Inhibition of Complement by Heterologous Hemoglobin. (27540)

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Complement fixation tests have been used for quantitative estimates of the concentration of antibody. This technic has been a prominent feature in certain investigations of the role of autoimmune mechanisms in disease(1,2,3,4,5). During some of our studies of autoimmune responses, the data suggested the possibility that specific complement fixation may be partially inhibited by adventitious, nonspecific proteins. Other investigators have noted a similar inhibition by gamma globulin in the absence of specifically induced immune response to these proteins (6,7,8,9,10). The purpose of this paper is to report the inhibitory effect of hemoglobin on complement. Hemoglobin was selected for this study for 3 reasons: (1) it can be easily obtained in a relatively pure state; (2) it can be easily measured, and (3) heterologous hemoglobin plays a prominent role as an indicator of available complement in complement fixation tests.

Materials and methods. Human and sheep red cells were freed of plasma proteins by repeated washings in saline. Hemolysis was produced by adding a volume of distilled water to an equal volume of packed cells. After shaking this mixture for a few minutes, the cell remnants and unhemolyzed cells were removed by centrifugation. A stock hemoglobin solution was then prepared by diluting one part of the clear red supernatant hemoglobin solution in 10 parts of physiological saline. Serial dilutions of hemoglobin solution were added to 0.15, 0.2, 0.25, 0.3, 0.35 ml of 1:30 diluted human or guinea pig serum which served as a source of complement (Fig. 1, A, B, C). The mixture was incubated at 37°C for 30 minutes. 0.5 ml of hemolysin and 0.5 ml of 2% sensitized sheep red cells were added as in the technic of complement titration in the quantitative Komer test(11) and incubated 1 hour.

In a second set of experiments, human gamma globulin solutions obtained from commercially prepared high titer immune gamma globulin were substituted for hemoglobin solutions as the interfering protein (Fig. 1, D).

The final hemoglobin concentrations of these mixtures were then measured in a Coleman Junior Spectrophotometer at 540 mμ. By subtracting the hemoglobin concentration of the control mixture, containing no complement, from the final hemoglobin concentrations, it was possible to measure the effect of hemoglobin on hemolytic activity of complement, hemolysin and sensitized red cells.

Results. Fig. 1 summarizes the effects of human hemoglobin, sheep hemoglobin and pooled human globulins on hemolytic activity. Each point on the graphs represents the hemolysis produced by the average of the 5 determinations of complement plotted against increasing titers of interfering proteins, *i.e.*, hemoglobin or gamma globulin. In each set of conditions, the heterologous protein inhibited or fixed complement. Increasing hemolysin concentration to 1 ml produced no increase of hemolytic activity. This excludes the possibility that hemolysin was inhibited. If the added protein did not inhibit the activity of the complement, these graphs (Fig. 1) would be horizontal lines.

Hemoglobin derived from the same blood sample as complement did not inhibit complement. There were no significant alterations in number of sensitized sheep red cells lysed. Thirty determinations using varying amounts of homologous hemoglobin with 3 levels of complement concentration revealed no inhibition of complement activity. The ranges of complement concentration and amounts of added hemoglobin were the same as those which revealed inhibition by heterologous hemoglobin. The mean hemolytic activity of the mixtures with homologous hemoglobin was 1.3×10^5 red blood cells. Standard deviation was 0.026×10^5 cells. Therefore, it can be stated that the probability of successive points in the graph falling by chance below the horizontal as observed in Fig. 1, is less than 0.01. The true shape of

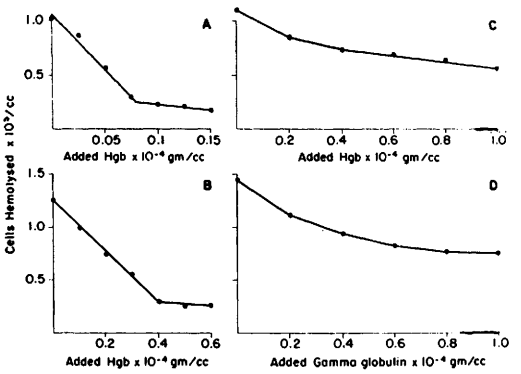

FIG. 1. Complement, hemolysin, and red cells are constant; the resultant hemolysis is plotted against concentrations of interfering protein. A. Complement source: Pooled human serum. Interfering protein: Sheep hemoglobin. B. Complement source: Guinea pig serum. Interfering protein: Sheep hemoglobin. C. Complement source: Pooled human serum. Interfering protein: Pooled human hemoglobin. D. Complement source: Pooled human serum. Interfering protein: Human gamma globulin.

the curves cannot be accurately defined from the data presently available.

The inhibition of complement by gamma globulin agrees with previous reports(7,8,9).

Summary and conclusions. Measurements of relative hemolytic activity of varying mixtures of complement, red cells, and hemolysin with added heterologous protein reveal inhibition or apparent partial complement fixation in the absence of known previous immunization. The added protein in these studies is either hemoglobin or human gamma globulin. This inhibition is absent when the hemoglobin and complement are derived from the same specimen of blood. These data reveal an important possible source of error when quantitative estimates of antibody formation are made using complement fixation tests.

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In vitro Effect of Mammalian Adrenocorticotropin on Secretion of Skate (*Raja erinacea*) Interrenal Tissue.* (27541)

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Although adrenocorticosteroids have been identified in plasma of lower vertebrates(1), the steroidogenic action of adrencorticotropin (ACTH) has not been investigated directly to any extent. *In vitro* stimulation of adrenocortical secretion by mammalian ACTH has been shown for the American bullfrog (*Rana catesbeiana*)(2,3) and suggested for the White Leghorn Cockerel (*Gallus domesticus*)(4). The steroidogenic action of ACTH has also been demonstrated *in vivo* in several avian species(5,6). The present report suggests an *in vitro* steroidogenic effect of mammalian ACTH on skate interrenal tissue. As a by-product, data were also obtained demonstrating differences in skate interrenal

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