STUDIES ON THE MATURATION OF MYELOBLASTS INTO MYELOCYTES AND ON AMITOTIC CELL DIVISION IN THE PERIPHERAL BLOOD IN SUBACUTE MYELOBLASTIC LEUCEMIA.

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PLATES 39 AND 40.

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INTRODUCTION.

Through the courtesy of the Medical Department of the Johns Hopkins Medical School and of the Hebrew Hospital and Asylum of Baltimore, we have had the privilege of studying the blood in several cases of leucemia during the past 2 years. The case which forms the basis of this report was from the Hebrew Hospital, and the clinical history has been contributed to this article by its medical director, Dr. Charles R. Austrian.

With the introduction of a new technique, by which differential studies of living blood cells can be made, it is of the utmost value to study the blood of leucemic patients from the standpoint of establishing the morphology of the blood-forming organs on such a basis as to open up an experimental attack on the mechanism, first, of the regulation, of the normal proportion of different types of cells in the circulating blood and, second, of the alteration in disease of the proportion and indeed of the types of cells appearing in the blood.

In the case reported here, we think that we were able to identify myeloblasts with the supravital technique, to demonstrate that the maturation of myeloblasts into myelocytes followed the transfusion of whole blood, and to identify the stages through which myeloblasts develop into myelocytes and finally into leucocytes. Also we saw in these living preparations some remarkable types of nuclear and cellular division, which must ultimately be taken into consideration in the analysis of the cause of leucemia.

Technique.

For the discrimination of myeloblasts in the supravital technique we used neutral red and Janus green.[1] The method already described by Sabin (5) was followed, but in general for the blood of leucemic patients or for smears from bone marrow, in which there were many more white cells involved, it was found that the strength of both dyes should be about twice the amount for normal blood. In regard to the use of Janus green three different reactions have been observed. When the dye was in the correct proportion for staining the cells without apparent injury the mitochondria were found colored a brilliant blue and certain substances in the platelets stained a dull green; but when the concentration of the dye was too great the cytoplasm of the young white blood cell, which is known to be strongly basophilic, showed a diffuse grey tone.

We are indebted to Mr. James F. Didusch, artist of the Department of embryology of the Carnegie Institution of Washington, for the drawings of the living cells.

For the case which is the basis of this report we have the following clinical history and pathological report.

Clinical Report.

C. M., a white youth aged 15 years, was admitted to the Hebrew Hospital Nov. 23, 1923 (Med. No. 2217), complaining of "weakness and constant vomiting." His family and past histories were negative. In June, 1923, 6 months before he came under observation, he noted a sense of weakness, and was frequently nauseated and vomited. In Sept., 1923, he had dysphagia and an exudative inflammation of the tonsils without fever. 3 weeks prior to admission to the hospital, "sore throat" recurred, and a week before symptoms of pharyngeal inflammation recurred but no epistaxis or stomatitis. Asthenia, vomiting without hematemesis or melena, dyspnea, and acroparesthesia developed during the past week and became progressively more distressing. The body weight decreased from 120 to 94 pounds in the 6 months before admission.

Physical Examination.—The patient is emaciated, obviously weak, and with striking pallor without icteric tint. No hemorrhagic spots and no areas of infiltration are present in the skin or mucous membranes. There is no exophthalmos and though there is anisocoria the pupils are regular and react normally. The ocular fundi show abnormal fullness of the retinal veins, slight edema of the nerve heads, and on the right side a small diamond-shaped hemorrhage. The tongue is heavily furred. There is slight gingivitis; the tonsils are enormous.

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[1] The preparation of Janus green put up by the National Aniline and Chemical Company gives especially brilliant results. We use the Grübler vital neutral red (*n.* Ehrlich).

No pharyngeal or tonsillar ulceration or exudation is present. There is a general enlargement of the lymph nodes. The lungs are clear and there is no evidence of a mediastinal mass. The heart is not enlarged. There is slight tachycardia; no arrhythmia. The first sound at the apex is not abrupt and the short systolic murmur accompanying it, though audible at the apex and transmitted into the axilla, is of maximum intensity in the second left interspace and the second pulmonic sound is not accentuated. The pulse is 108 per minute, regular in force and rhythm, of fair volume and low tension. There is no sclerosis of the peripheral arteries. The abdomen shows nothing abnormal except that the edge of the spleen is just palpable. There is no edema. Neurological examination is negative.

Laboratory Examinations.—Blood examinations are shown in Table I.

Fragility test: Hemolysis begins in hypotonic solution 0.42 per cent—complete in solution 0.26 per cent.

(Control): Hemolysis begins in hypotonic solution 0.42 per cent—complete in solution 0.28 per cent.

Urinalysis.—Clear amber, acid, albumin 1 plus, no sugar, acetone, diacetic acid, or urobilin. Urobilinogen trace. Microscopic examination: numerous granular casts; red blood cells and epithelial cells, few white blood cells.

Chemical Examination of Blood.—Urea nitrogen, 15.12 mg. per 100 cc. Urea, 32.3 mg. Uric acid, 3.2 mg. Creatinine, 1.5 mg. Carbon dioxide, 28 volumes per cent. Wassermann reaction—negative. Blood grouping—Group IV.

Spinal Fluid.—Clear, colorless, under increased pressure. Cells, 5 per c.mm. Wassermann reaction—negative (0.2 and 1 cc.). Globulin—not increased. Colloidal gold and gum mastic curves—negative.

Gastric Analysis.—Ewald test meal removed after 30 minutes. Small amounts of fluid material with well digested particles recovered. No gross blood, mucus, or pus. Free hydrochloric acid, 10 acidity per cent. Total acidity, 18 acidity per cent. No lactic acid. Microscopic examination negative. Stool examination negative. No ova, parasites, pus, or blood.

The history of the patient, the clinical manifestations, and the increase in the number of white corpuscles with the large number of immature cells established the diagnosis of acute leucemia. The extent of the adenopathy and the predominance of cells, apparently of the lymphocytic series in the blood first examined, indicated an acute lymphatic leucemia but this view was modified by all subsequent studies of the blood and a diagnosis of subacute myelogenous leucemia was the final one.

Clinical Course.—During the next 6 weeks in spite of intensive treatment with neoarsphenamine, sodium cacodylate, Blaud's mass, and two transfusions of blood, the progress of the disease was unarrested. The superficial lymph nodes and spleen enlarged. Epistaxis occurred on Dec. 1, hemorrhagic spots appeared in the mucous membrane of the lips, and retinitis became more extensive, hemorrhages large and small appearing in the retina of both eyes. These

TABLE I.

Differential Counts, from Fixed Smears.

Date.	Red blood corpuscles.	Hemoglobin.	Color index.	White blood corpuscles.	Differential count—200 cells.						Oxidase.	Remarks.
					Polymorphonuclear neutrophils.	Polymorphonuclear eosinophils.	Polymorphonuclear basophils.	Lymphocytes.	Monocytes.	Path cells.		
		per cent			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Nov. 23	1,416,000	25	0.85	81,550	3.5	0	0	0	0	95.5		Bleeding time 3 min. Coagulation time 3.5 min. Anisocytosis—poikilocytosis.
" 26	1,368,000	25	0.9	111,300	1.5	0	0	15.0	0	83.5		
" 28												Blood CO_2 40 volumes per cent.
" 29	1,304,000	25	0.9	152,450	1.0	0.5	0	9.0	7.5	82.0		Myeloblasts and normoblasts.
" 30											6.0	Many myeloblasts. Some with dividing nuclei.
Dec. 1	1,216,000	23	0.9	160,000	0.5	0	0	3.0	0	96.5		
" 2											8.0	
" 3	1,048,000	20	0.95	108,250	6.0	0	0	18.0	0	76.0	9.25	Red cells show polychromatophilia and anisocytosis.
" 5	764,000	17	1.3	195,000							12.0*	Intravenous injection of 400 cc. of citrated blood from his mother.
											12.0†	
" 6	1,512,000	26	0.88	110,700	4.0	0	0	23.0	0	72.0		Red blood cells less pale. Myeloblasts 15, normoblasts 13.
" 7											55.5	
" 8	1,192,000	20	0.9	109,450							46.0	

Dec. 10											46.25	
" 11	1,440,000	20	0.8	134,550							42.5	
" 12											46.5	
" 13	1,506,000	19	0.6	162,200							52.6	
" 14											52.5	A second transfusion of 500 cc. citrated blood.
" 15	1,928,000	23	0.6	177,400							58.5	
" 17	1,600,000	25	0.8	230,400							58.6	
" 18											68.0	
" 19	1,680,000	20	0.65	308,000							60.0	

* Count made before transfusion.

