erol acetate, 0.9% sulfur amino acids, and 167 ppm ethoxyquin. The chicks developed exudative diathesis as early as the 9th day of experiment when fed the ethyl esters or reconstituted triglycerides obtained from the most unsaturated fractions of fish oil. The observed disorders are not due to the ethyl ester form of the fatty acids or to the oxidation of the oil in the feed.

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Received May 18, 1964. P.S.E.B.M., 1964, v116.

Effect of Red Cell Age on Its Susceptibility to Lysis by Rabbit Hemolytic Factor. (29477)

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A hemolytic factor capable of lysing human and other mammalian red cells *in vitro* has been previously isolated from rabbit erythrocytes(1-3). This factor requires exogenous ATP, Mg^{++}, and Na^{+} or K^{+} for its lytic activity, but is not dependent on glycolysis, choline esterase, or active ion transport. In addition, the action of this factor on intact erythrocytes is completely inhibited by preincubation with purified human stroma in the presence of the above cofactors. It appears, therefore, that its substrate is some component of the red cell membrane and that lysis of the cell is the result of membrane degradation.

Since it has been demonstrated that some relationship exists between the age of the red cell and its susceptibility to osmotic(4), immunologic(5), and primaquine-induced hemolysis(6) it was of interest to investigate the effect of red cell age on its lysis by rabbit hemolytic factor.

Materials and methods. *^{59}Fe citrate:* Abbott Laboratories; 100 microcuries per ml.

Glycine-2-^{14}C: Prepared by the Isotopes Specialties Co.; specific activity of 4.2 mC per mM.

Buffered saline: 99 parts isotonic saline + 1 part isotonic Tris (hydroxymethyl) aminomethane-Histidine · HCl buffer, pH 7.0.

Incubation medium. Sucrose (0.25 M) containing adenosine triphosphate (0.001 M), $MgCl_2$ (0.002 M), glutathione (0.0016 M), Ethylenediamine tetraacetic acid (0.0002 M), and potassium phosphate buffer, pH 7.0 (0.01 M).

Hemolytic factor from rabbit erythrocytes. Partially purified rabbit hemolytic factor was prepared by ammonium sulfate fractionation of hemolyzed rabbit red cells as previously described and the hemolytic titer of each preparation was determined before use as described under "Hemolysin Test"(2), substituting washed rat for human red cells.

Glutamic oxaloacetic transaminase activity (GOT). Assayed by the method of Steinberg and Astrow(7).

Specific radioactivity of hemoglobin (Hgb). Hgb concentration was determined by measuring the optical density of stroma-free hemolysates at 540 mμ in a Beckman model DU Spectrophotometer. The Hgb was precipitated (carrier Hgb added where necessary) 3 times from CO_2-saturated water with acetone at 0°. ^{59}Fe was measured in a Tracer Lab well-type scintillation counter and ^{14}C in an automatic Nuclear D-47 gas-flow counter. Radioactivity was expressed as CPM per mg of Hgb.

Experimental and results. Radioactively tagged red blood cells were obtained from

TABLE I. 4 Days Post-Injection of ^{59}Fe-Citrate.

Fraction	Progressive hemolysis (%)	Specific activity (cpm/mg hgb)	GOT activity (units/mg hgb)
"Young" cells	100	1,460	4.7
1st hemolysate	1.0	1,941	12.1
2nd "	41.0	1,695	5.0
3rd "	58.0	1,285	4.4
"Old" cells	100	446	3.3
1st hemolysate	2.0	1,069	7.8
2nd "	58.0	603	3.9
3rd "	40.0	303	3.0

male albino rats 4 to 40 days post intravenous injection of 11 to 20 μc of either ^{59}Fe-citrate or glycine-2-^{14}C. The blood was mixed with an equal volume of ACD solution, plasma and buffy coat were removed and the cells were washed with buffered saline at 4°C until no radioactivity remained in the supernatant wash fluid.

