Acta Medica Okayama

Volume 12, *Issue* 2 1958 *Article* 3

JULY 1958

Study on sideroblasts as a determi-nation method of the erythropoietic function of bone marrow

Kiyoshi Hiraki* Ikuro Kimura†

*Okayama University,

†Okayama University,

Study on sideroblasts as a determi-nation method of the erythropoietic function of bone marrow*

Kiyoshi Hiraki and Ikuro Kimura

Abstract

With the purpose to study sideroblasts as a means of diagnosing blood diseases and to pursue the metabolism of non-hemin iron in erythroblasts we investigated sideroblasts (erythroblasts containing iron granules stainable by Pruian blue) under various erythropoietic conditions in the human and rabbits, and obtained the following results: 1. In blood diseases the proportion of sideroblasts in the case of low erythropoietic condition is higher and in the case with accelerated erythropoietic condition and of iron deficiency it tends to be lower than that in normal persons. Further, obtaining sideroblastogram and sideroblast ratio (S. r.) from the claification of Types I, Ⅱ, and Ⅲ according to the iron granule content, it has been proven that abnormal conditions can be clearly distinguished from the normal, indicating that sideroblasts are closely aociated with erythropoietic function. This is proven to be a far superior method for the diagnosis as well as for the prognosis of blood diseases than the determination of serum iron. 2. In experimental anemic rabbits the relationship of sideroblasts to the condition of erythropoietic function is still more clearly recognized, and it has been found that variations in the sideroblast count is dependent upon the condition of the equilibrium between the iron supply from serum iron and the iron utilization controlled by the erythropoietic function. 3. In addition, in the iron-treated rabbits under various erythropoietic conditions we have been able to confirm that there are a certain mechanism and a limitation to the iron intake by erythroblasts, and that erythroblasts take eentially three steps of metabolic procees, namely, intake, retention, and utilization of iron almost simultaneously, in the latter half of the maturation stage.

Acta Med. Okayama 12, 127—138 (1958)

STUDY ON SIDEROBLASTS AS A DETERMINATION METHOD OF THE ERYTHROPOIETIC FUNCTION OF BONE MARROW

Kiyoshi HIRAKI, Ikuro KIMURA

Department of Internal Medicine, Okayama University Medical School
Okayama, Japan (Director: Prof. Kiyoshi HIRAKI)

Received for Publication, March 7, 1958

Recently a great interest has been focused on the iron metabolism of erythrocyte series, and a conspicuous progress is gradually being made in research from red corpuscles in peripheral blood to erythroblasts in bone marrow by tissue culture or by radioautography. However, the iron movement in erythroblasts, especially the question involving the iron movement during the non-hemin iron phase, remains to be an unsolved mystery harboring many unclarified points.

In this connection ever since GRÜNEBERG's report[1] (1941) on siderocytes the existence of stainable iron in the erythrocyte series has attracted interest, and KAPLAN[2] designated those erythroblasts containing stainable iron as sideroblasts, and found such sideroblasts in all normal children and pointed out its correlation with serum iron. Pursuit of such stainable iron, differing from radioautography, is worthy of a special consideration in that it will afford a clearer view on non-hemin iron phase in erythroblasts.

In our research of sideroblasts we have classified sideroblasts into three types, and obtained the sideroblastogram and sideroblast ratio; and applied these for the differential diagnosis of blood diseases. We have also made an attempt in rabbits to elucidate the properties of sideroblasts or the iron metabolism in the non-hemin iron phase of erythroblasts.

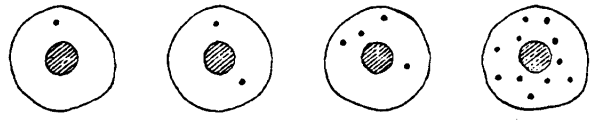
EXPERIMENTAL RESULTS

We have carried our research in patients with various blood diseases, various experimental anemic rabbits as well as in rabbits given iron compounds. Namely, by preparing bone marrow smear specimens from these subjects, and after staining iron with Prussian blue synthesis method after KAPLAN[2], and counterstaining the specimens with basic fuchsin so

as to detect sideroblasts, and at the same time the serum iron was determined by BARKAN's method[3].

