Erythroleukemia

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IT IS THE PURPOSE of this paper to report on a case in which routine investigations suggested a disorder of the red cell precursors. A bone marrow culture was prepared in order to determine the maturation rate and behavior of these cells. It is later suggested that such a culture method has proved helpful in establishing the true nature of the disease.

Case History

A pattern maker, Mr. X., aged 27 years, was admitted to the Royal Infirmary, Sheffield, on July 6, 1948, under the care of Dr. A. W. D. Leishman. Five weeks previously he had been treated for tonsillitis with penicillin and sulfonamides by his own doctor. After returning to work in two weeks' time, he complained of lack of energy and of exertional dyspnoea and palpitations. He expectorated a little mucopurulent sputum in the mornings. Slight bilateral tinnitus was noticed, and soreness below the right angle of the jaw was present on swallowing. Later he complained of shivering and sweating.

There was no previous history of respiratory infections. Three years had been spent in the Army in the Middle and Far East, where he was thought to have had malaria and infective hepatitis. There was no other significant past or family history.

On admission the patient appeared pale and sallow, but not truly icteric. His temperature was 100 F., pulse rate 96 per min., and respiratory rate 22 per min. There were no changes in the mouth and throat. The right tonsillar lymph node was enlarged and tender, but no other lymph nodes were significantly enlarged. Apart from soft systolic bruits over the whole precordium, the heart and lungs appeared normal. The spleen was palpable, extending 1 inch below the left costal margin. No other abnormal physical signs were detected in the abdomen or on rectal examination. The limbs and central nervous system showed no abnormality. No purpura was detected.

Investigations

Peripheral Blood (see table 1). Hb. 41 per cent (5.8 Gm. per cent), R.B.C. 1.92 M/cu.mm., C.I. 1.07, M.C.D. 8.0 μ, P.C.V. 19 per cent, M.C.V. 100 cu., M.C.H. 30.5 $\gamma\gamma$, M.C.H.C. 30.5 per cent, W.B.C. 3,200/cu.mm., Myeloblasts 32/cu.mm., Promyelocytes 32/cu.mm., N. myelocytes 64/cu.mm., N. band cells 160/cu.mm., N. polymorphs 1,696/cu.mm., Lymphocytes 1,152/cu.mm., Monocytes 64/cu.mm., Nucleated red cells 224/cu.mm. The red cells showed slight anisocytosis and poikilocytosis, tending towards macrocytosis and appearing normochromic. No spherocytes seen. Some nucleated red cells showed a megaloid nuclear pattern. No malarial parasites found in both thick and thin films. Platelets 77,000/cu.mm. (Lempert). Reticulocytes less than 1 per cent. E.S.R. (Westergren) 135 mm. in one hour. Bleeding time (Ivy) two and three-quarter minutes. Clotting time (Lee and White) five minutes. Capillary fragility (Rumpel-Leede) normal. Serum bilirubin 0.2 mg. per 100 ml. Direct and indirect van den Bergh reaction negative. Plasma prothrombin content (1-stage,

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It is a pleasure to acknowledge our indebtedness to Dr. A. W. D. Leishman for access to the case and case notes, and for constant encouragement and help. We also thank Dr. A. Piney for permission to quote from his notes and Dr. R. G. Macfarlane for his most helpful criticism.

Quick) normal. Plasma fibrinogen 0.38 Gm. per 100 ml. (Kjeldahl). Serum uric acid 5.7 mg. per 100 ml., Serum calcium 10.0 mg. per 100 ml. Serum inorganic phosphorus 4.2 mg. per 100 ml. Serum alkaline phosphatase 6 King-Armstrong units. Serum acid phosphatase 3

