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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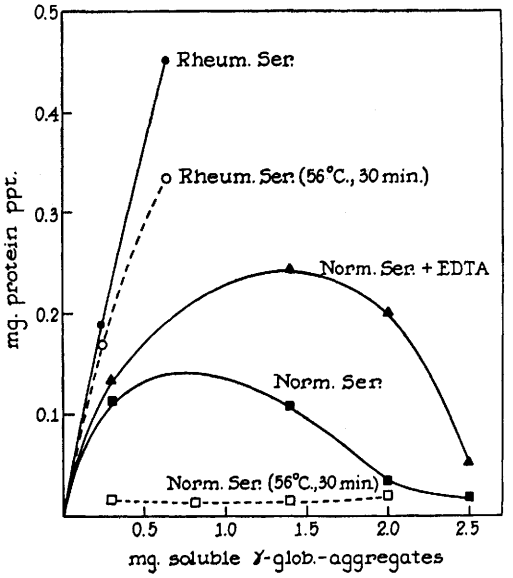

FIG. 1. Precipitin curves of normal human serum and of serum containing rheumatoid factor with soluble γ-globulin aggregates, showing the effect of heating and of EDTA upon precipitin reaction.

Preparation of 11 S component: 25-30 ml of a solution of γ-globulin aggregates were added to 200 ml fresh normal serum. Both parts contained 0.01 M Na_3EDTA. They were pre-cooled and after combination held at 4°C for 5 hours or overnight. The resulting precipitate was separated from the supernatant serum, and washed 3 times in 10 volumes phosphate buffer, pH 7, T/2 = 0.1. The washed precipitate was twice extracted in 6.5 ml cold phosphate buffer, pH 5.3, T/2 = 0.3. A thorough suspension was obtained by pipetting the precipitate for 5-10 minutes. The insoluble residue was then removed by centrifugation and the extracts filled into 6.5 ml lusteroid centrifuge tubes. After centrifugation at 114,000 × g in a Spinco rotor 40.3, for 40 min at 1°C, the supernatants of both extracts were combined and concentrated to 0.5 to one ml by ultrafiltration. The pH was adjusted to pH 7, using 0.2 M Na_2HPO_4.

Preparation of an R_{11S}: 3.5 ml of fresh serum containing 0.01 M Na_3EDTA were combined with 2.5 ml soluble γ-globulin aggregates containing 25-30 mg protein per ml and 0.01 M Na_3EDTA. One ml barbital buffer, pH 7.3, was also added. This mixture was held at 4°C overnight and centrifuged at 114,000 × g at 1°C for 1½ hours, using a Spinco rotor 40.3. The top 5 ml, which were recovered from the centrifuge tube, were diluted 1 → 2.5 to obtain a final dilution of 1 → 5 with respect to the original serum concentration, and were stored at 4°C for use within the subsequent 5 days. Ca^{++} and Mg^{++} were added to overcome the EDTA effect.

Quantitative precipitin reactions: To 0.2 ml portions of serum soluble γ-globulin aggregates were added in amounts ranging between 0.5 and 2.5 mg and final volume was adjusted to 0.4 ml with normal saline. After 15-20 hours at 4°C, amount of protein precipitated was determined by the Folin method.

Analytical ultracentrifugation was performed in a Spinco model E machine at 52,640 rpm at 20°C. For their analysis washed precipitates of serum, induced by soluble γ-globulin aggregates, were dissolved in glycine buffer, pH 3, T/2 = 0.1.

Results. Fig. 1 illustrates the precipitin reaction of normal human serum with soluble γ-globulin aggregates. For comparison the precipitin curve of a serum with rheumatoid factor activity was included. Although there was a striking quantitative difference between the reaction of rheumatoid serum and that of normal serum, the latter definitely precipitated soluble γ-globulin aggregates. While the activity of rheumatoid serum, as expected, was little diminished after heating at 56°C for 30 min, this treatment resulted in complete loss of activity of the normal serum. Depletion of Ca^{++} and Mg^{++} by EDTA on the other hand augmented the precipitation reaction of normal serum. More than 80 normal sera examined in this fashion were found to resemble each other, especially with respect to heat lability and augmentation of precipitating activity by EDTA. Regarding the extent of the reaction, considerable variation between different sera was encountered. Sera from children, especially umbilical cord serum, gave low precipitin curves. Three sera from agammaglobulinemia patients showed similarly low values, while some pathological sera, primar-