In the beginning of this study the cells were then separated into a relatively "young" and a relatively "old" fraction after centrifugation in 2 volumes of buffered saline at 2,000 *g* for 30 minutes, the young erythrocytes being less dense than older cells(8). This was done in an attempt to emphasize any differences in behavior of the hemolytic factor toward cells of different ages. However, this was found to be unnecessary, and therefore in later experiments the total cell population was used without prior fractionation.

The packed cells were then resuspended to 25% in saline and a 3 ml aliquot was subjected to progressive fractional lysis by incubation for 30 minutes at 37° with 7 ml of incubation medium and rabbit hemolytic factor. Since the degree of lysis per unit of red cells is directly proportional to the concentration of rabbit enzyme used, any desired percentage of the total population could be lysed at a given time. The surviving cells were separated from the resultant hemolysate by centrifugation and reincubated with 7 ml of fresh medium and additional hemolytic factor, the process being repeated until all of the cells had been hemolyzed.

The relative age of each cell fraction lysed was determined by measuring the specific radioactivity of the hemoglobin of the stroma-freed hemolysates and by the activity of glutamic-oxaloacetic transaminase (GOT), an enzyme which has been shown to occur in higher concentration in young red cells(9,10). At the same time, a small aliquot of the cells was completely lysed in one step in order to obtain the activity of the whole preparation.

Fractional hemolysis by rabbit factor on red cells obtained 4 days after injection of ^{59}Fe label (Table I) showed that as hemolysis progressed the specific activity of hemoglobin decreased. Since the cells most heavily labelled 4 days post injection are the youngest cells of the total population, it appears that rabbit factor preferentially lyses young cells, the older cells being the most resistant. This is confirmed by the GOT activities which also were lower in each subsequent hemolysate. Similar results were obtained used red cells tagged with glycine-2-^{14}C (Table II). It should be noted that in these experiments the first step was calculated to lyse only 1 or 2% of the total cells since it has been shown that GOT activity is markedly enhanced in only the very youngest of the erythrocytes. The progressive decrease in enzyme activity associated with red cell aging has been demonstrated for several other enzymes as well(11).

The results obtained on blood drawn 18 days after injection are shown in Table III. After this period of time, younger, unlabelled cells had entered the circulation and the cells originally tagged were aging. The specific activity of the hemolysates was relatively constant, decreasing slightly in the case of the last hemolysate. The GOT activity, however, was highest in the first 1% and lowest

TABLE II. 4 Day Post-Injection of Glycine-2-^{14}C.

Fraction	Progressive hemolysis (%)	Specific activity (cpm/mg hgb)	GOT activity (units/mg hgb)
"Young" cells	100.0	15.6	5.1
1st hemolysate	.7	27.6	8.6
2nd "	4.1	23.8	5.4
3rd "	26.5	18.8	5.6
4th "	49.4	18.4	5.9
5th "	19.3	14.8	5.2
"Old" cells	100.0	7.4	5.0
1st hemolysate	.8	14.0	6.0
2nd "	5.5	12.4	4.5
3rd "	26.4	6.9	5.1
4th "	50.0	6.7	4.7
5th "	17.3	5.9	4.0

TABLE III. 18 Days Post-Injection of ^{59}Fe-Citrate.

Fraction	Progressive hemolysis (%)	Specific activity (cpm/mg hgb)	GOT activity (units/mg hgb)
Original cells	100.0	1,366	4.1
1st hemolysate	1.0	1,344	6.9
2nd "	13.0	1,391	4.8
3rd "	69.0	1,406	5.2
4th "	17.0	1,020	3.5

in the last 17% of the cells to lyse, again indicating that the youngest cells had been preferentially attacked.

After 25 days (Table IV), the labelled cells had aged to the point where they now constituted the older fraction of the population. As hemolysis progressed, specific activity increased, whereas GOT activity decreased.

