activity than does GOT I and remains elevated for a considerably longer time after hepatic injury. Similar observations with regard to (total) GOT and GPT also have been made in patients with acute viral hepatitis or jaundice due to chlorpromazine(4-8), but a detailed study of the distribution of GOT I and GOT II in these conditions has not been made.

Another reason for the very low levels of serum GOT II in carbon tetrachloride poisoning may be sought in the recently discovered fact(9) that this enzyme is bound within the mitochondrion, in contrast to GOT I and GPT which appear to be present exclusively in the soluble part of the cell. While GOT II is readily released from its confinement inside the mitochondrion in the preparation of aqueous homogenates, we have noted that the enzyme is quite tightly bound in mitochondria isolated by standard procedure, and is then difficult to solubilize. It is conceivable that also under physiologic and pathologic conditions this enzyme may be released with some difficulty.

Summary. Serums of dogs poisoned with carbon tetrachloride exhibited high activities of GOT I and GOT II with maxima 48 hours after administration of the toxic agent. At no time, however, did GOT II contribute to total activity more than 16%. In control serums the presence of GOT II could not be established with certainty. Disappearance rates of intravenously administered purified preparations of transaminase also were studied in dogs. Activity of GOT II decreased very much faster than that of GOT I. This difference may explain the uneven distribution of the 2 serum transaminases in carbon tetrachloride poisoning.

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Glycogen Content of Normal Lymphocytes.* (26311)

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The glycogen content of the formed elements of the blood is said to reside in the leukocytes, red cells and platelets containing little or none of this polysaccharide(1). Furthermore, it has been assumed that the major portion of that present in the white blood cells is contained in the myeloid elements. These assumptions are based on histochemical examination of the blood from normal individuals and leukemic patients(2,3) and from chemical determinations on the leukocytes from patients with acute and chronic lymphocytic leukemia(4,5). Glycogen assays of the lymphocytes found in these states have revealed extremely low levels. It has therefore been deduced that the lymphocyte of the normal individual contains a meager amount of this polysaccharide. However, direct measurements of normal lymphocytes have not been possible because of the difficulty of separating these cells from the other formed elements of the blood except when they are present in elevated and ex-

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tremely pure numbers. Recently a method has been devised for isolating lymphocytes from the total white cell population of normal individuals thus making possible determination of their glycogen content(6). The results will be described below.

Material and methods. Venous blood was collected in heparinized tubes from normal adults with normal peripheral leukocyte counts and differentials. For obtaining all white cell elements whole blood was allowed to settle for 30 minutes in a freshly prepared saline solution of fibrinogen. The supernatant leukocyte-rich plasma was then withdrawn. The lymphocytes were obtained from whole blood by the method of Jago in a refrigerated centrifuge at 4°C, and total cell yield was determined by white cell count. The percentage purity was determined by differential counts done on 100 white cells from smears made from this lymphocyte rich plasma.

The leukocyte and lymphocyte-rich plasma was then centrifuged at 3000 rpm for 15 minutes and supernatant cell free plasma was decanted. The button was treated with 2 ml of boiling KOH for 30 minutes. Glycogen was precipitated by adding 2 cc of 95% ethanol to the KOH solution and allowed to stand for a minimum of 3 hours at 37°C.

After precipitation, the tubes were centrifuged at 3000 rpm for 15 minutes. The clear supernatant fluid was gently decanted from the packed glycogen and the tubes allowed to drain in an inverted position. The glycogen was dissolved in 2 ml of distilled water and was determined by the anthrone method using distilled water as a blank and a glucose standard in a Beckman DU Spectrophotometer at 620 mμ.

Results. The glycogen content of total white cells from the blood of 10 normal individuals was determined. Mean leukocyte glycogen per 10^{10} total leukocytes was 50.9 mg with a range of 41.2 to 56.6 (Table I). These results are in close agreement with those of Valentine, Follette and Lawrence (3) for mean total leukocyte glycogen using a similar technic.

Glycogen content of normal lymphocytes alone was determined. A minimum of 10^4 cells containing at least 90% lymphocytes was used for each test. The mean of 20 determinations on normal adults was 113.67 mg/10^{10} cells. In 5 instances the determinations were performed in duplicate. The difference between paired analysis expressed as percent of their average value did not exceed 2.75%. The complete results are shown in Table I.

TABLE I. Glycogen Content of Leukocytes.

	No. deter.	Mean mg per 10^{10} cells	Range	S.D.
Total leukocytes	10	50.9	41.2- 56.6	5.85
Total lymphocytes	20	113.67	65.0-189.8	29.79

To confirm that the reducing substance obtained after precipitation with alcohol was glycogen, it was treated with a 1:10 dilution of saliva in saline for one hour at 37°C at pH 6.5. An equivalent portion of alcohol precipitate was treated with saline for a similar period. Both samples were then boiled with one ml of KOH and reprecipitated with ethyl alcohol. The glycogen remaining was determined by the anthrone method. In 2 experiments it was found that approximately 80% of the carbohydrate was digested.

Discussion. It has been shown that a considerable amount of glycogen is present in normal human lymphocytes. We have determined that lymphocytes comprise 40% of the cellular population of "total cells" as obtained by the fibrinogen separation method. On the basis of this and the glycogen values mentioned above it is apparent that the lymphocyte contributes the major portion found in "total cell" value. This finding is in contradiction to the previous assumption of low lymphocyte glycogen content based primarily upon histochemical examination of blood smears after periodic acid-Schiff staining. The visual method reveals that all of the mature granulocytes contain PAS positive material in the cytoplasm whereas only a few of the lymphocytes present contain granules of glycogen. The chemical determination described above casts doubt on conclusions as to quantity of glycogen present in the lymphocytes determined by the PAS method. The reasons for the variances be-

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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