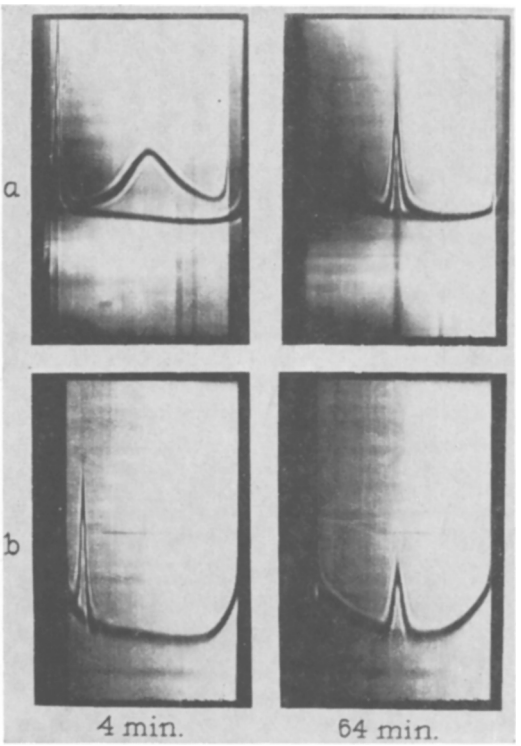

FIG. 2. Ultracentrifugal patterns (a) of a dissolved precipitate formed by normal human serum upon addition of soluble γ-globulin aggregates in presence of EDTA, (b) of the isolated precipitating factor (11 S component) of normal serum.

ily those from patients with hepatitis, exhibited a strikingly increased precipitating activity.

Precipitates of normal serum induced by soluble γ-globulin aggregates were dissolved in glycine buffer, pH 3, and analyzed in the ultracentrifuge. Invariably, 2 main components could be distinguished. One of these was heterogeneous and sedimented with an average s-rate of approximately 100 S, the other was homogeneous and sedimented much more slowly. The former represented γ-globulin aggregates and the latter an unknown constituent of serum. Variable amounts of 2 minor components with s-rates of 7 and 19 S were also seen. Fig. 2a depicts the ultracentrifugal pattern of a dissolved precipitate which was obtained from serum previously depleted of bivalent cations.

The main component derived from serum was extracted from precipitates with phosphate buffer, pH 5.3, ionic strength 0.3, at which conditions the aggregates remained largely insoluble. After additional purification which included density gradient ultracentrifugation, some highly homogeneous preparations were obtained consisting of more than 95% one component. The ultracentrifuge pattern of one preparation is shown in Fig. 2b. The $S^{\circ}_{20,w}$ of this material was 11.1 S, the electrophoretic mobility was similar to that of γ-globulin.

All of 15 different preparations of the 11 S component proved highly active in precipitin tests with soluble γ-globulin aggregates. The activity of the isolated 11 S component was, like that of whole serum, thermolabile and independent of bivalent cations. To investigate whether the 11 S component was essential for complement activity of human serum, a serum rendered deficient in this component (R_{11S}) was analyzed for hemolytic activity. The R_{11S} was virtually devoid of hemolytic activity while comparable amounts of the original serum afforded lysis up to nearly 100% (Fig. 3). When a preparation of 11 S was added to an R_{11S}, its activity was fully restored. The required amount was less than 1 μg which corresponded to approximately 0.1% of total serum protein present. The activity associated with preparations of 11 S could not be identified with any of the classical components of complement as such preparations failed to reconstitute serum lacking either the first, second,

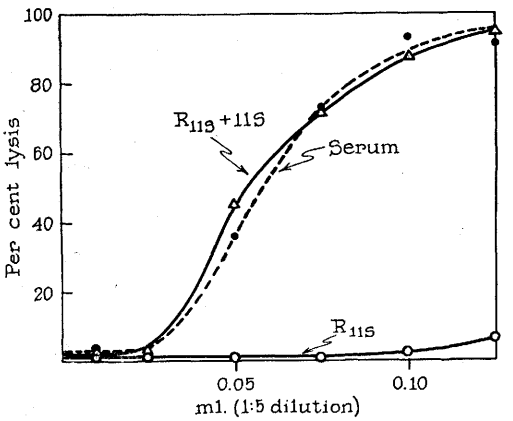

FIG. 3. Hemolysis curves demonstrating loss of hemolytic activity by depletion of a serum of 11 S component (R_{11S}) and regaining of full activity upon addition of a preparation of isolated 11 S.

third, or fourth component. Similarly, the lack of hemolytic activity of an R_{11S} could not be attributed to loss of any of the classical components, as none of them appeared to be significantly reduced. This included the first component, provided it was determined by an R_1 plus 11 S. In addition, preliminary evidence was obtained indicating that an intermediate complex, consisting of erythrocyte, antibody, and 11 S component can be formed and that this complex is capable of being lysed by an R_{11S} but not by other R's.

Discussion. Thermolability and independence of bivalent cations are intriguing features characterizing the activity associated with the 11 S component. The ability of this component to combine with γ-globulin aggregates in absence of bivalent cations suggests qualities more typical for an antibody than for complement. The similarity of its electrophoretic mobility with that of γ-globulin appears to lend further support to this view. However, the 11 S component is undoubtedly different from ordinary antibody protein. The sedimentation coefficient clearly distinguishes it from known types of antibody which have been shown to belong to the 7S and 19S class of serum proteins. Moreover, the 11 S component is markedly thermolabile while antibodies in general, with the possible exception of reagins, are resistant to heating at 56°C. Thus, it appears highly improbable that this protein represents an antibody, although this possibility cannot be ruled out at present.

