Changes in α-Macroglobulin During Malaria in the Duckling. (31543)

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The plasma proteins commonly associated with the response of a vertebrate host to malaria are the haptoglebins(1) (proteins which bind the hemoglobin released from red blood cells injured by the malarial parasite), and the immunoglobulins(2) or antibodies (formed in response to antigens introduced by the parasite). Reported here is the effect of malarial infection in the duckling on the concentration of another serum protein identified as a high molecular weight α-globulin.

Changes in plasma proteins. Doses of *Plasmodium lophurae** were injected into the jugular vein of 900-1100 g Pekin White ducklings. Heparinized plasma samples, obtained each day during the infection, were frozen immediately and finally, on the death of the animal, examined as a group by vertical gel electrophoresis(3). The number of parasitized red cells, counted each day, varied from individual to individual. Death usually occurred on the 6th day. The marked depletion of a slowly moving protein component, provisionally designated Component I, was observed in 7 of the 9 cases examined. No changes in Component I were observed in control ducklings during these experiments. The extent of decrease in relative concentration of Component I varied from duckling to duckling as did the time after infection when the decrease was first noticeable (compare duckling 1, Fig. 1 and duckling 2, Fig. 2). The general trend of depletion is progressive,

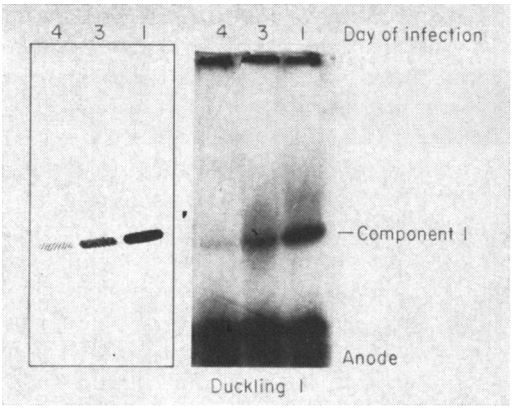

FIG. 1. Starch gel electrophoresis pattern showing major decreases in a component of duck plasma during malarial infections.

however, with the advance of the disease in an individual duckling.

In the advanced disease a "new component," migrating more slowly than Component I, appeared concurrently with the decrease in Component I in 3 of the 9 cases (Fig. 2, duckling No. 2). The origin of the new electrophoretic component is not known: it may possibly be derived from Component I by degradation or by the binding of some other macromolecule which could hinder its migration through the gel.

Characterization of Component I. Duck plasma was fractionated by starch block electrophoresis(4) (Fig. 3) and Component I identified as an α-globulin by starch gel electrophoresis of the fraction (Fig. 2). After centrifugation of pooled protein fractions (extracted from the starch block) through 10-40% sucrose density gradients(5) (Fig. 4), Component I was located by gel electrophoresis (Fig. 2) in the 40% sucrose region of the gradient, where human 19S and 20S plasma components are known to equilibrate. By comparison with a standard of human 7S γ-globulin, Component I has a molecular weight far greater than 160,000 and may therefore be considered an α-macroglobulin.

* Infected blood was drawn from ducks with 80-90% parasitized red cells and was mixed with 1/10 volume of heparin (Connaught, 5000 units/ml in 0.90% NaCl). The size of the inoculum in milliliters was calculated for each duck from the formula:

$$\frac{\text{Body weight of duck to be inoculated (g)}}{1000}$$

The strain of *P. lophurae* used in these experiments is maintained in the laboratory of Wm. Trager at the Rockefeller University as described in J. Exp. Med., 1958, v108, 753.

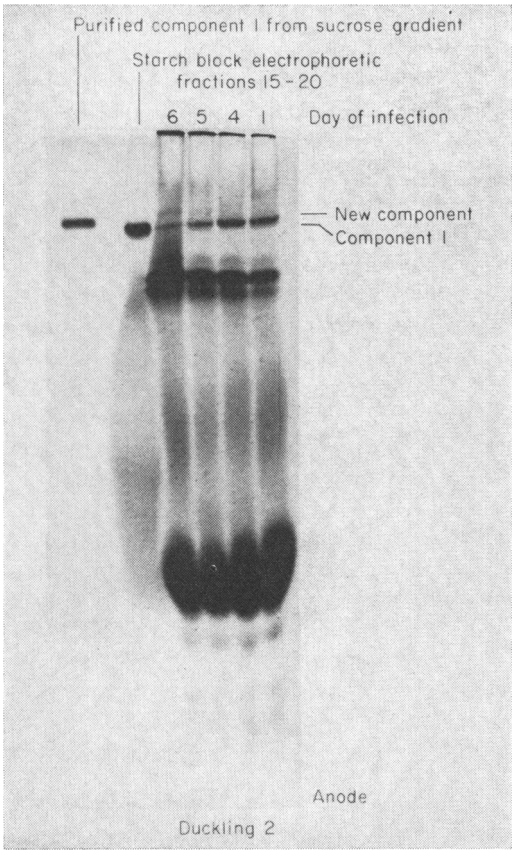

FIG. 2. Starch gel electrophoresis pattern demonstrating a further case of the depletion of Component I during malaria, the occurrence of a new plasma component during malaria, and the purification and isolation of Component I.