Calculation of sideroblasts was taken mainly on the cells in the latter half of maturation stage, and they were classified into three categories according to the number of iron granules as follows:

Fig. 1 Classification of Sideroblasts



Type I·····with 1 to 2 iron granules
Ia·····with a single iron granule
Ib·····with two i on granules
Type II ····· with 3-5 iron granules
Type III·····with more than six iron granules
(Type 0 ·····without iron granule)

Then studying 100 erythroblasts, the percentage of each type was calculated; and placing the percentage of the type at ordinate and type of siderobalst at abscissa, a sideroblastogram was made. In addition, using the following equation the ratio of sideroblasts in relation to each type was determined and called it "sideroblast ratio" (S. r.).

$$\text{S. r.} = \frac{\text{Type II}\,(\%) + \text{Type III}\,(\%)}{\text{Type I}\,(\%)}$$

RESULTS

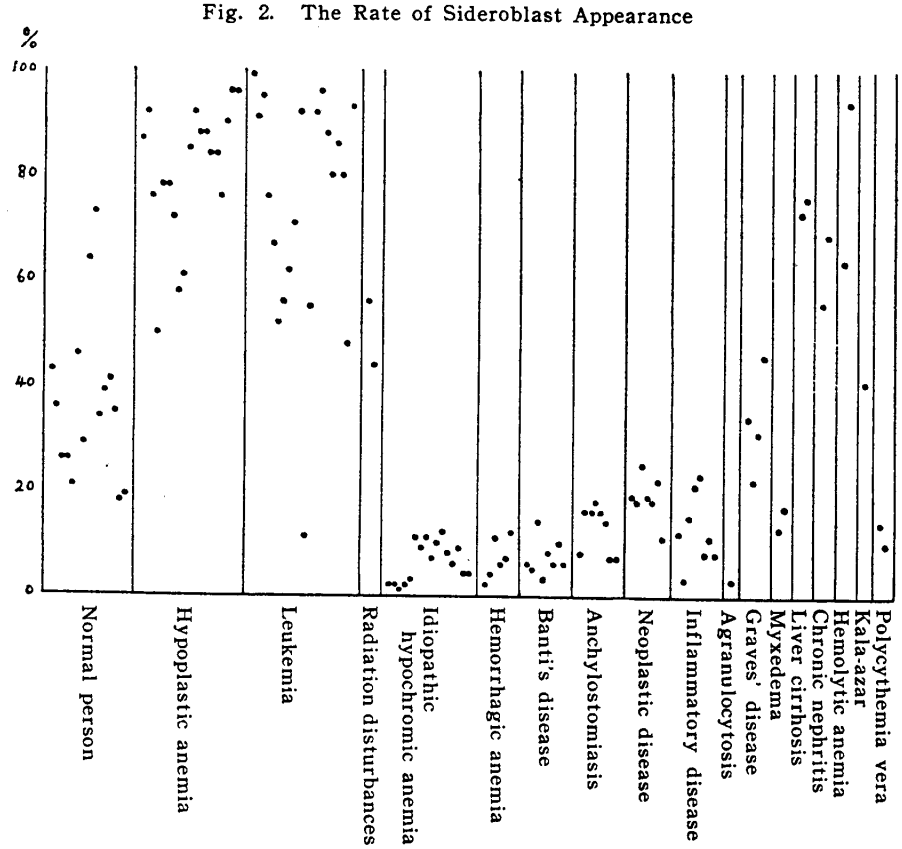
Patients with various blood diseases

Appearance of sideroblasts (Fig. 2): Stainable iron granules appear mostly during the latter half of maturation stage, especially most abundantly in polychromatic erythroblasts, and they are scattered in the cell in such positions as to surround the nucleus. These iron granules are of round, oval or irregular shape and vary in size and deepness in color. Moreover, at the time when there appear more granules, a greater cytoplasm tends to have a greater holding capacity of granules. In addition, even in what appears to be young red cells in specimens, granule appearance can be detected similarly as in erythroblasts.