† Count made after transfusion.

were the only evidence of a hemorrhagic tendency. Puffiness of the eyelids and edema of the dependent parts were noted on Dec. 5. There was never tenderness of the bones. Occasional pain in the abdomen, nausea, and vomiting increased the discomfort of the patient, but there were no intestinal disturbances. On Dec. 5, small areas of ulceration developed on the gums. Superficial, white, circumscribed, and only slightly infiltrated at first, these areas slowly increased in size, were covered by a yellowish exudate, caused much pain and marked fetor oris. By Dec. 15, the mucous membrane of the entire roof of the mouth was necrotic. Bacteriological studies of the buccal lesions showed no fusiform bacilli but streptococci and staphylococci. Concomitantly with the extension of the buccal lesions, the submaxillary and submental lymph nodes increased in size.

The circulatory disturbances increased during the 2nd week of observation; he had increasing dyspnea, a sense of substernal tension, edema, throbbing of the peripheral arteries, and capillary pulsation, a to-and-fro hum over the cervical veins, and systolic and diastolic murmurs over the precordium were heard by Dec. 14. There were no signs of increasing cardiac dilation. The systolic blood pressure varied between 125 and 105 mm. Hg and the diastolic pressure was 50 mm. or so low it could not be determined. The output of urine varied between 450 and 2,300 cc. and, after the first examination, urinalysis showed no abnormalities except albuminuria. The entire course in the hospital was febrile. The fever was remittent between 99.6° and 101.6°F. to Dec. 1, after which the general level was higher, the maxima varying between 102.2° and 105°F. with daily remissions of from 0.5–4°F. The more marked pyrexia accompanied the spread of the necrosis in the mouth. There were no chills but sweating was profuse. The pulse rate varied with the variations of temperature but the rate was never slower than 100 beats per minute. Except for dyspnea of short duration on Dec. 5, and polypnea during the last 10 days of the illness, there were no respiratory disturbances. After Nov. 23, the patient was dull mentally and apathy gradually increased. Though there were short intervals after the transfusions when the patient seemed more alert, lassitude was replaced by somnolence during the week of Dec. 10, but the patient was never comatose. Several blood cultures were sterile. X-ray examination of the chest showed enlargement of the mediastinal shadow. The patient died on Dec. 20; he had increasing dyspnea but was conscious until the end. The autopsy was performed by Dr. Arnold Rich of the Department of Pathology of the Johns Hopkins Medical School, who gave the following report.

Pathological Diagnosis.—Myeloblastic leucemia with green chloromatous coloration of all lymph glands and of all macroscopic foci of myeloblastic infiltration but without any isolated chloromatous tumor. Myeloid hyperplasia of bone marrow. Splenomegaly. Generalized enlargement of lymph glands and solitary lymph follicles of digestive tract. Extensive myeloid infiltration of all lymphoid tissues, spleen, liver, kidneys, adrenals, epicardium, lungs, retroperitoneal tissues, dura mater, testis, and choroid coats of the eyes. Infiltration of intestinal mucosa with ulceration. Slight bilateral hydroneph-

rosis from pressure of enlarged lymph glands upon ureters. Petechial hemorrhages in skin and retina. Terminal aspiration of stomach contents. Pulmonary edema. Ulceration of gingiva.

Report of the Vital Studies on Blood Cells.

Myeloblasts.—The first supravital differential count of the blood of this case is given in Table II.

The predominating cell we diagnosed a myeloblast. Our criteria for the discrimination of this cell were largely obtained from the study of a fatal case of myeloblastic leucemia from Dr. Longcope's clinic in the Johns Hopkins Hospital (B. R., Med. No. 50043, Path. No. 7716). From this case the cell of Fig. 1 was drawn. This was a cell of intermediate size whose cytoplasm contained great numbers of tiny mitochondria; nothing in the cytoplasm reacted to neutral red. The nucleus was large in proportion to the cytoplasm. The cell of Fig. 2 was from another case of myelogenous leucemia (J. F. F., Med. No. 50256), Johns

TABLE II.

Vital Differential Count of 300 Cells, November 27.

	per cent		*per cent*
Myeloblasts	92.33	Lymphocytes, small	0.66
Myelocytes	2.33	" intermediate	0.33
Polymorphonuclear neutrophils, very sluggishly motile or with some movement of granules and no locomotion	2.66	Giant cells	1.33

Hopkins Hospital. In this case, the abnormal cells had many mitochondria but in addition every cell had a clump of particles that stained in neutral red as do the neutrophilic granules. This type of cell we have identified as the earliest form of the myelocyte and have designated it as *myelocyte, Type A.* In some experimental work on the bone marrow of pigeons and rabbits, we (1) have identified similar cells as myeloblasts and early myelocytes.

In the study of the case of C. M. from the Hebrew Hospital the cell shown in Fig. 3 was the type we have identified as the myeloblast. In regard to the rest of the cells in the differential count, the small lymphocytes were wholly typical; and the one intermediate lymphocyte showed the characteristic type of motility (5). Throughout the course of the case there were about 2 per cent of small lymphocytes and at autopsy the lymph glands, although almost wholly transformed into myeloid tissue, nevertheless showed a few typical lymphoid

follicles. The myelocytes in this count were of the type shown in Fig. 8, which is the form that precedes the stage of the leucocyte. We made the diagnosis of myeloblastic leucemia on the presence of 92.33 per cent of cells which we identified as myeloblasts and on the myelocytes. The giant cells which were occasionally present were probably clasmatocytes.

The majority of the myeloblasts were like the one shown in Fig. 3. This cell had a diameter about twice that of a red blood cell. In general the myeloblasts throughout the course of the disease could be divided into three groups; a very large cell, not shown on our plates, whose diameter was two and a quarter times the diameter of the red cell, intermediate types with a diameter nearly twice that of a red cell, and small cells like that of Fig. 11, with a diameter about the same or less than that of a red cell. In proportion there were always fewest of the small types; for example, on December 5, in a count of 100 cells taken 2 hours after the transfusion there were 79 myeloblasts, of which 2 were small, 50 intermediate, and 27 large; while on the next day taking myeloblasts and myelocytes together there were 8 small, 37 intermediate, and 48 large types in 100 cells.

It seems to us, therefore, that the myeloblast is a cell that has a cytoplasm containing nothing that reacts to vital dyes except many small mitochondria. The nucleus is large, contains a coarse chromatin network and one or more definite nucleoli. It is usually placed centrally but may become as eccentric as the nucleus of the very early myelocyte of Fig. 5. Such myeloblasts are illustrated in Figs. 1, 3, 9, *a*, 9, *b*, 10, *a*, 10, *b*, and 11.

In some experimental work on the bone marrow of birds and rabbits, we (1) have found a cell which we consider to be the progenitor of all the white blood cells. It was impossible to identify this cell in normal bone marrow but in bone marrow which had been so simplified by experimental procedures that no cells remained between the vessels except the fat cells and the undifferentiated mesenchyme or reticular cell, it has been possible to follow the differentiation of such a *primitive white cell*. The reticular cell contained nothing in its cytoplasm that reacted to vital dyes, while the primitive white cell had a more basophilic cytoplasm which, when vitally stained, showed a few large, scattered mitochondria. The myeloblast developed from this cell by an increase in the number and a decrease in the size of the mitochondria. These cells are illustrated in the article quoted above (1).

