tween these two methods are being investigated.

The present findings emphasize the difference between normal lymphocytes, which are relatively rich in glycogen, and leukemic lymphocytes and lymphoblasts which contain little of this carbohydrate. Unfortunately, until recently this was the only practical source of large numbers of cells of pure yield. By adoption of the above described method satisfactory numbers of lymphocytes can be separated from the blood of normal individuals. Glycogen determinations on these cells reveal appreciably different results than those found in the above mentioned malignant conditions. These findings emphasize the danger of drawing conclusions concerning metabolic activity and chemical constitution of normal cells from observations of abnormal cells.

Summary and conclusion. Lymphocytes were isolated from the blood of normal individuals. Glycogen content of these cells was determined by the anthrone technic. A significant amount of this polysaccharide was found in these cells suggesting that normal lymphocytes contain more of this substance than the granulocyte. These findings are in contradiction to observations made on these cells by PAS method and assumptions based on chemical glycogen determinations of leukemic lymphocytes.

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Incorporation of Manganese into Duck Erythrocytes.* (26312)

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The appearance of intravenously-administered, labeled manganese in human erythrocytes follows a time-course like those for the hemoglobin precursors, iron and glycine. The administered manganese in erythrocytes, in both man and rabbit, is recoverable from the cells mainly in association with hemin, thus the provocative hypothesis has been advanced that the metal is present in the cells as a porphyrin complex(1). In the present work the erythrocytes of duck blood were found to take up labeled manganese reversibly *in vitro,* but no evidence for association of the metal with porphyrin was obtained. After x-irradiation of the animal, *in vitro* uptake decreased to a low level, which suggests that the uptake is a function mainly of reticulocytes. *In vivo,* intravenously-administered manganese appears to be taken up by erythrocytes during formation or maturation of the cells in bone marrow and also after the cells enter the circulation. The latter uptake likely reflects that observed *in vitro.* Some of the manganese entering the cells *in vivo,* presumably that taken up in marrow, was recovered in association with heme. This finding supports the hypothesis indicated above.

Experimental. Adult, male, Pekin ducks fed a mixture of commercial food pellets and corn were used. Blood was collected in heparin. The labeled manganese used, essentially carrier-free, divalent Mn^{54} in hydrochloric acid, was estimated by means of a well-type scintillation detector(2). The results and details of representative tests *in vitro* are given in Table I.

The packed-cell fraction of blood incubated with Mn^{54} contained an appreciable fraction of the metal, relatively little of which

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TABLE I. Uptake of Labeled Manganese by Duck Erythrocytes *In Vitro*. In each test, blood was mixed with .025 vol of a solution containing about 5×10^5 counts/min. of carrier-free, divalent Mn^{54} per ml of 0.05 N HCl. Except where indicated otherwise, the mixture was shaken at 37° under 5% CO_2 in O_2 for 2 hr and cells were collected and washed twice with 2.5 vol of 0.156 M NaCl in the cold. Cells and plasma plus washings were assayed for Mn^{54}. In Exp. 7, animal was exposed to 1000 r of X-rays (250 KVP, 1.9 mm Cu HVL, 85 r/min.) shortly after initial collection of blood.

Exp.	Fraction of Mn^{54} in cells	Treatment
1	.38	Cells not washed
	.35	Cells washed once with 4 vol of plasma at 25°
	.34	Cells washed twice with 4 vol of plasma at 25°
	.34	Cells washed twice with 4 vol of saline at 25°
2	.18	Buffy coat not removed
	.17	" " removed
3	.01	Cells collected immediately after mixing blood and Mn^{54}
	.16	Cells collected after incubating blood and Mn^{54}
4	.02	Blood plus Mn^{54} kept at 4°
	.16	" " " incubated
5	.15	Blood-Mn^{54} mixture incubated 2 hr
	.22	Blood-Mn^{54} mixture incubated 4 hr
6	.19	Cells collected after 2-hr incubation
	.11	Cells as above, but incubated 2 hr in unlabeled plasma
7	.13	Before irradiation of animal
	.16	20 hr post-irradiation
	.06	42 hr *idem*
	.03	64 hr "
	.02	164 hr "