The slower electrophoretic migration of Component I in starch gels *vs* starch blocks is most likely caused by the retardation of this large molecule by the matrix of the gel.

Duck haptoglobins and hemolysis. To identify the hemoglobin binding proteins, excess duck hemoglobin(6) was added to normal duck plasma before gel electrophoresis; the gel was subsequently stained with a benzidine-peroxide reagent(7). Peroxidase activity, indicative of bound hemoglobin, was associated with albumin and with a component presumed to be duck haptoglobin which migrated in advance of Component I. Therefore Component I may be distinguished from haptoglobin, an α-globulin known to decrease in plasma during the mammalian response to malaria(1).

The hemolysis which accompanies the malarial infection was shown to be insufficient in causing the decrease in Component I by itself. Five ml of 2% aqueous phenylhydrazine hydrochloride was injected into 3 ducklings to mimic the hemolytic anaemia caused by malarial infections. Another group of 3 was simultaneously infected with parasites. Although both groups developed extensive hemolysis after 3 days, there was no decrease of Component I in animals treated with phenylhydrazine as judged by gel electrophoresis patterns.

Survey of other vertebrates. Other vertebrates were infected with their specific malarial parasites. Heparinized plasma samples were obtained from the same individuals before and during infection, frozen immediately, and examined as a group by gel electrophoresis.

The relative concentration of the corresponding Component I in the different electrophoretic patterns did not change during *P. berghei* infections in rats and mice and during *P. lophurae* infections in chickens.

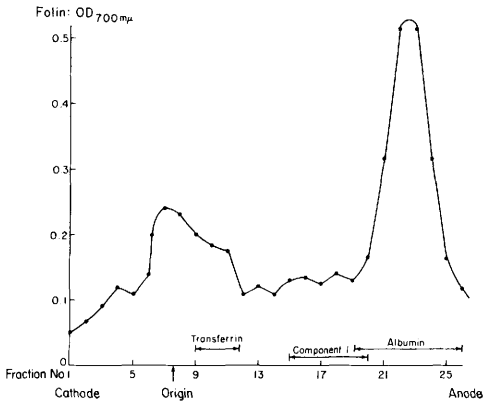

FIG 3. Electrophoretic fractionation of duck plasma. Samples of heparinized plasma from a 24 day old duckling were dialyzed overnight against 0.1 M sodium barbital buffer at pH 8.6. Electrophoretic fractions were prepared by starch block electrophoresis(4) in the same buffer, and their protein content estimated by the Lowry method.(13)

On examination by starch gel electrophoresis, the relative electrophoretic mobilities of certain easily identified duck plasma proteins appeared to correspond to their mammalian homologues. The transferrins, identified by Fe^{59} labeling, migrated in the β-zone, albumin migrated in the most rapid group, and Component I migrated immediately behind albumin in the α-zone (Fig. 2).

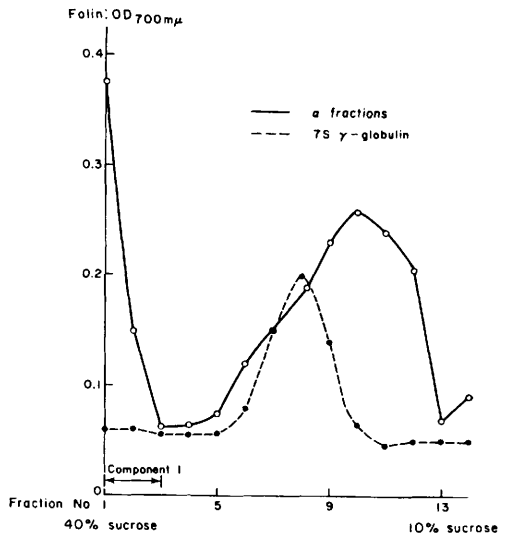

FIG. 4. Sucrose density gradient identification of Component I.

Aliquots (0.25 ml) of the pooled starch block fractions 15-20, inclusive, were layered over 4.20 ml of a continuous 40-10% sucrose gradient in 0.14 M saline.(5) A molecular weight standard of a 1% solution of 7S human γ-globulin was layered over an identical gradient. After 18 hours of centrifugation in the Spinco 39 SW head at 35,000 rpm at 5°C, fractions were collected and their protein contents estimated by the Lowry method.(13) The 7S γ-globulins were located in the middle of the gradient. Component I, identified by gel electrophoresis, was found only in the first 3 fractions (40% sucrose) (Fig. 2).