When myeloblasts are stained with Wright's or Wilson's stain, in the usual concentration, the cytoplasm proves to be uniformly basophilic. For the study of myeloblasts in smears of leucemic blood, or for the study of the cells in bone marrow, we have found it best to dilute the original stain with equal parts of methyl alcohol.[2] In the differential counts made with fixed smears in this case there was difficulty in discriminating small myeloblasts from small lymphocytes and reference to the counts in the clinical history will show that they could not be discriminated with the use of concentrated stain. The fact of the amitotic division in a small cell such as the one shown in Fig. 11, the small myeloblast, indicates that the supravital technique had an advantage. With dilute Wright's stain the myeloblasts had a basophilic cytoplasm and many of them showed a small acidophilic spot beside the nucleus, a possible acidophilic centrosphere. This spot did not show at all in the living cells. It gives an additional point in the discrimination of myeloblasts from lymphocytes. Cells with such a pale spot in the cytoplasm are shown in Plates xiiia and xiv of the Atlas of Pappenheim and are identified by him as *Lymphoidocyten*. On his Plate xiiia, the entire range of cells to be considered as myeloblasts is shown, those in the fourth and fifth rows all having centrospheres, while Cells 1, 3, and 17 in Plate xiv are also typical myeloblasts. Thus one of the types of cells called by Pappenheim the *Lymphoidocyte* we identify as the myeloblast.

Myelocytes.—In following the case of C. M. we were able to trace the development of myelocytes from myeloblasts. The first step in this transformation was the development in the cytoplasm of a small clump of neutrophilic granules or their precursors, Fig. 4. These granules stained more intensely in the neutral red than the neutrophilic granules of the adult leucocytes and it is well known that in smears of blood in Wright's stain the neutrophilic granules of the early forms have a bluish tint in contrast to the pink shade of neutrophilic granules in leucocytes. This difference is well shown by Pappenheim and it is on this evidence that we refer to the first granules that stain with neutral red as the precursors of the neutrophilic granules. Pappenheim called these cells *Lymphoidocyten mit myeloischer Azurkörnung*. Since we could not see the centrosphere in the living cell we could not decide definitely that the neutrophilic granules had no relation to it but it was clear that the original clump of granules did not make a rosette around it. This development in the cytoplasm of granules that stained with neutral red was the first evidence of neutrophilic

[2] We use the methyl alcohol put up especially for blood stains by the National Aniline and Chemical Company.

granulation and we have designated cells having a small clump of not more than ten to fifteen such granules, as *myelocytes, Type A*. We are showing three cells of this type in Figs. 2, 4, and 5. They correspond to Cell 4 on Plate xiv of Pappenheim's Atlas. In his figure it can be seen that the entire clump of granules is practically identical with that of those staining in neutral red as shown in our Figs. 2 and 4. In the cell of Fig. 5, it will be seen that there was a group of four granules, which stained with neutral red, in a large mass of mitochondria on one side of the nucleus and three neutral red granules on the other side. We have classified this cell with the myelocytes, Type A, on account of the small number of neutrophilic granules, and because the arrangement of the mitochondria is that of the myeloblast. It might be taken as a transition toward, or as the earliest phase of, the next stage in which the neutrophilic granules become dispersed throughout the cytoplasm. Such a cell brings up the question as to how constant an original clump of the neutrophilic granules is, and whether it is the rule rather than the condition in which the granules are diffusely scattered from the start. We consider that the clump of granules represents the more typical early form, first on account of the greater number of types like that of Fig. 4, and second on account of certain observations given under the subject of the oxidase reaction.

The second stage in the development of the myelocyte is shown in Figs. 6 and 7, which we call *myelocyte, Type B*, and which consists in the increase of neutrophilic granules, with a decrease of mitochondria. This decrease in mitochondria may not be in inverse proportion to the increase in neutrophilic granules, but it is evident that when the granules that react to neutral red become numerous the mitochondria are markedly reduced. In the living cell it was possible to see how the cells of Type A became Type B because there was a slow movement of the individual neutrophilic granules in the cytoplasm so that the original clump of granules became dispersed and intermingled with the mitochondria. Throughout the second stage no pattern whatever could be made out either of the mitochondria or of the neutrophilic granules.

In the cells of Figs. 6 and 7, neutrophilic granules and mitochondria were in about equal proportions. The phase which we have called

Type B must be considered as including every possible variation in the proportion of neutrophilic granules to mitochondria, between the two extremes which we have called Type A, with the first small clump of neutrophilic granules and masses of mitochondria, and the cells of Type C, with a maximum of neutrophilic granules and a minimum of mitochondria. A group of cells which we should term myelocytes, Type B, is shown in the first two rows of Pappenheim's Plate xiiib, and on Plates xiiic, and xiv. By comparison of these three plates of Pappenheim it will be seen that the cytoplasm of the cells in this phase may be more or less basophilic. In the cases that we have studied, the cytoplasm of the cells in this phase was faintly basophilic.

The third stage of the myelocyte, or Type C, as we have termed it, is shown in Fig. 8; in this stage neutrophilic granules predominate and the mitochondria have been reduced to a narrow rim in the periphery in the cell. This is the type of myelocyte that predominates in normal bone marrow. The earlier leucocytes have a few mitochondria which are intermingled with the neutrophilic granules and move in the cytoplasm with them; such young leucocytes are occasionally found in the circulating blood but most of the leucocytes of the blood are without mitochondria.

In the transformation of myelocytes into leucocytes, we think that the well known changes in the nucleus preclude any further initiation of cell division; that there is usually an entire loss of the mitochondria from the cytoplasm; and that there is a decided change in the cytoplasm so that the cell becomes capable of ameboid movement. This last change involves a streaming of the cytoplasm correlated with movement of the cell. Indeed in the vital technique it becomes clear that both myeloblasts and myelocytes are cells without ameboid activity. In the myelocyte there may be no movement or a slight shifting of granules, but any flowing or streaming of the cytoplasm, carrying the granules along in the current, is to be seen only when the myelocyte is becoming a leucocyte. In the study of films of blood by the vital technique this lack of ameboid activity of the myelocyte is an important point in discriminating it from the leucocyte. Thus we have followed the changes which represent the maturation of myeloblasts through the stages of the myelocytes into leucocytes by the vital technique.

Maturation of Myelocytes.—In following this case we have given our results in two charts.