In those of normal persons the proportion of the sideroblast appea-

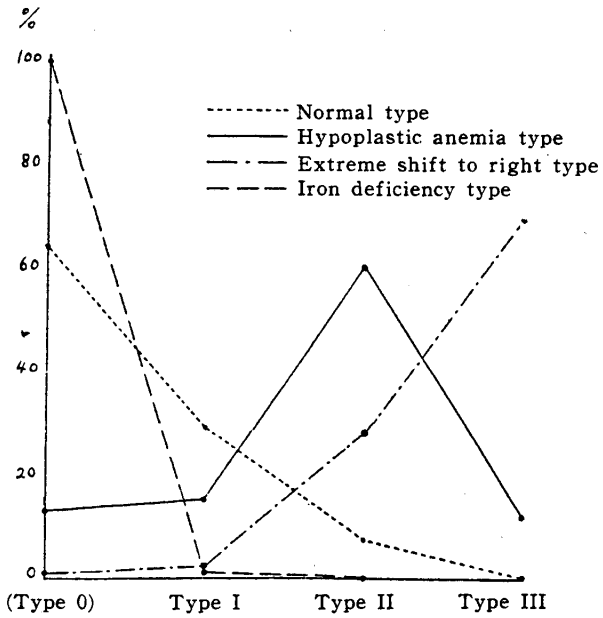
rance (average of 36.6%) was fairly in a wide range and sideroblasts were observable in all cases. It tended to be a little lower in females. Generally the proportion of the sideroblast appearance has been found high in the patient with various erythropoietic disturbances such as hypoplastic anemia, leukemia, radiation disturbances, as well as hemolytic anemia, at the same time granules tend to be greater in size and more deeply stained. In iron deficiency cases in broader sense, such as idiopathic hypochromic anemia, hemorrhagic anemia, Banti's disease, anchylostomiasis, neoplastic diseases, and inflammatory diseases, the proportion of sideroblast appearance is low and the granules tend to be smaller and stained lightly. Moreover, the majority of them show a transient increase in sideroblasts when given iron compound. Besides these, Graves' disease and kala-azar demonstrate normal level of sideroblasts; chronic nephritis and liver cirrhosis higher values; and myxedema, agranulocytosis and polycythemia vera show a low proportion of the appearance. Furthermore, between the proportion of the sideroblast appearance and the color index there exists more or less a parallel relationship.

Fig. 2. The Rate of Sideroblast Appearance



Sideroblastogram (Fig. 3): The sideroblastogram is roughly classified into three types, namely, Left Type (shift to the left), Right Type (shift to the right), and Intermediate Type (between these two types). In normal persons sideroblasts appear in a descending order of Type I, Type II, and Type III, and all of them show the sideroblastogram of Intermediate Type(normal type). In the case of hypoplastic anemia irrespective of the sideroblast appearace sideroblasts appear in the descending order of Type II followed by Type I and Type III; and Type II demonstrates a characteristically marked increase as to be called the hypoplastic anemia type, showing a peculiar shift to the right; and the same tendency can be observable in the case of radiation disturbances. In the case of leukemia all shifting types can be observed; and namely, extreme shift to right type (assuming the descending order of Type III, Type II and Type I), hypoplastic anemia type, the normal type and further a type that can be considered as identical with an iron deficiency type to be described later. It is characteristic of this disease that there exist various shifting types such as described above.

Fig. 3. Sideroblastogram



Those diseases belonging to the iron deficiency anemia in broader sense as has been already mentioned and polycythemia vera all show a marked decrease in Type I, disappearance in Type III and often the dis-

appearance of Type II, presenting the Left Type what might be called an iron deficiency type.

Sideroblast ratio : As for the sideroblast ratio, none in normal persons shows the value over 1 nor zero, and all show the value between zero and 1, practically most of them below 0.5. Sideroblast ratio in the case of hypoplastic anemia stays in between 1 and 5, never less than 1. Leukemia shows various values ; namely those of extreme shift to right type show over 5 with the maximum approaching 50, values for hypoplastic anemia type and normal type are as already described, and in the iron deficiency type sideroblast ratio is close to zero. Most of those that belong to the iron deficiency anemia in broader sense or to polycythemia vera show the value zero the like of which never occurs in normal persons.

Relationship between sideroblast and serum iron : There is often a parallel relationship between the percentage of sideroblast and the serum iron content but not always so. In general an increase above the normal in sideroblasts can be recognized when there is an increase in the serum iron content ; but an increase in sideroblasts does not necessarily accompany the increase in serum iron, on the contrary, in some cases there is a decrease in serum iron instead.