was removed by washing the cells at room temperature with plasma or salt solution (cf Exp. 1). Removal of the buffy coat from packed, labeled cells after initial collection and after each of 2 washings with saline in the cold did not markedly decrease the amount of Mn^{54} associated with the cells (Exp. 2). Therefore, erythrocytes account for most of the manganese taken up by the blood cells. Cells collected immediately after mixing blood and manganese (Exp. 3) or from mixtures kept in the cold (Exp. 4) contained but a small fraction of the added metal. Thus, the uptake depends upon incubation and its duration (Exp. 5). Incubation of labeled cells with unlabeled plasma removed a large fraction of the Mn^{54} (Exp. 6). Consequently, the process involved in the uptake appears reversible. X-irradiation of the animal was followed by decrease in manganese uptake to a negligible level (Exp. 7); however, irradiation of blood *in vitro* had no effect on uptake. Iron uptake by the erythrocytes of duck blood *in vitro*, as the manganese uptake, decreases after irradiation of the animal, due to disappearance of reticulocytes from the blood(3). Thus, although other possibilities are not ruled out, it seems reasonable to ascribe the decrease in manganese uptake after irradiation of the animal to disappearance of reticulated erythrocytes from the blood.

Solutions of heme in acid ethyl acetate(4) obtained from mixtures of blood and Mn^{54} immediately and after incubation contained about the same fraction of the isotope (Table II, Exp. 1 and 2). Crystalline hemin preparations(5) obtained from cells of blood incubated with the isotope and from unlabeled cells mixed with isotope just before treatment for hemin isolation contained about the same fraction of isotope (Exp. 3 and 4). These results indicate no appreciable complex formation between cell porphyrin and manganese taken up *in vitro* and contrast with indications for such complex formation obtained in man and rabbit *in vivo*(1).

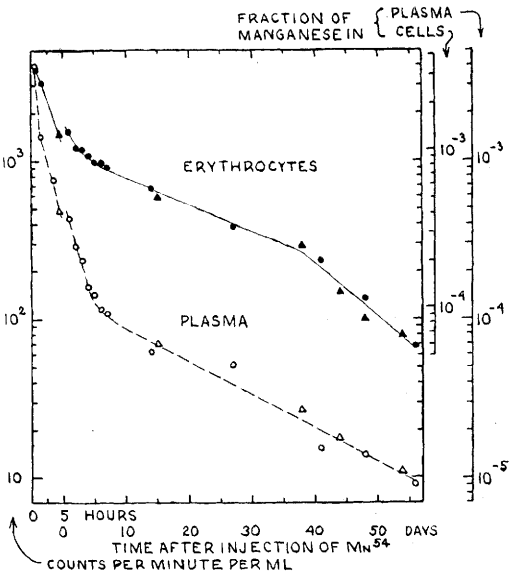
The distribution of intravenously administered Mn^{54} between erythrocytes, plasma, and excreta is illustrated in Fig. 1. The prompt appearance of the isotope in the cells and relatively rapid loss of isotope from the cells during the first few days of test period presumably reflects a reversible uptake by circulating erythrocytes like that observed *in vitro*. Since the lifetime of the erythrocytes is approximately 5.5 weeks (6-8), the marked loss of the manganese from the cells after about Day 40 appears referable to breakdown of cells labeled earlier. It seems likely that this early labeling occurred mainly in bone marrow rather in the circulation because the prompt initial uptake appears readily reversible and in man(1) incorporation of manganese into erythrocytes rather certainly

TABLE II. Mn^{54} in Heme and Hemin Preparations from Erythrocytes Labeled with Isotope *In Vitro* and *In Vivo*. *In vitro*-labeled erythrocytes were obtained by incubation of isotope with blood (Table I). *In vivo*-labeled cells were those of tests described in Fig. 1. Heme preparations (4) were made from whole blood, hemin preparations(5) from packed cells. Blood- or cell-isotope mixtures treated immediately for heme or hemin preparation respectively served as controls in *in vitro* tests.

Material from which heme or hemin was derived		Mn^{54} activity in or added to cells, counts/min./ml	Mn^{54} activity of: Cells / Blood	Heme / Cells	Hemin / Cells
Exp. 1. Blood.	Not incubated	1.2×10^4	.01	.015	
	Incubated	2.6×10^5	.21	.010	
" 2. "	Not incubated	2.0×10	.02	.013	
	Incubated	1.5×10^2	.15	.012	
" 3. Cells.	Not incubated	2.5×10^5			.002
	Incubated	3.1×10^5	.25		.003
" 4. "	Not incubated	1.8×10^2			.003
	Incubated	2.6×10^2	.18		.001
Animal 1 Blood, day 14		6.8×10^2	.92	.160	
" " 56		6.8×10	.88	.220	
Cells, " "		" × "	"		.045
Animal 2 Blood, " 0.2		4.5×10^3	.76	.020	
Cells, " "		" × "	"		.003
" " 15		1.8×10^3	.90		.029
" " 48		3.0×10^2			.058
Blood, " 54		2.4×10^2	.89	.150	

takes place during cell formation and/or maturation. Removal from the plasma was much faster, incorporation into cells much less, and early excretion was greater for the Mn^{54} than for labeled iron(6).