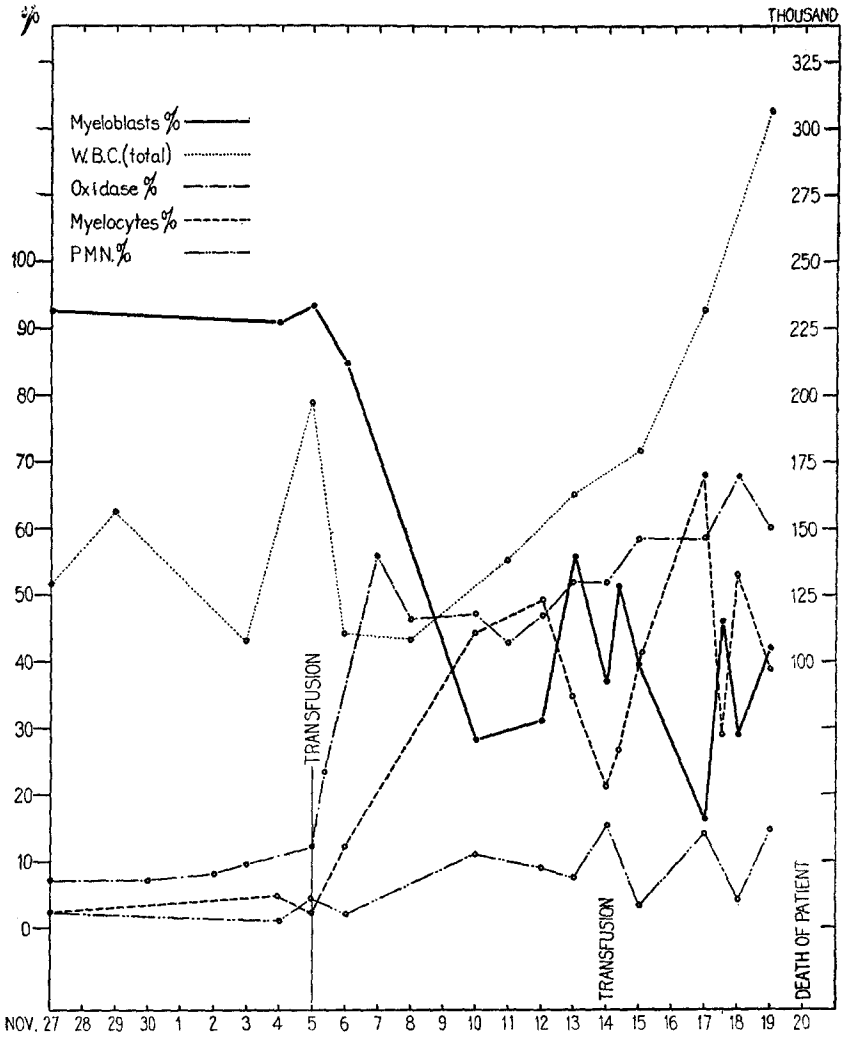


CHART 1. Graph of studies on the blood of C. M., Hebrew Hospital, Baltimore. The line of the total counts of the white blood cells is to be referred to the column of thousands along the right hand margin. The other data are given in percentage and represent myeloblasts, Types A, B, and C taken together, polymorphonuclear neutrophilic leucocytes, and the percentage of cells showing the oxidase reaction.

The first graph shows the total white count, the myeloblasts, the total myelocytes, the polymorphonuclear leucocytes, and the oxidase reaction. For the total white counts and the oxidase counts we are indebted to Dr. Ann Purdy, in charge of the clinical laboratory of the Hebrew Hospital; the rest of the data on the charts were taken from our vital counts. All of the data plotted on the charts are in terms of percentage except the one line of the total count of the white cells. This we found more feasible because our counts did not always coincide with the hours on which the total counts were made. The number of white cells was 81,550 on admission Nov. 23; our chart begins on the 27th, but the first few days are representative of conditions between Nov. 23 and Dec. 4. The total white count varied during the first few days shown on the chart, with a marked rise in the count on the day of the first transfusion, Dec. 5, reaching 195,000. The next day the number of cells returned to about the previous level, but from then there was a steady increase to a maximum of 308,000 on Dec. 19,

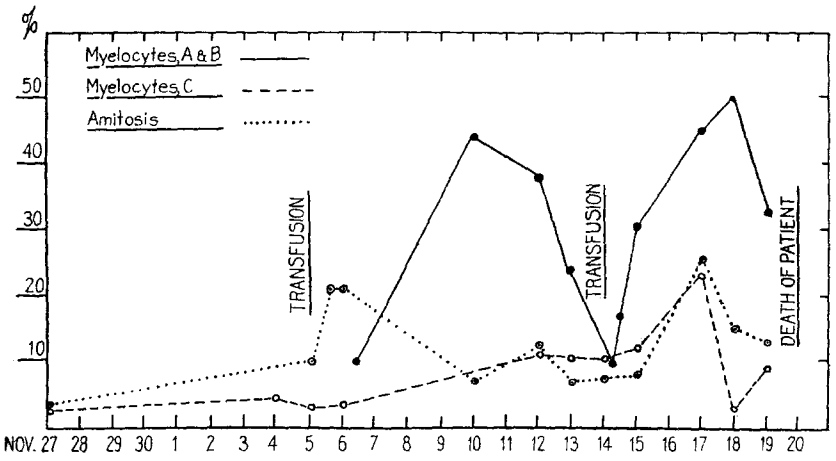


CHART 2. Graph of studies on the blood of the same case as Chart 1. The data represent the percentage of myelocytes, Types A and B together, in contrast to the myelocytes, Type C; and the percentage of cells showing amitosis.

the day before death. During the early days the myeloblast was the predominating cell with few leucocytes and few myelocytes. Throughout the course of the disease the leucocytes were mostly of the neutrophilic variety, but occasionally basophilic and eosinophilic leucocytes were seen, and one basophilic myelocyte, but these forms were too few to be considered in the chart. The curve of the myelocytes on this chart represents the combined number of all three types together.

Chart 1 shows that following the first transfusion there was a drop in the proportion of the myeloblasts accompanied by a slight increase in leucocytes and a marked increase in myelocytes. In other words,

there was a shifting of the cells toward more mature types. The effect was more marked after the second transfusion when the curve of the myelocytes went considerably above that of the myeloblasts, but the course of the chart indicates that this shifting of the types of cells reflected no actual gain in the nature of a clinical remission.

On the second chart we have presented three compilations, also given in percentages, the myelocytes, Type C, as compared with the more primitive forms, the myelocytes of Types A and B combined, and the cells showing amitosis. It will be recalled that on Nov. 27, the day of the first vital count, there were 2.33 per cent of myelocytes, Type C. We saw none of the myelocytes of Types A and B in the vital preparations until Dec. 5, the day after the first transfusion

TABLE III.

Vital Differential Count, 300 Cells, December 13.

	per cent		*per cent*
Polymorphonuclear neutrophils	7.3	Myelocytes, Type C	10.6
Myeloblasts	55.0	Lymphocytes	3.0
Myelocytes, Types A and B	24.0		

though there were a few cells of Type B identified in fixed smears. On the day of the first transfusion, Dec. 5, we made three vital counts, one before the transfusion, the others an hour, and 2 hours after. In the first count there were 93 per cent myeloblasts as shown on Chart 1, in the other two counts 77 and 79 per cent respectively. This difference was made up in an increase in leucocytes, "non-motile" cells, and damaged cells.[3] This immediate result we thought without special significance since it included the donor's blood and it has therefore not been shown on the chart; but on Dec. 6, we found three marked changes. First, the most significant change was that 10 per cent of the total white cells were myelocytes of Type A, which had appeared at the expense of the myeloblasts. This indicated that the myeloblasts had been stimulated to maturate into myelocytes. Second, though the percentage of the leucocytes did not increase markedly, being 2, 6, and 10 per cent respectively, in three counts, there was a decided increase in their motility. Third, there was an increase in the percentage of cells showing amitosis, Chart 2.

By the time of the second transfusion there had been another shift in the proportions of the myeloblasts and myelocytes, as will be seen in Table III.

[3] We now regard the name "non-motile cell" (5) as wholly inadequate, because the essential change is not from motility to non-motility, but in the appearance of the neutrophilic granules. They become highly refractive, swollen, and wholly resistant to vital dyes. In leucocytes, it is true, the motility ceases entirely, and both cell and nucleus become edematous; but in this study we have seen myelocytes, which of course have never developed the type of rapid ameboid activity characteristic of leucocytes, showing this same change in the nature of the neutrophilic granules.

On Dec. 14, counts were made a half hour and an hour and a half after the transfusion, as shown in Table IV. ½ hour after, there were 23 per cent of cells that we interpreted as old leucocytes, and thought that they had probably come from the donor's blood. They had a vacuolated cytoplasm and few neutrophilic granules and their nuclei did not look like the nuclei of the myeloblasts. In Table IV it will be seen that the blood taken 30 minutes after the transfusion

TABLE IV.

Vital Differential Counts, after the Second Transfusion, December 14.

