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Agglutination of Erythrocytes *in vivo* in Mice after Injection of *Ascaris* Extracts, Related to Immunization with Human Erythrocytes. (24792)

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In previous studies(1,2), it was found that extracts from the isolated tissues of *Ascaris lumbricoides*, such as cuticle, muscle, intestine, eggs, sperm, and coelomic fluid, possess certain immunologic properties briefly summarized as follows: 1. When such extracts are added in specific amounts to human serums of Groups O and B, α_2 isoagglutinin titer is reduced to negative. 2. When injected intravenously into dogs and guinea pigs that contain α_2 isoagglutinins in their plasma, when tested against human erythrocytes of subgroup A_2 , *in vivo* agglutination of erythrocytes occurs, leading to formation of large clumps of erythrocytes, seen chiefly in the liver. When dogs are utilized, there occur severe reactions suggestive of anaphylaxis and followed by death. 3. When injected intravenously into normal mice with no α_2 titers in their plasma, agglutination of erythrocytes was not observed in liver and kidney, but agglutination takes place when animals develop α_2 titers by a previous artificial immunization with human erythrocytes of subgroup A_2 . It has been postulated that upon injection of ascaris tissue extract, the substance with A_2 isoagglutininogen-like property, adheres to the erythrocytes. Agglutination is then accomplished by union of α_2 antibody in animal's plasma with erythrocytes which had the A_2 -like antigen induced by the substance present in the extract. Further studies were therefore necessary to determine

the specificity of the reaction; that is, whether by using the erythrocyte-tissue extract combination agglutination of erythrocytes would be also observed in mice immunized against human erythrocytes of subgroup A_1 , and Groups B and O, as seen in those immunized against erythrocytes of subgroup A_2 . In addition, the pathological reactions in the liver and other organs of all animals needed more detailed consideration. Mice of A strain, of both sexes, and weighing 20 to 30 g, were injected intraperitoneally with 1% suspension of human erythrocytes of Groups B and O, and subgroups A_1 and A_2 , in physiological saline. Each animal received injections of 1 cc of the particular cell suspension for 3 consecutive days, followed by another series of 3 injections administered after interval of 4 days. Ten to 15 days after last injection, the mice were injected into tail vein, with 1 mg of ascaris tissue extract suspended in 0.5 cc of saline. The extract prepared from the cuticle was the only one utilized in this study, since previous experiments showed that extracts from other ascaris tissues elicited the same reactions(2). Mice were killed 24 hours following injection. Normal mice, as well as mice injected with A_2 cells alone, and with cuticular extract alone, were utilized as controls. Organs of all animals were fixed in Zenker-formol and stained with hematoxylin and eosin after paraffin embedding.