TABLE 1.—*Peripheral Blood Findings Throughout the Course of the Disease*

Date	Hb. %	Hb. Gm. %	R.B.C. M/cu. mm.	W.B.C. /cu. mm.	Myeloblasts /cu. mm.	Promyelocytes /cu. mm.	Myelocytes /cu. mm.	Metamyelocytes /cu. mm.	Polymorphs /cu. mm.	Lymphocytes /cu. mm.	Monocytes /cu. mm.	Bare nuclei /cu. mm.	Nucleated red cells /cu. mm.	Reticulocytes
7/ 6/48	41	5.8	1.92	3,200	32	32	64	160	1,696	1,152	64	—	224	1%
7/10/48	46	6.5	—	—	—	—	—	—	—	—	—	—	—	—
7/12/48	58	8.2	—	—	—	—	—	—	—	—	—	—	—	—
7/14/48	60	8.5	2.75	3,800	76	266	266	988	1,444	760	—	+	228	—
7/16/48	63	8.8	2.95	—	—	—	—	—	—	—	—	+	+	1%
7/17/48	58	8.1	2.60	—	—	—	—	—	—	—	—	—	+	2%
7/19/48	55	7.7	2.57	—	—	—	—	—	—	—	—	—	—	1.5%
7/21/48	55	7.7	—	—	—	—	—	—	—	—	—	—	—	2%
7/22/48			—	—	—	—	—	—	—	—	—	—	—	2%
7/23/48	50	7.0	2.48	—	—	—	—	—	—	—	—	+	++	1%
7/26/48	48	6.7	2.07	8,000	2,000	480	320	—	2,720	1,200	—	1,280	4,160	1%
7/28/48			—	—	—	—	—	—	—	—	—	—	—	1%
7/29/48	47	6.7	—	—	—	—	—	—	—	—	—	—	—	1%
7/30/48	44	6.2	2.10	—	—	—	—	—	—	—	—	—	—	1%
7/30/48	72	10.1	—	—	—	—	—	—	—	—	—	—	—	1%
8/ 3/48	71	10.0	—	—	—	—	—	—	—	—	—	—	—	1%
8/ 4/48			—	4,400	176	836	1,012	704	660	1,012	—	+	396	—
8/10/48	68	9.5	3.20	—	—	—	—	—	—	—	—	—	+	1%
8/12/48	55	7.7	—	—	—	—	—	—	—	—	—	—	—	1%
8/16/48	50	7.1	2.46	5,000	300	300	—	1,150	1,500	1,250	50	450	250	—
8/23/48	39	5.5	1.70	5,000	1,025	200	25	125	1,575	1,375	—	675	+	—
8/26/48	40	5.6	—	—	—	—	—	—	—	—	—	—	—	—
9/ 9/48*	60	9.35	2.90	9,600	432	—	336	1,056	2,016	4,512	1,200	—	+	—
9/16/48*	56	8.75	2.89	6,900	345	—	—	138	1,863	4,347	207	—	345	—
9/22/48*	76	11.85	3.46	3,480	69	—	—	35	1,492	1,288	626	—	++	—
9/29/48*	72	11.25	2.69	4,400	396	—	88	—	2,112	1,584	220	—	132	—
10/ 6/48*	100	15.69	4.75	2,900	348	—	58	—	1,566	783	145	—	+	—
10/12/48*	98	15.3	4.50	3,600	36	—	180	36	2,700	432	216	—	108	—
10/19/48*	100	15.69	4.80	3,200	—	—	—	—	2,240	864	96	—	—	—
11/ 2/48	33	4.6	1.65	2,600	624	364	—	104	520	676	52	260	++	—
11/ 4/48	50	7.2	—	—	+	+	—	—	—	—	—	—	+	—
11/ 5/48	72	10.1	—	—	—	—	—	—	—	—	—	—	+	—
11/29/48	55	8.1	—	—	+	—	—	—	—	—	—	—	+	—
12/14/48	38	5.6	—	—	+	—	—	—	—	—	—	—	+	—
12/15/48	60	8.5	—	—	+	—	—	—	—	—	—	—	+	—
12/16/48	64	9.0	—	—	+	—	—	—	—	—	—	—	+	—

* From Dr. Piney's findings.

Gutman units. Formaldehyde stable acid phosphatase 2 Gutman units. Serum iron 200 μμg. per 100 ml. Serum total lipoid fatty acids 330 mg. per 100 ml. Total serum proteins 6.8 Gm. per 100 ml. Total serum albumin 3.6 Gm. per 100 ml. Total serum globulin 3.2 Gm. per

100 ml. Paul-Bunnell test negative. Repeated blood cultures sterile. Wassermann reaction negative.

Fractional Test Meal. Normal curve. Free HCl present. No blood detected.

Urine. Normal. No Bence-Jones protein.

Fecal urobilinogen. Twenty-four hourly specimen of feces (wet weight 97 Gm.) contained 340 mg. urobilinogen.

X-ray examinations. Chest (including bony thorax), pelvis, femora, skull, no abnormality.