The lack of change in Component I of the chicken corroborates the paper electrophoresis studies of Sherman(8) which indicated no statistically significant alterations of α-globulins during infections with *P. lophurae*. However, the corresponding component of human plasma, known to be an α_2-macroglobulin, did undergo changes in relative concentration during malarial infections. A decrease in α_2-macroglobulin occurred in the case of one 25-day *P. vivax* infection; no changes in this protein occurred in the case of 2 other 21- and 27-day *P. vivax* infections; and an increase of this protein was observed in the case of a 27-day *P. cynomolgi* infection.† These fluctuations in human α_2-macroglobulin were smaller than those generally observed in duck α-macroglobulin.‡

Discussion. A plasma protein, characterized as an α-macroglobulin, is shown to be decreased strikingly during malarial infection in the duckling. However, preliminary screening of the plasma of rats, mice, chickens, and humans by the same starch gel electrophoresis method did not reveal any consistent or progressive concentration changes of the corresponding electrophoretic components during malarial infections. The failure to observe such changes may be due to the difficulty, inherent in the gel electrophoresis method, of perceiving small quantitative changes (quantitative immunologic methods employing specific antibodies are more suitable). Alternatively, sharp changes in concentration could have occurred during stages of the infections not represented in the sampling.

The major high molecular weight (19S-20S) plasma proteins of mammals are γ_1-macroglobulin, an immunoglobulin, and α_2-macroglobulin, a protein whose function is currently unknown. The information about avian serum proteins is insufficient to identify duck α-macroglobulin as a chemical homologue of either of the major mammalian macroglobulins. Nonetheless, the fact that duck α-macroglobulin migrates among the most acidic duck plasma proteins during free electrophoresis (on starch block) suggests that duck α-macroglobulin is more closely related to human α_2-macroglobulin than to the immunoglobulins which characteristically have an isoelectric point near to pH 7.0.

Observations of the levels of human α_2-macroglobulin during various physiologic and

† The generous donation by Dr. John Tobie, Nat. Inst. Health, Bethesda, Md., of the human serum samples is much appreciated.

‡ Recently published studies by Coatney and coworkers(2) have demonstrated major (50%) decreases in α_2-globulins after the onset of patent parasitemia in human malaria. These workers estimated from paper electrophoresis profiles that the decrease in the α_2-globulins amounts to 4-5 mg/ml plasma. If the haptoglobins, which are α_2-globulins present up to 1.5 mg/ml, were to disappear from circulation, an additional 2.5-3.5 mg/ml of protein would remain unaccounted for according to these calculations. Thus α_2-macroglobulin, present in 1.4-2.1 mg/ml[12] may well be among those α_2-globulins diminished during malaria in humans.

pathologic states are rare in the literature. Notable are the reports that a_2-macroglobulin levels are elevated in newly born humans through the first year(9) and in sufferers from rheumatoid arthritis and ankylosing spondylitis(10). There is also a report that a_2-macroglobulin levels are depressed in maternal plasmas toward the end of pregnancy(11). The most thorough study to date by Brown and associates(12) demonstrates major increases in human a_2-macroglobulin during nephrosis. Consequently, it has been suggested that a_2-macroglobulin and other large plasma proteins may aid in maintaining blood osmotic pressure in pathologies accompanied by depletion of smaller plasma proteins.

This report is the first to document the diminution of a vertebrate a-macroglobulin during a specific pathology. The progressive decreases of a-macroglobulin found during malaria in the duckling suggest that a-macroblubulin levels may be subject to influences, yet undescribed, which are not directly related to osmotic regulation. The possibility thus occurs that a-macroglobulins may be primary components of the defensive reaction of the vertebrate host to infection.

Summary. A plasma protein, identified as a high molecular weight a-globulin is shown to decrease strikingly during malarial infection in the duckling.

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Staphylococcal Infection in Normal and Splenectomized Monkeys.* (31544)

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Previous studies from this laboratory have demonstrated similar responses in normal and splenectomized monkeys following streptococcal(1) and pneumococcal(2) infections. Despite the great number of studies in experimental staphylococcal infections(3,4), little information on the reaction of monkeys to this organism is available. Verlinde and Makstenieckс(5) observed mild clinical reaction in monkeys following either intranasal or intratracheal inoculation with *Staphylococcus aureus;* slight bronchiolitis and limited foci of bronchopneumonia were observed. It is the purpose of this report to compare the results following aerosol and intravenous

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