women and 71 women in labour were examined for C-reactive protein. 25% to 32% of the pregnant women and 66% of the women in labour showed positive results. 2. Only one of 73 cord bloods examined showed the presence of C-reactive protein. 3. Antistreptolysin O was present in all 50 cord bloods examined, frequently at a titer higher than that in maternal blood. 4. C-reactive protein was found in blood of very young babies suffering from bacterial infections. 5. It is suggested that C-reactive protein is not transferred across placental barrier.

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Received March 27, 1956. P.S.E.B.M., 1956, v92.

Cytoautoradiography Fe^{59} Uptake by Bone Marrow Cells. I. Normal Human Erythroblasts and Young Erythrocytes.* (22367)

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In vivo Fe^{59} uptake was first demonstrated in rat bone marrow by stripping film cytoautoradiography of marrow smears after injection of considerable amounts of radioiron (1).

The present paper reports the *in vitro* uptake of Fe^{59} by normal human erythroblasts and young erythrocytes during incubation with small amounts of radioiron.

Method. Human bone marrow from normal and pathological subjects was obtained by sternal aspiration with oxalated or heparinized syringes. Samples of 0.5-1 ml marrow were quickly suspended in a buffered solution of Ferrous^{59} citrate containing 3-5 μc, specific activity 3.79 μc/μg Fe. The tubes were incubated at 37° for 1-2-4-6-9-12-24 hours, then centrifuged at low speed. The supernatant fluid was then discarded and the cells washed twice with normal saline solution. This procedure usually removed the excess radioiron but sometimes damaged the cells. The following alternative procedure was later adopted. The marrow-radioiron solution was put into vertical sedimentation rate pipettes and incubated at 37°. After the desired time, thin smears were prepared from the upper part of the cellular layer, where the marrow particles are easily recognized. With this procedure the Fe^{59} background obtained is only slightly higher than with the washing method. The smears, dried at room temperature and fixed with pure methanol, were treated with the usual stripping film pro-

* These researches were initiated at University of California, when author held a Fulbright-Smith-Mundt Fellowship, and were completed at Institute of Medical Pathology in Padua. The work done in the Donner Laboratory was partially supported by the Henry Stevens Kiersted Memorial Fund.

The author is grateful to Professor John H. Lawrence for his encouragement and assistance.

TABLE I. *In Vitro* Fe^{59}-Uptake by Normal Human Erythroblasts. Percent of *labeled* and *non-labeled* cells and their *activity* (expressed by addition of plusses of the marked erythroblasts of each stage) after incubation with 5 μc of Ferrous^{59} citrate $\left(\text{spec. activity } \frac{3.79\ \mu c}{\mu g\ Fe}\right)$.

Kodak permeable base stripping film (experimental) exposure: 17 d. Case 76: C. Corrado, 30-yr-old healthy man. Hb. 96, RBC 5.2 million, C. I. 0.93.

Cell types	% non-labeled cells	% labeled cells				% total labeled cells	Relative activity (total +'s)
		1+	2+	3+	4+		
						After 2 hr incubation	
Proerythroblasts	57	31	11	1	0	43	56
Basophilic erythroblasts	42	45	12	1	0	58	72
Polychromatic	40	53	7	0	0	60	67
Orthochromatic	55	38	7	0	0	45	52
						After 4 hr incubation	
Proerythroblasts	52	38	8	2	0	48	60
Basophilic erythroblasts	39	35	15	10	1	61	99
Polychromatic	38	41	14	7	0	62	100
Orthochromatic	28	41	18	12	1	72	117
						After 9 hr incubation	
Proerythroblasts	48	45	6	1	0	52	60
Basophilic erythroblasts	47	43	6	4	0	53	67
Polychromatic	37	51	11	1	0	63	76
Orthochromatic	29	54	13	3	1	71	93

The 12 and 24 hr incubation samples yielded defective preparations and were discarded.