Sternal marrow. Differential count of 200 cells (per cent):

Myeloblasts	4.5	Lymphocytes	1.0
Paramyeloblasts	3.5*	Plasmocytes	2.0
Promyelocytes	6.5	Reticulum cells	0.5
N. myelocytes	11.5	Cells in mitosis	2.5
N. band cells	2.5	Pronormoblasts	7.5
N. polymorphs	1.5	Basoph. normoblasts	37.5
Eosinophils	2.5	Polychr. normoblasts	16.5
		Orthochr. normoblasts	—

Leuko-erythrogenetic ratio = 0.42. Cellularity increased. There are some megaloid changes in the nucleated red cells. No megaloblasts seen. No parasites seen.

Clinical Course

This is summarized in table 2. Repeated blood transfusions, including exsanguino-transfusion under the care of Dr. A. Piney, and empirical treatment with iron, liver, folic acid, urethane and penicillin proved of no avail, the patient dying six months after his first admission to hospital.

Bone Marrow Culture in Vitro.

One of us (L. G. L.) has performed over the past three years more than 120 routine marrow cultures. From these it has been possible to recognize a standard rate of maturation in normal marrows. Marked differences from normal are always found in megaloblastic anemia and in the various forms of leukemia. Thus we were stimulated to investigate the maturation rate and path of this marrow.

A modified Osgood and Brownlee technic[9] was used. A homogenous suspension of the marrow cells ("initial suspension," see table 3) was distributed into 4 culture bottles, 2 of which contained 70 per cent normal human serum + 30 per cent Ringer solution as a culture medium, while the other 2 bottles contained an additional 0.005 mg. of folic acid per 3 ml. of the above culture medium. After twenty-four and forty-eight hours' incubation respectively, the bottles were opened and the contents centrifuged. Smears were made. One thousand cells were counted on 3 successive smears from each bottle. The results are shown in table 3.

* Blast cells slightly more differentiated than primitive myeloblasts which have some monocytoid characteristics in their nuclear structure and shape.

TABLE 2.—*Clinical Course*

Date	Semeiology	Hb. in Gm. %	Blood Transfusions	Drug Treatment
7/10/48	Temp. 101 F. Pulse 86 per min. Clinical state as on admission (see text).	6.5	1,080 ml. of packed cells given. No reaction.	
7/12/48	Afebrile. Subjective improvement.	8.2		
7/14/48	Splenomegaly increasing, (3 in. below costal margin).	8.5		Anahemin commenced, 4 ml. then 2 ml. daily intramuscularly.
7/15/48	Again febrile.			
7/22/48	Still low intermittent pyrexia.	7.7		Folic acid commenced, 100 mg. daily, by mouth.
7/30/48	Marked clinical deterioration.	6.2	1,080 ml. of packed cells given. No reaction.	Penicillin commenced, 60,000 units every 3 hours, intramuscularly.
7/31/48	Subjective improvement. Temperature reverted to normal. Spleen 2½ inches below costal margin.			
8/10/48		9.5		1 ml. Ferrivenin intravenously. No reaction.
8/11/48	Tonsillar lymph node unchanged.			Urethane 1 Gm. t.d.s. commenced.
8/12/48	Nausea, but no vomiting.	7.7		
8/17/48	Clinical deterioration. Still febrile.	7.1		Penicillin stopped.
8/22/48	Further deterioration. Spleen 1½ inches below costal margin.			2 ml. Ferrivenin intravenously. No reaction.
8/23/48		5.5		5 ml. Ferrivenin intravenously, on alternate days commenced.
8/27/48	Discharged home.	5.6		Anahemin and Ferrivenin stopped. Continue urethane 1 Gm. t.d.s. To have ferrous sulphate 6 Gm. t.d.s. and folic acid 20 mg. b.d.
9/ 7/48	Admitted to St. Mary's Hospital, Plaistow, under care of Dr. A. Piney.			All drug therapy stopped.
9/ 9/48		9.35	Intermittent replacement transfusions commenced: 2,700 ml. of whole blood given, 1,080 ml. removed.	
9/15/48	Slow rise in temperature.		540 ml. of whole blood given.	
9/16/48	Subjective improvement.	8.75		