	3.55 p.m.	4.45 p.m.
	per cent	*per cent*
Polymorphonuclear neutrophils	15	10
" " old, possibly from donor's blood	23	6
Myeloblasts	37	51
Myelocytes, Types A and B	10	17
" Type C	11	10
Lymphocytes	3	3
Giant cells	0	3

TABLE V.

Vital Differential Counts, December 15.

	11 a.m.	11.30 a.m.
	per cent	*per cent*
Polymorphonuclear neutrophils	5	3
" basophils	0	1
" eosinophils	1	0
" neutrophils, damaged	0	1
Myeloblasts	25	53
Myelocytes, Type A	5	13
" " B	29	14
" " C	11	14
Special phagocytic cells	21	0
Non-motile cells, leucocytes and myelocytes	3	0

was quite out of alignment with our other counts. We interpreted this as an irregular mixing of the blood of the donor and recipient and that the leucocytes marked old were those of the donor. It was quite striking that in an hour and a half after the transfusion (4.45 p.m.) the differential count was quite like those which had preceded. Again on the next morning there was one very irregular differential count, and two records taken a half hour apart are shown in Table V. On the first count of Dec. 15, the cells were markedly clumped and the group called "special phagocytic cells" was different from any others that were seen throughout the case. They had indented nuclei, their cytoplasm had fine gran-

ules equally distributed between mitochondria and neutral red bodies, but they showed marked signs of phagocytosis. Some had taken up red blood cells and others masses of debris. We saw these cells but once and were not sure of their identity; they were more like monocytes than any other type.

In general it will be noted on Chart 2 that, following each transfusion, there was a marked rise in the number of myelocytes of Types A and B. After the first transfusion the peak of these myelocytes was reached on the 5th day with a return on the 9th to almost the previous level. A second even higher peak was reached on the 4th day after the second transfusion, followed by a sudden drop on the following day, which was the day before the patient died. The greatest number of myelocytes of Type C occurred on the 3rd day after the second transfusion.

Oxidase Reaction.—The study of the oxidase reaction has proved interesting. We have used the Goodpasture (3) technique, which demonstrates the "peroxidase ferment."

From the first chart it is obvious that as the number of myelocytes increased at the expense of the myeloblasts, the proportion of cells positive to the oxidase reaction increased. In general, it will be noted that the entire oxidase curve runs a little low when correlated with the total myelocytes and leucocytes in vital counts. This we interpret as due to the loss of fragile cells in making smears We found in making the correlation between differentials on fixed smears and vital counts that all injured cells in fixed preparations must be included. On the other hand, it was well nigh impossible to make mathematically correct, differential counts of any of these preparations, especially during the latter part of our studies, because the number of white cells involved was so large.

In studying the slides stained by the oxidase method the cells showing the positive reaction could be divided into three groups; first, with one large blue granule in the cytoplasm; second, with fine scattered granules; and third, with massive granulations. The three types of cells classified by the oxidase granules corresponded so well with the three types of myelocytes outlined above, that it seemed feasible to consider cells having one large oxidase granule as myelocytes of Type A, those with scattered, fine, blue granules as myelocytes of Type B, leaving the cells with the massive oxidase reaction to correspond with the myelocytes, Type C, plus adult leucocytes.

In Table VI we have given the correlation between the vital counts and the oxidase counts on 4 different days. Note that the total vital count of all the myelocytes and polymorphonuclear leucocytes represents an estimate of what the oxidase reaction ought to be.

Each column represents 100 cells and it is clear that there is considerable variation in the counts of blood taken at the same time. The most interesting variation is in the vital counts of Dec. 18, in which it will be seen that the myelocytes, Type A, are far in excess of the corresponding cells with one oxidase granule

in the fixed smears; but the sum of the myelocytes of Types A and B agrees closely with the sum of the first two numbers in the oxidase count. We think that such a cell as that shown in Fig. 5 is an adequate explanation of such a discrepancy, for this cell would appear as a cell with the scattered granules in the oxidase reaction but as a myelocyte, Type A, in the vital count. It will be seen in Chart 1 that the correlation between the vital counts and the counts of the specimens stained to show the oxidase reaction is not exact, but, in view of the difficulties in making the counts, is suggestive enough to be regarded as presumptive evidence that the myeloblasts give a negative reaction to the oxidase stain; that cells with a small clump of neutrophilic granules or their precursors give one relatively large granule; that myelocytes of the intermediate phase of mingled neutrophilic granules and mitochondria give a positive oxidase reaction of fine, blue granules, while the myelocytes, Type C, give the same type of massive reaction as do the leucocytes.[4] It is entirely demonstrated that the maturation of myeloblasts into myelocytes was accompanied by an increase in the oxidase reaction. In following this case we had practically no monocytes to complicate the picture unless there were a few after the second transfusion. Thus from the sum of the myelocytes and leucocytes of the vital counts in this case we could give a fair estimate of the percentage of cells showing a positive oxidase reaction.

Amitosis.—One of the most striking features of the case was the amount of amitosis in the blood stream. There were also cells undergoing mitosis in the blood stream, since we found them in fixed smears, but we did not see any spindles in the living preparations.

We are showing in our figures five examples of amitosis drawn from the living preparations. The drawings shown in Figs. 9, *a* and 9, *b* were made on Dec. 4; when the cell, which was a large myeloblast, was first seen there were one large nucleus in the upper part of the cell and two small ones, widely separated, in the lower part. There was no question but that the three nuclei were distinct; one large and two small, unequal ones. By the time the drawing was started, 15 minutes later, the two small nuclei had approached each other and there was a slight constriction of the cytoplasm as shown in Fig. 9, *a*. From this time on, the entire process took 25 minutes. As shown in the drawings, the two small nuclei approached, fused, and then the cell divided, giving two cells of approximately equal size but with unequal nuclei.

The process by which nuclei fused in the cells of this case is shown better in Figs. 10, *a* and 10, *b*. When this cell was first seen it had two nuclei (Fig. 10, *a*) and it was selected with the idea that cell division might follow. Instead of that, in 18 minutes the two nuclei touched, and then without any loss of nuclear

[4] These observations confirm and extend those of Rosenthal (Rosenthal, N., *Arch. Int. Med.*, 1917, xx, 184).

TABLE VI.

Correlation between Counts Made with Vital and Oxidase Stains.

	Vital counts.					Oxidase counts.				
	Counts	1	2	3	4	Counts	1	2	3	4
		per cent	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Dec. 15	Myelocytes, Type A	13	5			Cells with one granule	16	18	19	25
	" " B	14	29			" " scattered granules	20	8	6	17
	" " C and polymorpho-					" " massive "	22	25	27	21
	nuclear neutrophils	19	17			Damaged cells	3	2	4	0
	Unclassified	0	21				—	—	—	—
		—	—			Total	61	53	56	63
	Total	46	72							
" 17	Myelocytes, Type A	4	6			Cells with one granule	8	9	3	
	" " B	17	10			" " scattered granules	6	9	17	
	" " C and polymorpho-					" " massive "	32 } 42	30 } 37	30 } 35	
	nuclear neutrophils	35	46			Damaged cells	10 }	7 }	5 }	
	Unclassified	0	0				—	—	—	
		—	—			Total	56	55	55	
	Total	56	62							
" 18	Myelocytes, Type A	29 } 50				Cells with one granule	5 } 46	12 } 46		
	" " B	21 }				" " scattered granules	41 }	34 }		
	" " C and polymorpho-					" " massive "	23	21		
	nuclear neutrophils	18				Damaged cells	0	0		
	Unclassified	0					—	—		
		—				Total	69	67		
	Total	68								