It appears that, in general, young cells are the most susceptible to hemolysis by rabbit hemolytic factor and old cells are the most resistant. This is in direct contrast to the lytic patterns obtained in osmotic and immuno-lysis where the preponderance of the young cells exhibit the least fragility. Previous studies have shown that as rabbit factor acts upon the red cell, the membrane becomes progressively and increasingly more permeable, first to small components such as Na and K ions, and finally, to large molecules such as hemoglobin(3). The mode of action of the enzyme appears to be one of combination with, or degradation of, some component of the red cell membrane. Therefore, changes in the composition or concentration of membrane constituents as the erythrocyte ages may account for the greater affinity of the rabbit hemolytic factor for young cells. It has been shown for example that young red cell membranes have a higher phospholipid content than older cells(12). Another possibility is that the relatively larger size of the young erythrocyte provides more sites for enzyme attachment leading to preferential hemolysis by the rabbit factor.

TABLE IV. 25 Days Post-Injection of Glycine-2-^{14}C.

Progressive hemolysis (%)	Specific activity (cpm/mg hgb)	GOT activity (units/mg hgb)
Original cells	23.6	6.0
1.0	14.1	7.8
7.5	15.0	6.5
9.2	19.3	6.7
25.3	20.7	6.1
13.7	22.8	5.5
28.9	25.2	5.5
12.4	27.3	5.3
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Received May 21, 1964. P.S.E.B.M., 1964, v116.

Coombs-positive Hemolytic Anemia and Generalized Amyloidosis in Mice Following Transmission of Subcellular Leukemic Material.* (29478)

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Recently we reported(1) the evidence of a murine leukemogenic virus present in a transplantable line (line 66) of plasma cell leukemia(2). This assumption was based on the transmission of the leukemia with subcellular material, on the finding of characteristic virus-like particles with "tail" with the electron microscope in negatively stained preparations of plasma, and finally on an inhibition of growth of the leukemia following "immunization" with virus extract of leukemic tissue. These findings prompted us to examine several of our other transplantable lines of leukemia for the possible presence of virus particles in negatively stained preparations of plasma. Virus-like particles were found, although in small numbers, in most mice examined, *viz* mice of the following transplantation lines: 3 sublines of line 68(3), namely a plasma cell leukemia without paraprotein in serum, one with gamma paraprotein and a mast cell leukemia, an amyloidosis-inducing reticulosarcomatosis(4), and a leukemia associated with macroglobulinemia(5). Consequently, attempts to transmit these lines of leukemia with subcellular material were carried out in addition to identical experiments using material from line 66 and from the 2 transplantation lines derived from line 66 by subcellular transmission(1). The experimental results using material from these 8 different lines were identical, and they will therefore be described collectively.

Three different *methods* were used for subcellular introduction of leukemic material (Table I). 1. In Exp. 24 0.1-0.2 ml of the supernatant from vigorously centrifuged minced leukemic tissue, spleen or plasma was injected subcutaneously or intraperitoneally. 2. In Exp. 25 0.05-0.1 ml of virus extract of leukemic tissue or plasma, prepared according to the method of Moloney(6) was inoculated along the same routes. In both experiments adult as well as new-born mice were injected. 3. In Exp. 22 attempts were made to transmit the virus through the placenta or with the milk from mice injected with subcellular leukemic material or from mice carrying whole cell transplants of the various above-mentioned leukemias.

The subcellular material was in all cases derived from (DBA/2 × CBA) F_1 mice, in which all our leukemias have developed(7); it was introduced into DBA/2 and (DBA/2 × CBA) F_1 mice; since our leukemias grow only in F_1 mice, those mice of Exp. 22 which were offspring of mice carrying whole cell transplanted leukemias were consequently F_2 mice (Table I). Recently, Balb/C mice were also included in the experiments.

The *results* were unexpected. They were the same irrespective of method of introduction of the leukemic material and of age of the mice at injection. Therefore they will be

* Supported in part by grant from The Danish State Research Foundation.