cedure(1). After 15-30 days, the autoradiographs were read by a semi-quantitative estimation method based on the qualitative evaluation of *non-labeled* (or negative) and labeled cells, and on the 1+, 2+, 3+, 4+ quantitative classification of the marked cells according to their relative autoradiographic activity. The sum of the plusses of the labeled elements of each erythroblastic stage reflects the cell radioactivity and the differential capacity of each erythroid element to take up iron *in vitro*. Determinations of *labeled haemoglobin* resulting from the above incubation experiments were conducted in a few cases, in order to measure the erythron capacity of synthesizing heme *in vitro*. The same incubation experiments were also performed with the circulating blood of a few subjects in order to ascertain if young erythrocytes are capable of synthesizing heme *in vitro*. For this purpose, after incubation and washing, haemoglobin was liberated from the cellular stroma with distilled water. The haemoglobin solutions were then tested for their radioactivity with a Geiger-Muller or scintillation counter. Direct autoradiographs were also taken of known amounts of dried haemoglobin. When a more purified Hb. was desired, electrophoretic separation at varying pH levels was also performed and the activity of the different fractions determined by counting or autoradiography. With the above technic the marrows and bloods of 15 cases, normal individuals or patients with various abnormal conditions, were examined.

Results. 1. *Cytoautoradiography.* Table I gives data of a typical experiment. As has been demonstrated by *in vivo* studies in the rat(1), the human normal erythroblast *in vitro* also shows the capacity of rapidly taking up considerable amounts of radioiron. All the erythroblastic maturation stages are more or less "labeled" (Fig. 1). The percentage of marked elements varies between 40 and 70%, according to the maturation stage, incubation time, specific activity of the isotope, exposure time and other non-identifiable factors. In other experiments all the elements are similarly labeled, allowing the conclusion that, potentially, all the immature elements of the erythron keep their capacity of utilizing iron *in vitro*. Also a certain number of medullary young erythrocytes with morphological aspect of reticulocytes, show a definite iron-uptake (Fig. 1). Only circulating blood with considerable reticulocytosis showed,

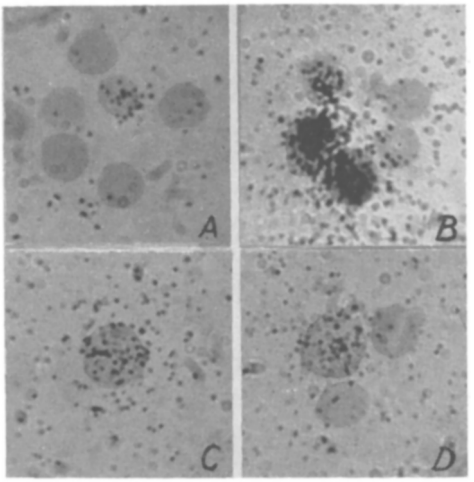

FIG. 1. Bone marrow "labeled" young erythrocytes: autoradiographic picture of the *in vitro* Fe^{59}-uptake by erythrocytes of a normal young woman after 2 hours incubation with 5 μc of Ferrous^{59} citrate. A) Small labeled erythrocyte; B) 2 labeled erythroblasts and a small labeled erythrocyte; C) and D) 2 large polychromatophilic labeled erythrocytes. (Stained autoradiographs; 1000 ×.)

after incubation with radioiron, significant autoradiographic evidence of erythrocyte labeling. A case of Marchiafava-Micheli disease with 96% reticulocytosis revealed considerable labeling. The maximum iron-uptake occurs. after 2 hours incubation, in the basophilic and polychromatic stages (58% and 60% with 72 and 67 plusses, respectively). But, after 4 hours, the orthochromatic elements show a much higher activity (72% with 117 plusses), while the more immature elements undergo a slighter increase. After 9 hours incubation, there is a slight decrease in all the stages, the maximum still occurring in the orthochromatic cells. The individual cell Fe uptake shows some quantitative variations. Sometimes it appears greater in the earlier phases, sometimes in the later stages of maturation. It may be suspected that this can be related to variations in technic (Fe^{59} specific activity and oxidation state, plasma iron binding capacity, incubation time, film exposure and developing, buffering procedure, washing, etc.). Definite conclusions can not be drawn, especially because it was not possible to evaluate many physiological factors which may be involved in the process. These variations are, in fact, much greater in pathological conditions.

Mature erythrocytes in bone marrow as well as in circulating blood are not able to take up radioiron as shown by the negative autoradiographs of such elements. This confirms the work of Hahn(2) and Gowaerts(3). In regard to the *non-erythroid marrow cells* it appears that, in general, there is no evidence of iron-uptake *in vitro*. Only *eosinophilic elements* are occasionally labeled (Fig. 2). This behavior has been observed only in a few cases, namely liver cirrhosis and chronic hepatitis. It cannot be stated that this observation is related to cellular iron turnover. The negative autoradiographs of the other myeloid cells indicate that heme enzymes and other iron compounds have a relatively low iron metabolism in these cells and that the iron quantities involved are below the sensitivity of this autoradiographic method.