Dec. 19	Myelocytes, Type A	1	1	0	2	Cells with one granule	2	5			
	" B	41	22	27	31	" " scattered granules	32	36			
	" C and polymorphonuclear neutrophils	16	33	29	25	" " massive "	24	13			
	Unclassified	0	0	0	0	Damaged cells	2	6			
		—	—	—	—	Total	60	—			
	Total	58	56	56	58			60			

membrane except at the point of contact a bridge was formed as is shown in Fig. 10, *b*. This process was apparently similar to the way in which two oil drops flow together. 5 minutes later the bridge began to narrow again so that the process did not go to a complete fusion of the two nuclei as in the cell of Figs. 9, *a* and 9, *b*, but after an hour the two nuclei separated completely so that the cell again looked as it did when first drawn. Complete division of the nucleus, not followed by cell division, is shown in the case of two myelocytes of Type B in Figs. 6 and 7. In the first cell, Fig. 6, the nucleus underwent complete division from the stage shown in the drawing in 6 minutes; the two nuclei remained separate for 10 minutes and then rejoined and returned to the original condition in 5 more minutes. This was a typical myelocyte, Type B, in which the neutrophilic granules were moving in the cytoplasm. The other cell, Fig. 7, showed a condition which we noted several times; namely, one in which there were irregular and uneven threads of chromatic material in the bridge of the nucleus. Instead of dividing equally these threads became unevenly distributed between the two daughter nuclei. In the material at autopsy we have also found this same appearance in some of the constricted nuclei; we concluded that some of the examples of amitosis were abnormal in showing an atypical formation of chromosomes. In another paper we (6) are giving our concept of normal amitosis. This point raises the question as to whether the appearance of these incomplete mitotic figures represents an abortive attempt at mitotic division, or a very abnormal type of amitotic division. The last drawing of amitosis is shown in Fig. 11, selected to show that the small cells could also divide. The special interest in this cell was that the first division, which was watched through to completion, was an even division of the nucleus, but while this division was taking place, one of the two resulting nuclei also started to divide at the point marked *x*, and when this division had taken place there were three unequal nuclei.

On Dec. 6, all of the cells showing amitosis were myeloblasts; out of the 21 per cent of the cells which were found dividing 1 per cent were small cells, 3 per cent were of intermediate size, and 17 per cent were large myeloblasts. Up to Dec. 6, all of the cells in amitosis were myeloblasts. From that time on both myeloblasts and myelocytes were found dividing but there were more examples of division among the myeloblasts, until Dec. 7, when twenty-three myelocytes and only two myeloblasts were found in division. We followed many other examples of amitosis which were not drawn. In one instance, studied on Dec. 6, we found a nucleus with four indentations and finally one part of the nucleus separated off entirely, leaving a large three lobed nucleus and a small oval one. We also found cells with two nuclei in which the larger mass seemed to be splitting into four parts, which resulted in a final division of the nucleus into five separate parts. Again we found cells with two nuclei in which the smaller had a diameter less than a fifth of the larger one.

On Dec. 10, a study was made on a slide which had no stain in order to test whether the toxicity of the Janus green was producing the nuclear changes. In the count of 100 cells five myeloblasts and two myelocytes were found undergoing amitosis. One of the myeloblasts, when first seen, had a nucleus which was evenly constricted as if in preparation for division; this division was completed and then one of the nuclei again divided asymmetrically, so that the resulting cell had three nuclei, two of them approximately equal and one smaller. This process took 37 minutes.

In one instance studied on Dec. 15, an example of amitosis was followed in a red blood cell. In this instance the nucleus divided and there were three abortive attempts at cell division in three entirely different planes, the nuclei having shifted their position in the cell so that each time the cytoplasmic plane was between the two nuclei. One of the cytoplasmic cleavages was almost complete; once, one of the nuclei approached the edge of the cell and caused it to bulge as if this nucleus was going to be extruded; this nucleus, however, returned to the center of the cell, which eventually rounded up and became quiescent.

TABLE VII.

Percentage of Amitosis.

Nov. 27	Dec. 5	Dec. 5	Dec. 5	Dec. 6	Dec. 10	Dec. 12	Dec. 13	Dec. 14	Dec. 15	Dec. 17	Dec. 18	Dec. 19
	10 a. m.	*1 p. m.*	*2 p. m.*									
2	10	8	21	21	7	12	7	7.5	8	25	15	13

In following the records of the percentage of amitosis seen throughout the case we have included both cells in which the nuclei were actually seen dividing and cells with two or more nuclei.

Our records are shown in Table VII.

These figures are shown graphically on Chart 2, in comparison with the curves of the myelocytes, Type C, and of the myelocytes, Types A and B, taken together. The most striking point is that the times at which the highest percentages of amitoses were reached were after the transfusions. The count on Dec. 5, made 2 hours after the transfusion showed an increase to 21 per cent which was maintained on the morning of Dec. 6; the second peak was on Dec. 17, reaching a maximum of 25 per cent, apparently a somewhat delayed reaction when contrasted with the former post-transfusion increase.

The process of amitosis was extremely varied and peculiar in many of the cells observed in this case. There were markedly uneven divisions of the nuclei with immediate, or delayed cell division, the fusion of two nuclei after complete separation, and the presence of threads of

chromatin in the zone of cleavage of some of the dividing nuclei. Such peculiarities in the process of amitotic division suggest strongly that the process in this case was a distinctly abnormal one.

DISCUSSION.

From the study of these cases of myeloblastic leucemia it has seemed possible to discriminate the myeloblast with the vital technique, and to follow the steps of the maturation of the myelocytes. We consider that the *primitive white blood cell* (1), from which the leucocytes, monocytes, and lymphocytes are derived, is a cell with a large nucleus in proportion to its cytoplasm, and containing a few unevenly distributed rod-shaped mitochondria. The especial characteristic of the myeloblast is the great increase in the number, and decrease in the size of the mitochondria; *i.e.*, the primitive cell becomes a myeloblast by the development of many small mitochondria in the cytoplasm. Thus, in our concept, an increase of mitochondria in developing blood cells is correlated with the phase of maturation of a *primitive white cell* into a myeloblast, while on the other hand the maturation of the myeloblast through the stages of the myelocyte into the leucocyte is correlated with a decrease in the number of mitochondria. These observations, on living cells, represent only one factor in the change in the cytoplasm; another factor, the degree of the reaction to basophilic dyes in fixed smears, has long been known. In our studies of the marrow in rabbits we have followed the changes of the mitochondria from the primitive cell toward the myeloblast but in this case there were no cells more primitve than the myeloblast. We speak of the myeloblast as a level or a stage in the development of the leucocyte, and it was obviously at this level or with this type of cell that the pathological involvement in this case occurred.

Considerable variations in the exact distribution of the mitochondria of the myeloblasts are shown in our Figs. 1, 3, 5, 9, *a*, 10, *a*, and 11. In general, we consider the distribution shown in Figs. 3 and 5 as the typical arrangement; here the nucleus is eccentric and the great mass of the granules is in the larger zone of the cytoplasm. In the cell of Fig. 3, the nucleus was more centrally placed, while in the stages of the myelocytes of Type B as shown in Figs. 6 and 7 there was an approximately equal distribution of stainable substances on the two sides of

the nucleus, these cells being at a stage when the granules were moving in the cytoplasm. Another variation in which the mitochondria are limited to one side of the nucleus is shown in Figs. 9, *a* and 9, *b*. We feel confident that the development of a large content of mitochondria characterizes the myeloblast, and is a condition which precedes the differentiation of neutrophilic granules in the cytoplasm.

The variation in the size of the myeloblasts from a cell whose diameter is more than twice that of a red blood cell to one even smaller we think correlated with rapid cell division, since immediately after division the resulting cells must be half the size of the original cell. In general we found more large cells undergoing division, but the fact that we found cells of all sizes, even so small as shown in Fig. 11, undergoing division indicated that there was some disturbance between the normal alternation of the phases of growth and division.

The cell which we call the myeloblast is at least one of the types of cells called by Pappenheim (4) the *Lymphoidocyte*. We consider that he also included in the term *Lymphoidocyte,* types that we would discriminate as monoblasts and lymphoblasts. In his Atlas, on page 84, Pappenheim postulated a still more primitive cell than his *Lymphoidocyte,* a form from which all of the blood cells must be derived.