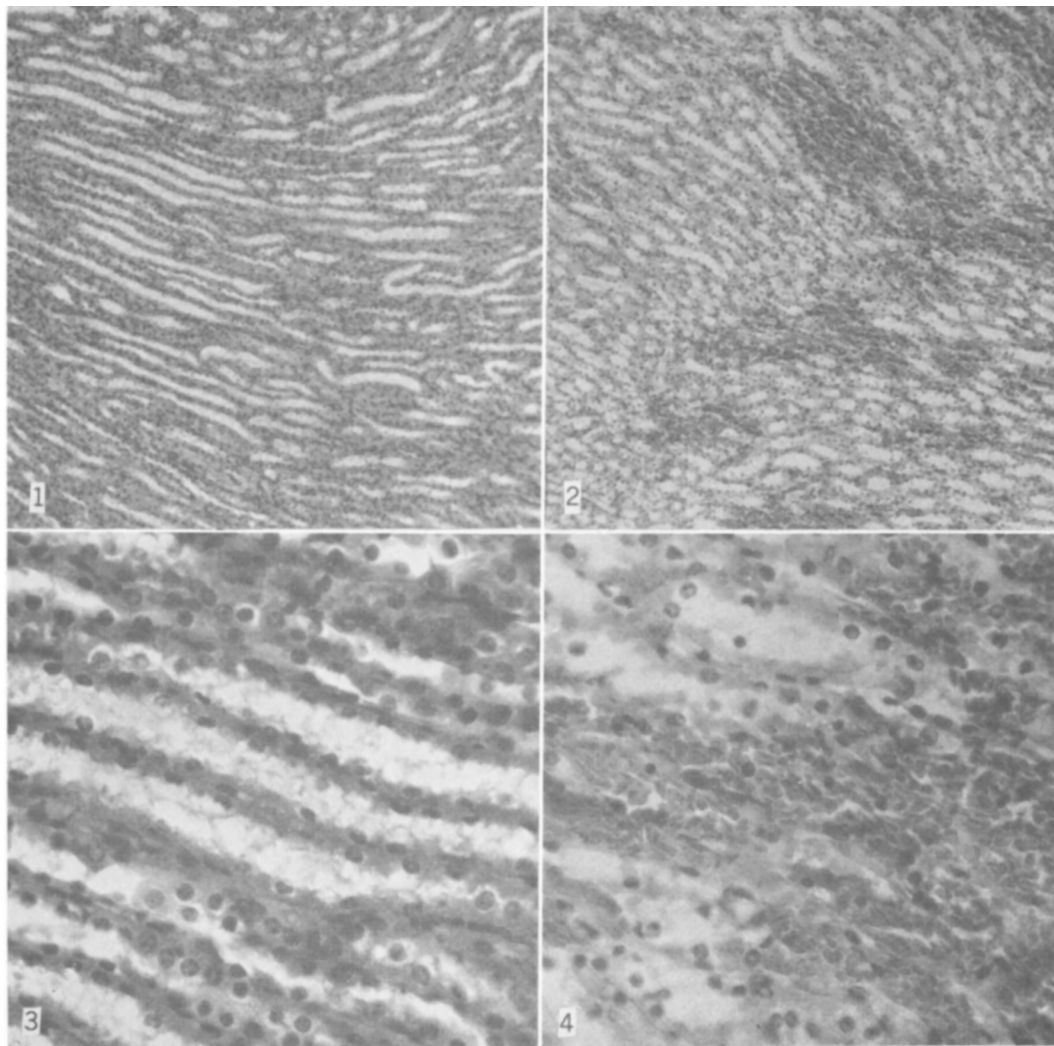


FIG. 1. Normal kidney. 100 $\times$.

FIG. 2. Intense congestion in experimental kidney. 100 $\times$.

FIG. 3. Normal kidney. 430 $\times$.

FIG. 4. Intense congestion and agglutination of erythrocytes in experimental kidney. 430 $\times$.

Results. Mice immunized against human erythrocytes of subgroup A₂, and then injected intravenously with the extract from ascaris cuticle, were the only animals that showed marked changes in liver and kidneys. The liver presented intense congestion of the sinusoids and marked agglutination of erythrocytes. In kidneys, capillary congestion in the medulla was very intense and diffuse, with compression of adjacent tubules (Figs. 1 to 4) also with agglutination of erythrocytes.

Livers and kidneys of mice immunized

against human erythrocytes of Groups B and O, and subgroup A₁ and then injected with the cuticular extract, did not show congestion of liver or kidneys. Similarly normal mice injected with A₂ erythrocytes, and with cuticular extract alone, did not show those changes.

Discussion. From above studies, it is apparent that agglutination of erythrocytes in liver and kidneys takes place chiefly when animals possessing α_2 agglutinins in blood are injected with ascaris tissue extract. It did not occur in animals with α_1 , and β and anti-

O isoagglutinins produced by immunization with respective homologous agglutinogens. This indicates that the antigen-antibody reaction may be a specific reaction between the a_2 antibody in animal's plasma and an A_2 isoagglutinin-like antigen present in tissue extract.

The A_2 isoagglutinin-like factor is also present in metabolic products of animal parasites and bacteria(1). The reaction between a_2 isoantibody and the A_2 isoagglutinin-like factor in infectious agents may also occur *in vivo* in humans of Groups O and B. This could well be related to the cause of certain diseases in which agglutination and hemolysis of red blood cells occurs regularly. Although the findings in mice indicate that agglutination occurs only in those that have a_2 antibody, in humans this reaction may not be so group-specific, or perhaps it may involve another agglutinin-agglutinin complex similar to the well known $a_2 - A_2$ one.

The relative ease with which congestion of liver and kidney, with large masses of agglutinated erythrocytes, is elicited, as shown above in mice, suggests that this experimental animal may be of use in studies in connection with infection and autoagglutinins in relation to acquired hemolytic anemias.

Summary. Mice of an A strain were injected intraperitoneally with suspensions of human erythrocytes of Groups B and O, and

of subgroups A_1 and A_2 . Ten to 15 days after last injection of erythrocytes the animals and normal controls were injected intravenously with 1 mg of an extract prepared from cuticle of *Ascaris lumbricoides*. The purpose of immunization with erythrocytes was to determine whether the a_1 , a_2 , β , and anti-O agglutinins, developing as a result of artificial immunization, would react *in vivo* with cuticular extract. Histopathological examination of livers and kidneys disclosed: 1. Agglutination of erythrocytes with formation of large clumps in liver and kidneys was seen only in animals immunized against A_2 erythrocytes followed by the cuticular extract. This did not occur in mice inoculated with erythrocytes of Groups B and O and subgroup A_1 , also injected with the cuticular extract. No such changes were seen in liver and kidneys of normal mice inoculated with A_2 erythrocytes or cuticular extract alone. Agglutination takes place, therefore, between a_2 agglutinins in plasma and the A_2 isoagglutinin-like factor in the extract. These findings indicate a definite relationship between blood groups and pathological changes produced by infectious organisms.

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Effect of Ribonuclease on Survival of Ascites Tumor Bearing Mice.* (24793)

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Klein(1) has demonstrated that ascites tumors treated with trypanflavine show definite retardation of growth coincident with drop in nucleic acid concentration. Ledoux confirmed nucleolytic action of ribonuclease when employed against both ascites(2) and solid tumors(3). Brachet(4,5,6) reported that RNase produces a strong inhibition of growth in plant

cells and a significant reduction in incorporation of labeled amino acids into proteins of growing onion root tips. He suggested that RNase, upon entering living onion root cell, inhibits protein synthesis. Roth(7) concurred in this concept and observed that since RNA synthesis and degradation are probably closely related to protein synthesis, the RNase system may possibly become an important controlling factor in growth and cell division.

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