TABLE 2.—*Continued*

Date	Semeiology	Hb. in Gm. %	Blood Transfusions	Drug Treatment
9/18/48			2,700 ml. of whole blood given, 3,500 ml. removed.	
10/ 2/48			750 ml. of blood removed. Then 2,700 ml. of whole blood given. Then 3,780 ml. of whole blood removed and 2,160 ml. of whole blood and 2,700 of packed cells given.	
10/ 6/48	Subjectively improved.	15.69		
10/19/48	Discharged home.	15.69		
11/ 2/48	Readmitted to the Royal Infirmary, Sheffield. Insomnia, anorexia, nausea. Very pale. Temp. 101 F. Pulse 126 per min. Small healing ulcer on the right shin at the site of a previous transfusion. Liver and spleen each 1½ inches below costal margin. No bony tenderness. No enlarged lymph nodes.			None
11/ 3/48			1,080 ml. of packed cells given.	
11/ 4/48			540 ml. of packed cells given.	
11/ 5/48		10.1	270 ml. of packed cells and 540 ml. of whole blood given.	
11/ 6/48	Subjectively much improved. Physical signs unchanged. Afebrile. Discharged home.			
11/27/48	Readmitted to hospital for epistaxis. Pulse 120 per min. Low intermittent pyrexia. No purpura. No bone tenderness. No lymph node enlargement. Liver and spleen not palpable. Epistaxis controlled by nasal tampons.			
11/28/48	Nasal plug removed.			
11/29/48	Discharged home.	8.1		
12/14/48	Readmitted to hospital. Critically ill. Temp. 102 F. Pulse 88 per min. Spleen just palpable.	5.6	1620 ml. of packed cells given.	
12/15/48	Low intermittent pyrexia.	8.5	540 ml. of whole blood given.	
12/18/48	Some clinical improvement. Discharged home.	9.0		
12/23/48	Died in his sleep at home. Permission for autopsy was not obtained.			

Interpretation of the Cultures.

The maturation rate of the red cell precursors of this marrow was delayed. It might even be considered questionable whether there was any maturation (figure 1). The abnormally large number of basophilic normoblasts decreased, but did not give place to any corresponding rise in the more mature forms. This re-

TABLE 3.—*Bone Marrow Culture*

	Initial Suspension (No. 421)	Twenty-four Hour Culture		Forty-eight Hour Culture	
		in normal serum (No. 422)	in normal serum and folic acid (No. 423)	in normal serum (No. 424)	in normal serum and folic acid (No. 425)
Myeloblasts	4.5	5.5	5.0	2.0	0.5
"Paramyeloblasts"	3.5	5.0	5.5	2.0	0.5
Promyelocytes	6.5	5.0	4.5	3.5	3.0
N. Myelocytes	11.5	16.0	14.0	23.0	26.5
N. Band Cells	2.5	3.5	4.0	10.0	9.5
N. Segmented	1.5	5.5	6.0	10.5	12.0
Eosinophils	2.5	2.0	1.5	2.0	2.0
Lymphocytes	1.0	2.0	2.5	2.5	3.0
Plasmocytes	2.0	2.5	4.0	3.0	4.5
Reticulum cells	0.5	1.0	1.0	2.5	2.0
Cells in Mitosis	2.5	2.0	1.5	0.5	0.5
Pronormoblasts	7.5	2.0	0.5	0.5	—
Basophil normoblasts	37.5	20.0	19.0	5.0	—
Polychromatic normoblasts	16.5	20.5	24.5	28.0	31.0
Orthochromatic normoblasts	—	7.5	6.5	5.0	5.0
Free pyknotic nuclei/100 cells	8.0	11.0	13.0	18.0	20.0
Smear cells/100 cells	3.0	8.0	10.0	22.0	24.0

Remarks on cell morphology

No. 421. Frequent "megaloid" nuclear patterns. No typical megaloblasts.

No. 422. Some atypical mitotic forms. Occasional "megaloid" cells still present. "Paramyeloblasts" more differentiated.

No. 423. No "megaloid" cells seen. Degenerative changes in the basophil normoblasts.

No. 424. Most of the normoblasts have pyknotic nuclei. Some mitoses seen. "Paramyeloblasts" differentiating to monocytoid forms.

No. 425. Nearly all the normoblasts have pyknotic nuclei. Remarkably high ratio of free (? extruded) pyknotic nuclei.

tarded maturation was not due to any deficiency in the serum, since the serum produced normal maturation in a normal marrow. The proportion of free extruded nuclei in the cultures was most unusually high. These free nuclei escaped the usual fairly rapid lysis which is the common fate of extruded nuclei in normal cases. They maintained their pyknotic character throughout the period of the culture (figure 2). There was also an increased proportion of smear cells

(degenerated dying cells) in the cultures. The effect of folic acid on the nucleated red cells was not marked.