We think that our studies (1) lead to an identification of the primitive cell which Pappenheim postulated, and that it is the forerunner of all three types of the white blood corpuscles. With the method of depletion of the bone marrow this cell can be found at will, so it is no longer a hypothetical type. The study of this case has brought out a factor fundamental to the identification of the myeloblast, the definite forerunner of the leucocyte. This factor is the development of large numbers of tiny mitochondria, which gradually disappear as the cell becomes the mature leucocyte. In the maturation of the myeloblast into the myelocyte, there can be discriminated three phases, whose correlation with the three types of the oxidase reaction is close enough to indicate that such a grouping is not accidental. We think that the significance in recognizing these three types consists in the value of knowing how primitive the type of myelocyte is which may appear in the circulating blood. For example we have found the myelocyte of Type B in the circulation in the case of experimentally infected rabbits.

There are three degrees of the reaction to neutral red of the neutrophilic granules in human blood; the original phase is found in the A and B types of myelocytes, in which the granules react more intensely to neutral red than in the stage of the leucocyte; the second stage is that of the myelocyte, Type C, and the leucocyte, which show a reaction which is clear but not intense; and the third phase is that of the *non-motile* leucocyte (5), in which the neutrophilic granules have become swollen, more refractive, and take no vital dye whatever. After the first transfusion we found a few myelocytes which showed the non-motile phase of the neutrophilic granule.

In regard to motility, myeloblasts and myelocytes show no ameboid movements. Individual granules may move in the cytoplasm, the cell itself may change its position slowly, but in these early forms there is none of that active streaming of cytoplasm, correlated with rapid movement of the cell, which characterizes the leucocyte up to the time of its death. In our preparations the leucocytes are always in ameboid activity under normal conditions of temperature, except during the first few seconds after making the preparations, and we are inferring that this is probably the case when these cells are in the tissues. In regard to the effects of the transfusions the matter is much too complex with our present state of knowledge to analyze, but three points of view may be presented; first, that the blood introduced contained a stimulant which caused the myeloblasts to become myelocytes; second, that the donor's blood cells supplied, for a brief interval, substances used by the tissues and thus relieved the demand on the blood-forming tissues; or third, that the new blood temporarily counteracted substances that were checking the maturation of the myeloblasts. Such a statement shows how completely we lack a knowledge of the forces that produce the maturation of the blood cells and the stimuli which bring them into the circulation.

The amitoses were the most striking feature of this case. In our vital counts we discriminated between the cells in which we actually saw the nuclei in the process of division and those in which we found two, three, or four nuclei, but in the percentage of amitosis we have included both types of nuclear changes. The marked variety in these phenomena, the uneven divisions of the nuclei, the usual delay in cell division following these nuclear divisions, the examples of abortive

attempts at nuclear division, as shown in many cases in which nuclei divided and reunited, the example of abortive cell division in which three different planes were tried and then abandoned, and the presence in some and the absence in others of atypical chromosomes make us regard the amitosis as evidence of a disordered cellular process. We are the more sure of this since, in following the type of amitotic cell division of normal cells in our vital preparations, we have found an orderly and characteristic sequence of events (6). We do not consider that this case was wholly unusual in the fact of amitosis though the amount of it may have been in excess of the average case.

CONCLUSIONS.

1. Myeloblasts can be discriminated in the supravital technique by the great numbers of tiny mitochondria in the cytoplasm and the absence of any other vitally stainable substance.

2. There are three stages in the maturation of myelocytes.

3. These three phases can be correlated with three types of the oxidase reaction.

4. One case of myeloblastic leucemia showed such an amount of an abnormal type of amitosis as to suggest the disordered cell division of neoplasms.

5. In this case transfusions were correlated with a maturation of myeloblasts into myelocytes, with an increase of the oxidase reaction, and with an increase in amitosis.

BIBLIOGRAPHY.

1. Cunningham, R. S., Sabin, F. R., and Doan, C. A., The development of leucocytes, lymphocytes and monocytes from a specific stem-cell in adult tissues, *Carnegie Institution of Washington, Contributions to Embryology*, 1924, xvi, 227.

2. Doan, C. A., Cunningham, R. S., and Sabin, F. R., Experimental studies on the origin and maturation of avian and mammalian red blood-cells, *Carnegie Institution of Washington, Contributions to Embryology*, 1924, xvi, 163.

3. Goodpasture, E. W., A peroxidase reaction with sodium nitroprusside and benzidine in blood smears and tissues, *J. Lab. and Clin. Med.*, 1918–19, iv, 442.

4. Pappenheim, A., Die Zellen der Leukämischen Myelose (Leukämie-Zellen), (Text and Atlas), Jena, 1914.

5. Sabin, F. R., Studies of living human blood-cells, *Bull. Johns Hopkins Hosp.*, 1923, xxxiv, 277.
6. Sabin, F. R., Doan, C. A., and Cunningham, R. S., The discrimination of two types of phagocytic cells in the connective tissues by the supravital technique, *Carnegie Institution of Washington, Contributions to Embryology*, 1924, xvi, 125.

EXPLANATION OF PLATES.

PLATE 39.

The cells on this plate were drawn from living specimens which had been supravitally stained with neutral red and Janus green. The magnification is about 3,300; a red corpuscle is given for comparison. Figs. 3 to 6 were from Case C. M., Hebrew Hospital, Baltimore.

FIG. 1. Myeloblast from the blood of a case of myeloblastic leucemia (B. R., Johns Hopkins Hospital, Med. No. 50043, Path. No. 7716). The nucleus of this cell showed a diffuse chromatin network and was slightly eccentric. In the cytoplasm there were tiny mitochondria.

FIG. 2. Myelocyte, Type A, from a case of myeloid leucemia (J. F. F., Johns Hopkins Hospital, Med. No. 50256). The chromatin network of the nucleus was dense; the small clump of granules stained in neutral red represents the first neutrophilic granules or their precursors and indicates that this cell belongs to Type A.

FIG. 3. Myeloblast from the case of myeloblastic leucemia, drawn Dec. 5, 2 hours after the first transfusion. The nucleus had a coarse chromatin network and three nucleoli. The cytoplasm contained great numbers of tiny mitochondria.

FIG. 4. Myelocyte, Type A, drawn Dec. 17. The nucleus had a dense chromatin network and one nucleolus. The cytoplasm contained many tiny mitochondria and a small clump of granules that stained in neutral red.

FIG. 5. Myeloblast, Type A, drawn Dec. 17. The nucleus showed a massive chromatin network without definite nucleoli. There was a clump of four neutrophilic granules on one side of the nucleus and three on the other.

FIG. 6. Myelocyte, Type B, drawn Dec. 18. When the drawing was started the cell was as shown in this figure; in 6 minutes the nuclear division was completed, after 10 minutes the nuclei started to rejoin each other, and in 5 minutes the nucleus had returned to its original condition. The granules in the cytoplasm were moving.

PLATE 40.

All the cells on this plate were from the case of myeloblastic leucemia (C.M., Hebrew Hospital, Baltimore). They were drawn from living cells, supravitally stained with neutral red and Janus green. Figs. 7, 8, 9, *a*, and 9, *b*, were drawn at a magnification of about 3,300. The cells of Figs. 10, *a*, 10, *b*, and 11 were drawn at a magnification of about 5,700 and a red blood corpuscle is given for comparison.

FIG. 7. Myelocyte, Type B, drawn Dec. 18. This cell showed atypical threads of chromatin in the line of cleavage of the nucleus. The nucleus of this cell divided completely in 32 minutes.

FIG. 8. Myelocyte, Type C, drawn Dec. 5, 2 hours after the first transfusion. The neutrophilic granulation was massive and the mitochondria were reduced to a few granules in the periphery.

