythroblasts, whereas in the case with low erythropoietic function like hypoplastic anemia the former values are higher and lower in radioactivity of erythroblasts.

There is an inverse correlation between the average number of stainable iron granules and the average rate of radioactive iron appearance in erythroblasts, and the observation on these factors will give an important clue for judging the utilization process of iron in each disease. The limitation of the iron uptake correlating with the hemoglobin synthesis have been discussed

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