The demonstrated participation of the 11 S component in immune hemolysis strongly suggests that it constitutes a component of complement. The fact that it is able to combine directly with γ-globulin, without the aid of another factor, and that it is absent from serum rendered deficient in the first component of complement(1,2), would relate the 11 S component to the first component. However, while fixation of hemolytic complement to immune aggregates requires Ca^{++} as has been shown by Levine *et al.* (9,10), combination of the 11 S component with γ-globulin aggregates does not. This, as well as the complete failure of preparations of 11 S component to restore hemolytic activity of an R_1 and the undiminished presence of C'_1 in an R_{11S} precluded identity with the first component.

The information at hand suggests that the interaction of the 11 S component with γ-globulin aggregates and with sensitized sheep cells precedes the action of the classical components. This concept is primarily supported by observations showing that interaction between this heat-labile component and soluble γ-globulin aggregates proceeds in the absence of Ca^{++}. It seems probable that, after the 11 S component has combined with aggregates, other factors of the complement system become able to interact with it, provided Ca^{++} and Mg^{++} are present. Certain combining sites of the 11 S component might thus be blocked by complement, preventing combination with other aggregates. In precipitation tests with aggregates, the amount of precipitate would therefore be expected to be considerably smaller in presence of Ca^{++} and Mg^{++} than in their absence. As pointed out above, this was actually observed. Further evidence will be needed to correct or to confirm the proposed concept according to which the 11 S component is part of human complement preceding in the reactions of complement the classical first component.

Summary. A protein of normal human serum capable of precipitating soluble aggregates of γ-globulin was isolated and found to be a euglobulin with an $S^{\circ}_{20,w} = 11$ S and the approximate electrophoretic mobility of γ-globulin. The activity of this protein proved thermolabile and independent of Ca^{++} and Mg^{++}. Serum rendered deficient in 11 S component showed drastically reduced hemolytic activity despite virtually unchanged titers for the classical components of complement, and regained full hemolytic activity upon addition of microgram amounts of preparations of isolated 11 S. The possible significance of this component for the early steps in complement reactions was discussed.

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Studies on the Metabolism of 5-Hydroxytryptamine (Serotonin). I. Effect of Starvation and Thiamine Deficiency. (26314)

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In spite of the significant number of important papers related to the metabolism of 5-hydroxytryptamine (5-HT) the effect of certain stresses notably those of starvation, dietary deficiencies and of obesity, are scarcely explored. An exception is the studies on the effect of Vit. B_6-deficiency which were shown to lead to reduced levels of 5-HT in the spleen(1), possibly through impairment of 5-hydroxytryptophan decarboxylase(2), and B_2-deficiency with concomitant reduction of monoamine oxidase (MAO) activity of the liver(3). The observed metabolic disturbances which appear during starvation(4) and thiamine deficiency (5,6) were considered to evoke possible changes in metabolism of 5-HT, a substance involved in functional control of the central nervous system. This paper derives from a research program concerning the influence of dietary stresses on certain aspects of 5-HT metabolism.

Methods and materials. Sprague-Dawley male rats were divided into 3 groups. The first 2 groups were kept on thiamine sufficient and deficient diet respectively for 4½ weeks as described elsewhere(7). The third group of rats was maintained on a daily ration of 2 g of Purina standard rat chow for the first 3 days, then starved for 4 days with only 1 ml of soluble vitamin mixture ("Berocca-C", Hoffmann-LaRoche, Inc.) and water given *ad libitum*. The animals were mechanically stunned and, after withdrawal of 1 ml of cardiac blood with a heparinized needle, killed by decapitation. Blood and tissues were immediately worked up for 5-HT content according to the method of Bogdanski *et al.*(8). In the experiments aimed at determining (MAO) activity the tissues were removed immediately and, with the excess blood blotted, chilled and worked up to isolate the mitochondrial fraction by the method of Schneider *et al.*(9). Mitochondrial fraction of the spleen was obtained by pooling samples from several animals. Measurement of MAO activity was carried out manometrically(10). The main compartment of the vessels contained 0.3 ml of 0.5 M phosphate buffer pH 7.3, 1 ml of mitochondrial suspension in 0.25 M sucrose, 0.1 ml of 3×10^{-2} M KCN solution and water to bring the volume to 3 ml, while the side arm had 0.4 ml of 0.05 M 5-HT solution adjusted to pH 7.4. The inner well contained filter paper, 0.2 ml of a 1:1 mixture of 20% KOH and 1 M KCN. Each experimental flask had a control in which the side arm had 0.4 ml of water instead of 5-HT. This served to correct for any oxidation in absence of substrate. The gas phase was O_2 and temperature 37°C. After 10 minutes of oxygenation and equilibration the contents of the side arms were tipped in. At the end of each experiment the content of main compartment was withdrawn for pH determination (average varied between pH 7.5 to 7.8). Enzyme activity is expressed as $Qo_2(N)$ and was calculated from the linear period of oxidation. Mitochondrial nitrogen was determined by micro-Kjeldahl method.

Results. Effect of starvation and thiamine