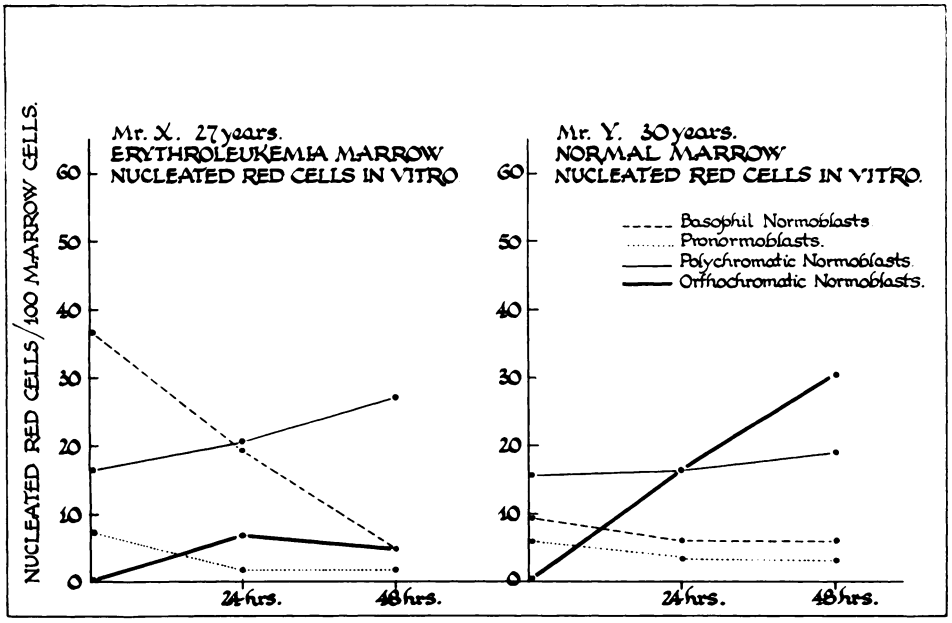

FIG. 1.—Graphs showing the maturation rate of red cell precursors

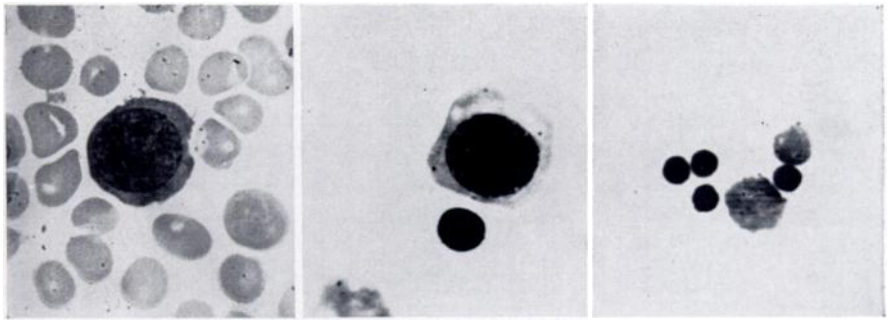

FIG. 2.—*Left:* Megaloid cell in Mr. X's marrow. *Center:* Erythroblast and free pyknotic nucleus in forty-eight hour culture. *Right:* Nuclear shadows and free pyknotic nuclei in forty-eight hour culture.

These observations are in accordance with those of Astaldi and Tolentino[1] who also describe the slower maturation rate of these cells in vitro. This mechanism they suggest as the main cause of anemia in these cases. They also indicate that the nuclear maturation is disturbed qualitatively, as the nucleus does not disappear from the polychromatic normoblasts (leaving a reticulocyte behind),

but continues to exist even when the cytoplasm is fully differentiated. Our results lend some confirmation to this statement, the extruded nuclei showing an unusually high resistance and stability in the cultures.

Discussion and Summary

Differential Diagnosis. An unresponsive anemia associated with a leukoerythroblastic peripheral blood picture may be seen in such varying clinical conditions as carcinomatosis, leukemia in early phase, Hodgkin's disease, toxic aplastic anemia, hemolytic anemia, myelosclerosis, myeloma, syphilis, tuberculosis, lipoidosis, Leishmaniasis and infective hepatitis. From the clinical course of the disease and routine methods of investigation, the majority of these diseases could be excluded. It was soon apparent from the appearance of the marrow and the result of the marrow culture that this disease was to be regarded as a primary blood dyscrasia.