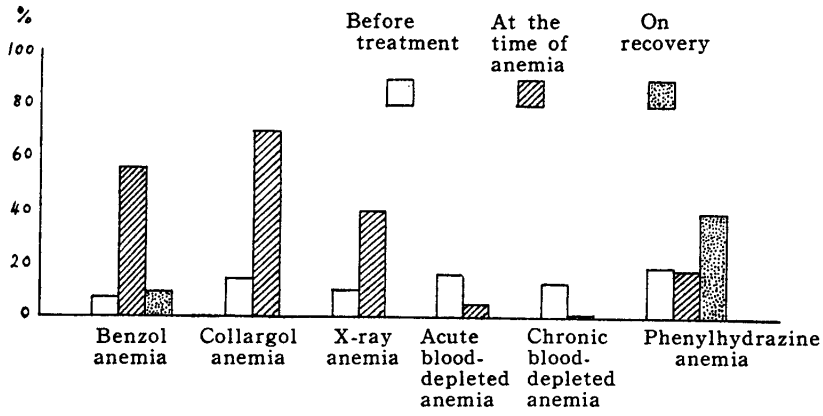
Relationship between sideroblast and bone marrow function (in experimental anemic rabbits, Fig. 4)

The proportion of the sideroblast appearance in normal rabbits is lower (average of 14.4%) than that in normal persons, and its range of fluctuations, being narrow, is fairly fixed. Sideroblasts as in the case of human appear in every case, and Type I occupies the majority of them, and a few of Type II can be recognized in some, but none of Type III in any. Moreover, iron granules likewise tend to be stained light.

In anemic rabbits exposed to benzol, collargol, or X-ray irradiation as to cause disturbances of bone marrow erythropoietic function a marked increase in sideroblasts from the normal conditions, and iron granules are large and stained deep ; and at the recovery stage of benzol anemia they all return to normal. In the rabbits whose bone marrow erythropoietic function is accelerated or iron deficiency is induced, by acute or chronic depletion of blood, sideroblasts in every case decrease, and in extreme cases even a complete obliteration of sideroblasts has been observed. Iron granules also tend to be smaller in size and stained lighter. In rabbits in which hemolytic conditions are induced such as phenylhydrazine anemia, sideroblasts differ in number at different stages, and showing no marked changes at the peak of anemia, they increase at the stage of recovery. Parallel relationship does not necessarily exist between sideroblasts and

serum iron content, but on the contrary these two might stand in opposition to each other.

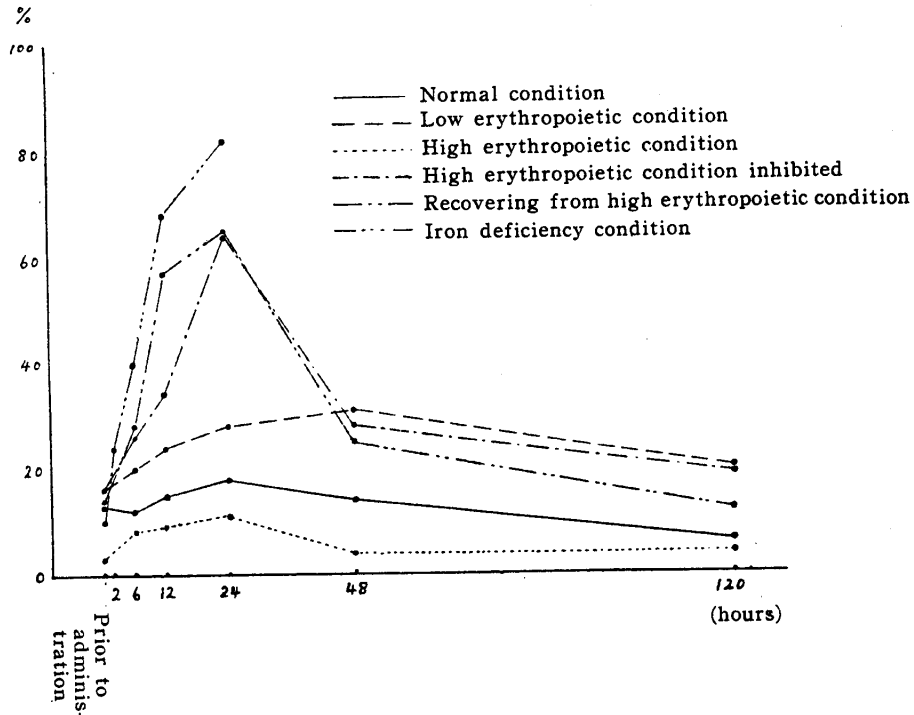
Fig. 4. Sideroblasts in Experimental Anemic Rabbits