2. *Labeled haemoglobin determinations.* a) *Bone marrow haemoglobin* from normal individuals shows, after 2 hours incubation, a specific activity of 0.5-1%. Hyperplastic marrows, as in cases of haemolytic anaemias, yield up to 3-4 times as much labeled Hb. Hypoplastic marrows, as in cases of aplastic or aregenerative anaemias, on the contrary, often show a marked reduction of Hb. synthesis. The Hb. production *in vitro*, as *in vivo*, seems to correspond with the functional state of the marrow.

b) *Peripheral blood haemoglobin* from normal individuals and cases of aregenerative anaemias is produced in very low quantities or not detectable at all. In patients with hyperplastic marrow, on the contrary, distinct amounts of labeled pigment (1-2%) are produced by incubating their blood. This seems to be related to the reticulocytosis present in these cases. Autoradiographs and paper electrophoresis determinations with scintillation countings gave results consistent with the above-mentioned data.

These results demonstrate that at least some part of the iron taken up by the bone marrow or blood reticulocytes is for haemoglobin formation. The work of Watson(4)

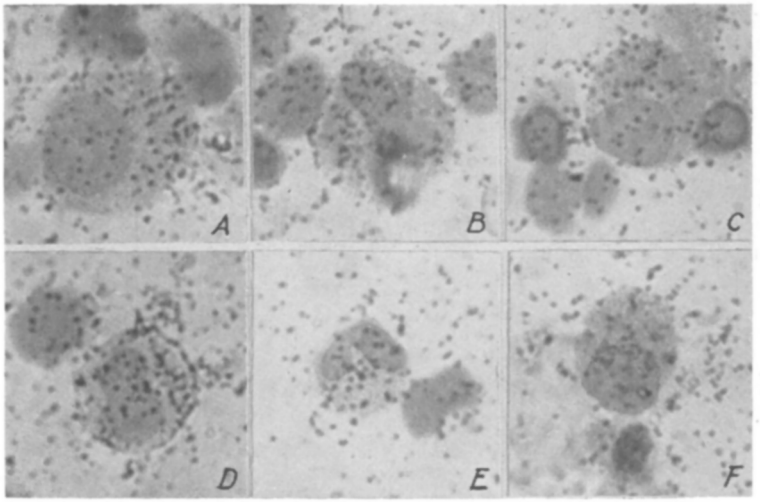

FIG. 2. "Labeled" eosinophils in a case of liver cirrhosis with slight macrocytic anaemia: A, B, C—earlier and D, E, F—later medullary eosinophils after 2 hours incubation with 5 μc of Ferrous^{59} citrate. (Stained autoradiographs; × 1000.)

has pointed out that in the erythron there are, as precursors of heme, protoporphyrin, globin and Fe. As long as these compounds are present the synthesis takes place. The anuclear mammalian erythrocyte is usually released into the circulation at a time which approximates the completion of haemoglobin formation. Presumably, no further Hb. formation occurs after that time. But, if for some reason, the erythrocyte is released when still immature, as a reticulocyte or erythroblast, further Hb. synthesis may take place in the circulating blood(5), provided that these red cells contain the other precursor materials. This happens also *in vitro,* as shown by the above experiments. The nucleus then is not necessary for this final step of Hb. synthesis, but it must be pointed out that the nucleus plays the directing role in synthesizing the porphyrin precursors.

Summary. The *in vitro* uptake of Fe^{59} by normal human erythrons is described. All the erythroblastic maturation stages and young erythrocytes in bone marrow as well as in circulating blood take up considerable amounts of radioiron. During the earlier phases of the experiment, the iron-uptake is maximal in the late basophilic and early polychromatic elements. Subsequently, the maximum is reached in the orthochromatic stage. Marrow reticulocytes and circulating young erythrocytes take up small quantities of isotope. Mature erythrocytes do not seem capable of so doing. Iron utilization for haemoglobin synthesis appears to be the main function of the erythron.

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Received November 17, 1954. P.S.E.B.M., 1956, v92.