The following indicates that the state of some of the manganese that entered the cells *in vivo* differs from that obtaining *in vitro*: Hemolyzed cells(4) from blood obtained on Day 37 were dialyzed at room temperature against 250 volumes of stirred 0.156 *M* potassium chloride for 3.5 days, 0.005 *M* disodium ethylenediaminetetraacetate for one day, and 0.001 *M* manganese dichloride for one day. After dialysis the cell preparation contained 0.44 of the Mn^{54} originally present while hemolyzates from cells labeled *in vitro* and cells of Day 0.2 contained respectively 0.20 and 0.29 of the activity. The fractions of cell Mn^{54} found in heme or hemin preparations derived from cells collected after Day 0.2 were appreciably greater than found in the *in vitro* tests (Table II). The recovery of Day 0.2 was about the same as *in vitro*.



Results for this animal are indicated by circles. Animal 2, wt 3.10 kg, given 1.39×10^8 counts/min. /kg body wt. Results, indicated by triangles, have been adjusted to expectation for the dose of isotope given Animal 1. Initial distribution of Mn in plasma (87 ml blood/kg assumed, hematocrit of 0.46) or in extracellular fluid (250 ml/kg) corresponds theoretically to activities of 9.81×10^5 and 1.86×10^5 counts/min./ml respectively. Animal 1 excreted 0.12 of Mn by Day 1, 0.14 by Day 5, and less than 0.01 between Day 5 and 17. Curves were drawn by inspection.

FIG. 1. Uptake of Mn^{54} by duck erythrocytes *in vivo*. Animal 1 weighed 3.64 kg and was given 4.65×10^7 counts/min. of carrier-free, divalent Mn^{54} in 5 ml of .03 N HCl in .156 M NaCl/kg of body wt.

This indicates again that the early appearance of the isotope in the cells is attributable to uptake like that found *in vitro*. Although the fraction of the labeled manganese in the duck erythrocytes that was recovered in association with the iron-porphyrin complex is much smaller than found in man and rabbit(1), the results support the hypothesis that erythrocytes contain a manganese-porphyrin complex.

Summary. Duck erythrocytes *in vitro* take up labeled manganese added to blood. The uptake is mainly ascribable to reticulocytes. Following intravenous administration, the manganese appears promptly in the cells apparently as a function of the readily reversible uptake observed *in vitro* and as a function of incorporation in bone marrow. Manganese taken up by cells *in vivo*, but not *in vitro*, is recoverable to some extent in association with heme. This recovery is considered to support the hypothesis that erythrocyte manganese is present in part at least as a porphyrin complex.

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Isolation of a Thermolabile Serum Protein which Precipitates γ-Globulin Aggregates and Participates in Immune Hemolysis.* (26313)

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Recently, the authors(1) and Taranta(2) reported that normal human serum contains a heat-labile factor capable of precipitating soluble γ-globulin aggregates. Gamma-globulin aggregates have proved in the past a useful means for demonstration of rheumatoid factor, a high molecular weight, heat-stable serum protein possessing a strong affinity for γ-globulin and occurring predominantly in patients with rheumatoid arthritis (3). The difference in heat stability precluded the identity of the precipitating factor of normal serum with rheumatoid factor and strongly suggested its relation to the complement system. A relation to complement appeared particularly likely in view of the observations of several investigators(5,6, 7,8) indicating that complement is capable of reacting with γ-globulin aggregates and that it undergoes inactivation during this reaction.

The present paper describes the method of isolation and some characteristics of the precipitating factor of normal serum. It also reports the finding that preparations of purified factor contained an activity distinct from the activity of any known complement component and which proved essential for immune hemolysis.

Materials and methods. Soluble γ-globulin aggregates were prepared according to a method similar to that described earlier(3,4). Three grams of Lederle F II γ-globulin, dissolved in 300 ml saline were heated at 63°C for 12 min. The aggregates were precipitated from this solution by addition of 20 g sodium sulphate. After one hour at 4°C the precipitate was collected, suspended in 20 ml distilled water, and dialyzed against 2 × 3 liter barbital buffer, pH 7.3. The resulting solution of γ-globulin aggregates contained 25-30 mg protein per ml. More than two-thirds represented material with an average s-rate of 90-110 S.

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