Iron intake in erythroblasts (*Fig. 5*)

By inducing various conditions in rabbits mainly involving erythroblasts, and after intravenous injection of iron compound 20 mg./kg. in the form of ferric gluconate solution, observations on the manner of iron movement in erythroblasts have been carried out by determining sideroblasts in bone marrow aspirated at frequent intervals. In the rabbit under normal conditions the proportion of sideroblasts shows only a slight increase, having its peak at 24 hours after iron injection. Under low erythropoietic condition (induced by X-ray irradiation) sideroblasts tend to show a slightly more increase than under normal condition, namely, a moderate increase, having its peak 48 hours after injection. In the case of high erythropoietic condition by blood depletion the sideroblasts increase a little more quickly reaching its maximum at 12 or at 24 hours after injection, a slight increase. In the case where high erythropoietic condition is inhibited (by X-ray irradiation after blood depletion), sideroblasts just keep on increasing, and show a striking increase having its peak 24 hours afterward. Under the condition just recuperating from the accelerated erythropoietic state (left alone for 2 weeks after blood depletion) sideroblasts likewise show a marked increase having its peak at 24 hours later. Under iron deficiency condition (left with iron deficient feed after several blood depletions), the increase in sideroblasts proves, indeed, to be the greatest of all in the present experiments.

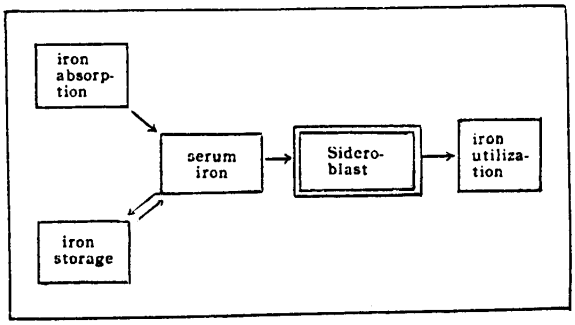
Fig. 5. Sideroblasts of Rabbits given Iron Compound



SUMMARY AND DISCUSSION

Viewing the iron metabolism *in vivo*, sideroblasts may be thought to be quite a novel and interesting existence. The very fact that sideroblasts tend to increase in blood diseases under the low erythropoietic condition, while they tend to decrease under the accelerated erythropoietic condition

Fig. 6. Diagramatic Description of Iron Movement in Relation to Sideroblasts



as well as in the iron deficient state, and that, when iron is administered to blood disease in the latter conditions, sideroblasts demonstrate a transient increase, signifies that this stainable iron exists in the course of iron utilization from serum iron to erythropoiesis and that it is destined to be synthesized for hemoglobin just prior to the serum iron. These can well be understood from the fact that sideroblasts, when the erythropoietic function is disturbed, especially in hypoplastic anemia, are much more reliable criterion for diagnosing conclusively the actual state of disturbances than serum iron. Moreover, it is assumed that the complexity in leukemia is, besides the disturbances in the erythropoietic function, due to a concomitant deficiency of iron supply to the bone marrow erythrocyte series by the disturbance in the course of iron utilization. And it seems that in experimental anemic rabbits the increase in sideroblasts under the low erythropoietic condition and the decrease in the accelerated state along with the increase in hemolytic condition and the decrease under the iron deficient state, are all dependent upon the state of the equilibrium existing between the iron utilization by the erythropoietic function and the iron supply from serum, and that these two factors are responsible for the changes of sideroblasts in number. Although sideroblasts and serum iron do have a relationship, actually demonstrated conditions reveal quite a different feature. This phenomenon seems to be due, aside from the serum iron content being dependent upon the state of erythropoietic function, to the influence of conditions of the reticulo-endothelial system function, intestinal absorption and iron loss. Therefore, the fact that an increase in sideroblasts does not necessarily accompany the increase in serum iron seems to suggest relationship of sideroblasts with the color index as well as this stainable iron being destined to be utilized in the process of hematopoiesis. Consequently this stainable iron not being of pure depot form, and further on comparing it with the iron fractionation (Yoneyama-Konno method[4]) of viscera used in our laboratory[5], it is associated with bone marrow PIII (the fraction derived from nucleoproteins and high molecular compounds); and the stainable iron appears in all likelihood to be contained in the bone marrow PIII. Furthermore, it has been proven by the experiments with radioactive iron Fe^{55} conducted in our laboratory[6] that this iron fraction is especially easily utilizable of depot iron in a broader sense; and when the existence of what GREENBERG and WINTROBE[7] call 'labile iron pool' is taken into consideration in the same vein of thought, it is deeply interesting.