There is a group of such dyscrasias which is characterized by abnormalities in both erythro- and leukopoiesis. All gradations between erythremic myelosis (Copelli,[2] Guglielmo,[3] Leitner et al.[6]) and myeloblastic leukemia may occur. The case here described is neither one of erythremic myelosis nor one of acute leukemia.

Heilmeyer and Schöner[5] describe a true chronic erythroblastosis of adults which, however, is accompanied by a leukocytosis, never present in this case.

Several transitional cases, where there is neither a predominant erythroblastic nor a proponderating myeloblastic hyperplasia, have been reported. In these there is, however, evidence of a neoplastic hyperplasia of both erythroid and myeloid tissue. Such cases have been described by Penati,[10] Moeschlin,[7] Moeschlin and Rohr,[8] Rohr,[11] Harvier et al.,[4] and Stahel.[12] The course of six months in the present case resembles that of Moeschlin.[7]

It is of academic interest to differentiate between a true aplastic anemia, an aplastic initial phase of leukemia and the mixed neoplastic forms of the erythroleukemic group. Erythroblasts in cases of this group frequently show "megaloid" changes (as do those in acute leukemia). A series of transitional forms from normoblasts to megaloblasts through "megaloid" forms may be seen. This could be due to a relative deficiency in nutritive factors consequent on the abnormal cellular proliferation. Similarly in many types of acute leukemia this type of cell, when cultured in normal serum, reverts to the normal maturation rate and morphology of the normoblast. So do the cells of aplastic anemia, where the ultimate defect is further removed. When, however, an abnormal normoblastic maturation rate is found, a qualitative, probably neoplastic, change in the nature of these cells is suggested, since the same changes may be found in the myeloid series in cases of myeloid leukemia. Hence marrow culture may prove of diagnostic value in differentiating these somewhat allied conditions.

This case is one of progressive, irresponsive anemia with a peripheral leukoerythroblastosis, and bone marrow showing a considerably lowered leukoerythrogenetic ratio with a relative myeloid predominance of early forms and of atypical blast cells (paramyeloblasts).

Culture of the bone marrow established that the rate of maturation of both the erythroid and myeloid tissue was abnormal, although the defect was more marked in the erythroid series. On these findings a diagnosis of erythroleukemia seems justified. Since this report, 2 similar cases investigated by marrow culture have shown comparable maturation abnormalities.

REFERENCES

[1] ASTALDI, G. AND TOLENTINO, P.: Studies in vitro on maturation of erythroblasts in normal and pathological conditions. J. Clin. Path. *2:* 217, 1949.

[2] COPELLI, M.: Di una emopatia sistematizzata rappresentata da una iperplasia eritroblastica. Pathologica *4:* 460, 1912.

[3] GUGLIELMO, G. DI: Eritremia acuta. XXIX, Congr. med. int. Roma, 1923.

[4] HARVIER, P., LE MELLETIER, J., LAVERGNE, G. H. AND LAMOTTE, M.: Erythroleucomyélose aiguë. Sang *15:* 272, 1942.

[5] HEILMEYER, L. AND SCHÖNER, W.: Die chronische reine Erythroblastose des Erwachsenen als Leukämie-paralleler Process des Erythrocytären Systems. Deutsche Arch. f. Klin. Med. *187:* 225, 1941.

[6] LEITNER, S. J., BRITTON, C. J. C. AND NEUMARK, E.: Bone marrow Biopsy, London, Churchill, 1949.

[7] MOESCHLIN, S.: Erythroblastosen, Erythroleukämien und Erythroblastämien. Folia haemat. Lpz. *64:* 262, 1940.

[8] — AND ROHR, K.: Klinische und morphologische Gesichtspunkte zur Auffassung der myelose als neoplasma. Ergebn. d. inn. Med. u. Kinderh. *57:* 723, 1939.

[9] OSGOOD, E. E. AND BROWNLEE, I. E.: Culture of human marrow. Details of a simple method. J. A. M. A. *108:* 1793, 1937.

[10] PENATI, F.: Leucemia megaloblastica acuta. Minerva Med. *28:* 401, 1937.

[11] ROHR, K.: Das Menschliche Knochenmark, Leipzig, G. Thieme, 1940.

[12] STAHEL, R.: Subakute Erythroleukämie ohne Splenomegalie. Helv. med. acta. *10:* 605, 1943.