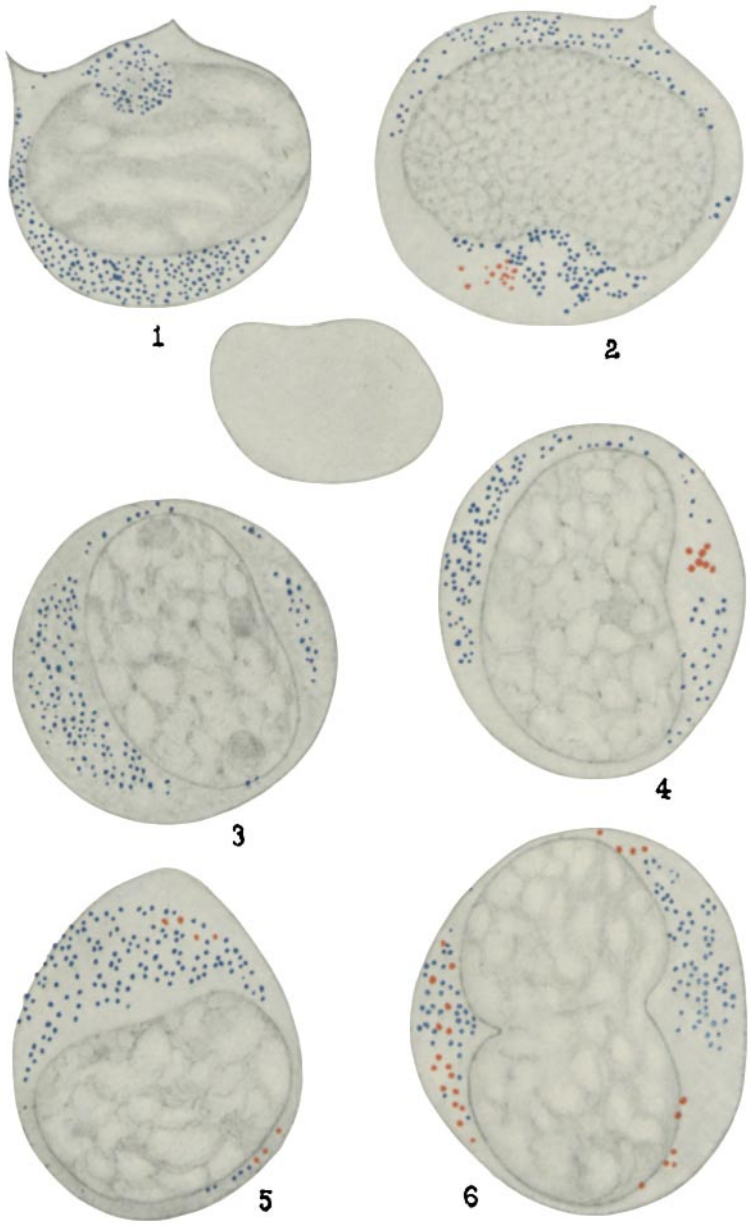
FIG. 9, *a*. Myeloblast, drawn Dec. 4, to show amitosis. When first seen the cell had three nuclei, the two smaller ones being widely separated and wholly distinct, and there was no constriction in the nucleus itself. 15 minutes later, the condition was as shown in this figure.

FIG. 9, *b*. The same cell as Fig. 9, *a*. While this cell was being drawn the two small nuclei fused into one and the cell divided. The processes of the fusion of the two small nuclei and the division of the cytoplasm took 25 minutes. During this entire time the cytoplasm was refractive and clear except for the mitochondria, which became redistributed as shown in the drawings.

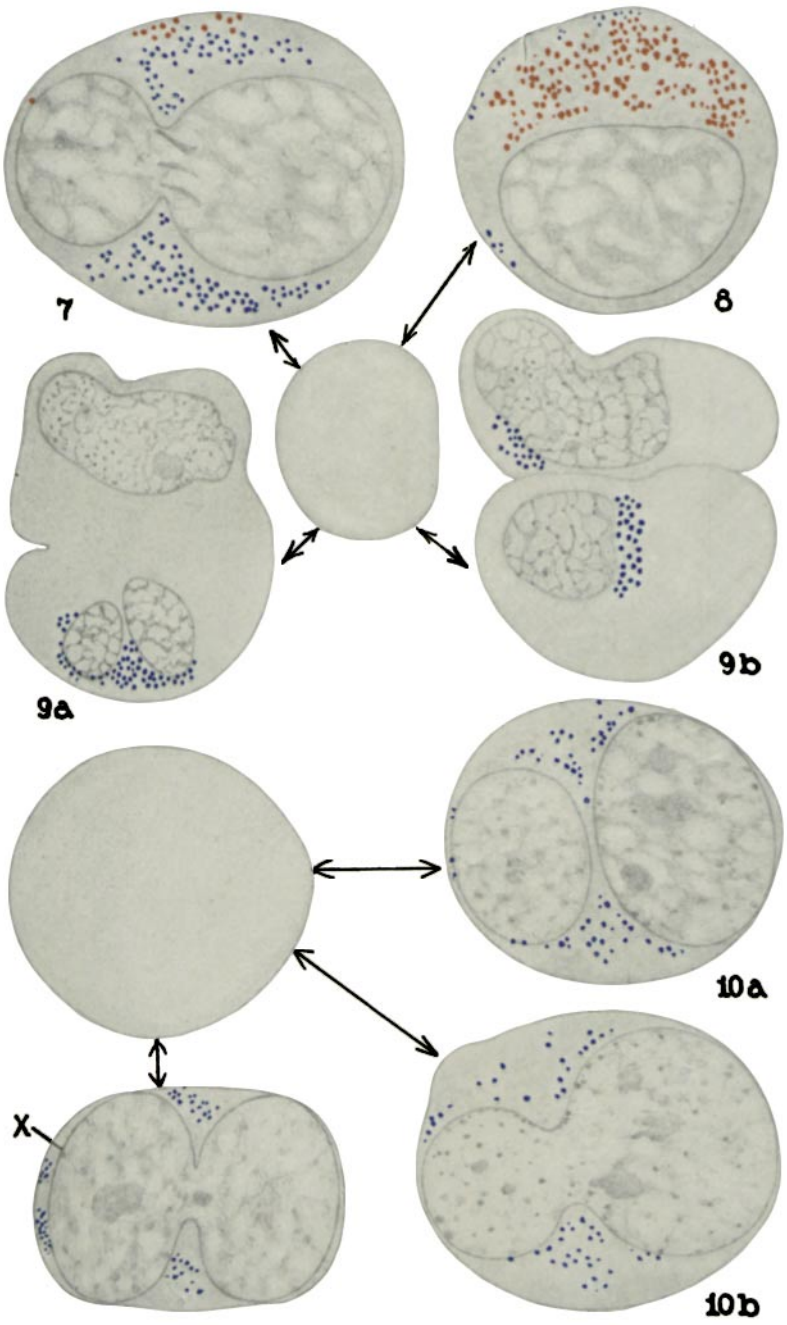
FIG. 10, *a*. Myeloblast of small size, drawn Dec. 5. The cell was from a preparation taken before the transfusion. When first seen the cell had two unequal nuclei, one of which had two or three nucleoli. The cell remained in the condition shown for 18 minutes.

FIG. 10, *b*. The same cell as Fig. 10, *a*. The two nuclei touched at one point and then flowed together as shown in this figure. After remaining in this condition for 5 minutes the bridge between the two nuclei began to narrow and in 52 minutes the nucleus divided completely so that the cell again looked as it did in Fig. 10, *a*.

FIG. 11. Myeloblast, drawn Dec. 5, before the first transfusion. When first seen the cell was as represented here. While it was being drawn there was a complete division of the nucleus in the line of the deep cleft and while this was taking place the segment on the left divided completely in the place indicated by the line marked *x*. This process took 12 minutes and resulted in a cell having three unequal nuclei.



(Sabin *et al.*: Maturation of myeloblasts into myelocytes.)



(Sabin *et al.*: Maturation of myeloblasts into myelocytes.)