In connection with iron intake by erythroblasts, the usual increase of sideroblasts in number and changes with the lapse of time according

to the conditions of erythropoietic function along with ever present stainable iron under normal conditions essentially indicate the existence of three metabolic steps, namely, the iron intake, its retention, and the utilization for hemoglobin in erythroblasts. It is understood that of the three steps for the iron intake there are two important factors controlling it, i. e., the one is the iron supply from serum and the other is non-hemin iron content which is controlled by the hemoglobin content in the erythroblast in the course of maturation. And it is assumed that the balance of concentrations between the two contents bordering the erythroblast membrane is chiefly responsible for the mechanism of the iron intake. Therefore, the mechanism of iron intake heavily tends to be relatively simple nature something like diffusion, and JENSEN[8] and NAKAO[9] have already pointed out this fact in the peripheral red corpuscles of birds. Consequently iron is taken up in the amount suitable for any given condition without any direct connection to its utilization for hemoglobin in erythroblasts, and the surplus amount unable to be consumed at once is stored up temporarily. Thus as the amount of hemoglobin gradually increases, non-hemin iron content, though small in amount, reaches its saturation and therefore, the amount of intake subsequently decreases gradually. It is thought that matured red cells are born without intake of surplus iron. Such a limitation in the iron intake on the part of erythroblasts can easily be understood from the iron holding capacity in the case of patients with blood diseases.

As regards the stage when iron is taken up by the erythroblast there are recent research works of AUSTONI[10] and LAJTHA[11] with radioautography, and these have become a source of conflicting opinions. This stainable iron can be recognized mainly in the latter half of maturation stage and it is quite difficult to detect in young erythroblasts. In other words, erythrocyte series are capable of taking up iron at any stage, but the greatest amount taken up is in the latter half of maturation stage, especially in the polychromatic phase, which seems to coincide with the differentiation stage where hemoglobin is produced as observed by THORELL[12]. Moreover, it is believed that erythroblasts at this stage assume a position able to take up iron in a great quantity and that intake is managed by the mechanism previously mentioned.

As regards the retention phenomenon such as shown by the stainable iron in erythroblasts, it is hard to believe that it assumes a pure storage form, but it seems in all probability there is iron maintaining a ferrous form that is readily utilizable and that it is stored up temporarily in the ferric form only in case of surplus iron supply.

For the iron intake by erythroblasts actually the mechanism and limitation above mentioned seem to exist, and this phenomenon is determined both by serum iron and non-hemin iron, which is controlled by the hemoglobin content in the erythroblast after a certain degree of maturation and probably is in the state of ferrous saturation. Therefore, this phenomenon seems to be a relatively simple nature something like diffusion.

Furthermore, the sideroblastogram and sideroblast ratio in patients with blood diseases are greatly useful in judging those pathological conditions not so uncommonly atypical in the appearance alone; and this study of sideroblasts is far superior to the serum iron in differential diagnosis or in deciding prognosis of the patients with blood diseases, and the ease with which this method can be manipulated far surpasses that of the serum iron.

CONCLUSIONS

With the purpose to study sideroblasts as a means of diagnosing blood diseases and to pursue the metabolism of non-hemin iron in erythroblasts we investigated sideroblasts (erythroblasts containing iron granules stainable by Prussian blue) under various erythropoietic conditions in the human and rabbits, and obtained the following results:

1. In blood diseases the proportion of sideroblasts in the case of low erythropoietic condition is higher and in the case with accelerated erythropoietic condition and of iron deficiency it tends to be lower than that in normal persons. Further, obtaining sideroblastogram and sideroblast ratio (S. r.) from the classification of Types I, II, and III according to the iron granule content, it has been proven that abnormal conditions can be clearly distinguished from the normal, indicating that sideroblasts are closely associated with erythropoietic function.

This is proven to be a far superior method for the diagnosis as well as for the prognosis of blood diseases than the determination of serum iron.

2. In experimental anemic rabbits the relationship of sideroblasts to the condition of erythropoietic function is still more clearly recognized, and it has been found that variations in the sideroblast count is dependent upon the condition of the equilibrium between the iron supply from serum iron and the iron utilization controlled by the erythropoietic function.

3. In addition, in the iron-treated rabbits under various erythropoietic conditions we have been able to confirm that there are a certain mecha-

nism and a limitation to the iron intake by erythroblasts, and that erythroblasts take essentially three steps of metabolic processes, namely, intake, retention, and utilization of iron almost simultaneously, in the latter half of the maturation stage.

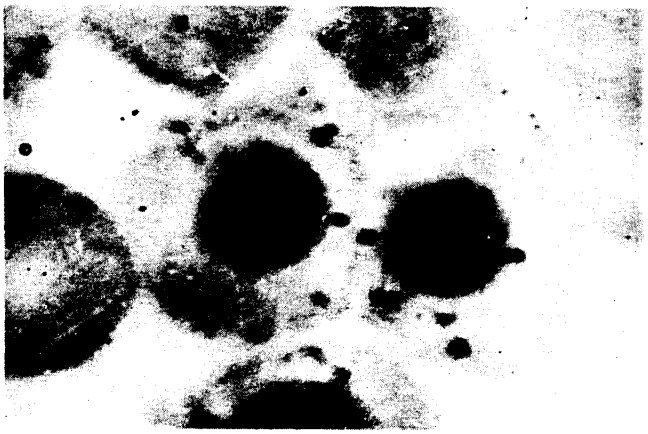
REFERENCES

1. GRÜNEBERG, H. : Siderocytes, a New Kind of Erythrocytes, Nature, **148**, 114, 1941
2. KAPLAN, E., et al. : Sideroblasts, a study of stainable nonhemoglobin iron in marrow normoblasts, Blood, **9**, 203, 1954
3. BARKAN, G. and WALKER, B. S. : Determination of serum iron and pseudohemoglobin iron with o-phenanthroline, J. Biol. Chem., **135**, 37, 1940
4. YONEYAMA, Y. and KONNO, K. : Studies on non-hemin iron, J. Biochem., **40**, 377, 1953
5. MAITANI, K., et al. : Quantitative studies on the iron storage in experimental anemias, Acta Haemat. Jap., **18**, 331, 1955
6. SHIOMI, F. and KIMURA, I. : Studies on bone marrow function and iron metabolism, Acta Haemat. Jap., **19**, 295, 1956
7. GREENBERG, G.R. and WINTROBE, M. M. : A labile iron pool, J. Biol. Chem., **165**, 397, 1946
8. JENSEN, W.N. et al. : The uptake *in vitro* of radioactive iron by avian erythrocytes, J. Labor. Clin. Med., **42**, 833, 1953
9. NAKAO, K. : The iron metabolism of erythrocytes, Saishin-Igaku, **11**, 129, 1956 (in Japanese)
10. AUSTONI, M. E. : Autoradiographic studies on iron-59 turnover by erythroid cells in rat bone marrow, Proc. Soc. Exper. Biol. Med., **85**, 48, 1954
11. LAJTHA, L. G. and SUIT, H. D. . Uptake of radioactive iron (^{59}Fe) by nucleated red cells *in vitro*, Brit. J. Haematol., **1**, 55, 1955
12. THORELL, B. : Studies on the formation of cellular substances during blood cell production, Acta Med. Scandinav., **129**, Suppl. 200, 1947

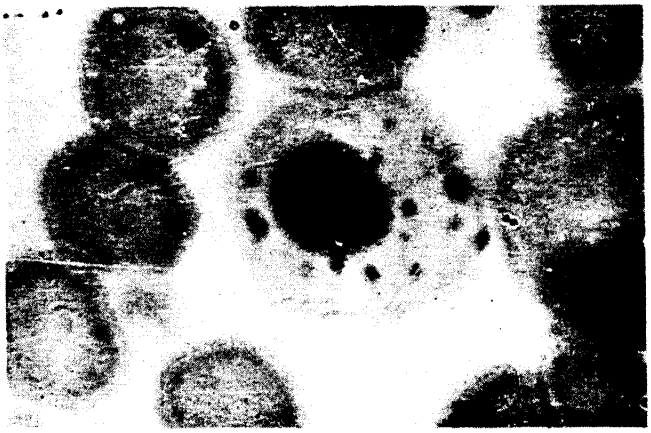
LEGEND FOR PLATE

Plate 1. A, B Sideroblasts in monocytic leukemia of extreme shift to right type.

Plate 1.



A



B