used. These results indicate that the reactions attending the use of citrate must be sought by other criteria. They may be associated with the disturbance of equilibrium causing the precipitate above mentioned.

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The antigenic properties of red-cell globulin.[1]

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Three theories have recently been advanced relative to the nature of the antigen which, on repeated injection of foreign red cells, gives rise to a specific hemolytic sensitizer in the blood stream of the immunized animal. Balls and Korns[2] have come to the conclusion that the antigen is contained in the stroma of the red cell and that it is neither a globulin nor an albumin but probably a nucleoprotein. While the presence of nucleic-acid residues in non-nucleated red cells cannot be denied, the careful work of Bloor[3] indicates that within the limits of experimental error of his method the presence of nucleoprotein in red cells appears doubtful. The experiments of Wooldridge[4] on the constituents of the stroma of red cells shows that although a protein combined with a molecule containing phosphorus (this may be lecithin) is present in small quantities, the greater part of the protein fraction consists of paraglobulin.

While it is not to be denied that immunization with stroma does lead to the appearance of a hemolytic sensitizer, the experi-

[1] Aided in part by a grant from the Research Board of the University.

[2] Balls, A. K., and Korns, J. R., *Jour. of Immunology*, 1918, iii, 375.

[3] Bloor, W. R., *Jour. Biol. Chem.*, 1918, xxxvi, 49.

[4] Wooldridge, L., *Arch. f. Anat. u. Physiol.* (Physiol. Abt.), 1881, p. 387.

ments of Ford and Halsey[1] as well as those of Bennett and Schmidt[2] nevertheless indicate that it is also possible to obtain a hemolytic sensitizer by immunization with the water-soluble portion of red cells. Moreover, the findings of Balls and Korns that the filtrate obtained by passing a solution of hemolyzed red cells through a porcelain filter does not bind hemolytic sensitizer do not appear to us as conclusive evidence that the antigen is contained wholly in the stroma. It is a well-known fact that the first portion of the filtrate obtained on passing a solution of proteins such as serum through a porcelain filter invariably shows a loss of protein and that the latter portions of the filtrate are relatively richer in protein than the first. The experiments of Muir[3] with the water-soluble portion of red cells clearly indicate that the first portion of the filtrate is unable to bind sensitizer while the latter portions can unite with increasing amounts.

Vedder[4] dissolved the stroma obtained from human red cells in dilute alkali, neutralized the solution with acetic acid and filtered off the protein precipitate. Rabbits were immunized with both this fraction and the protein contained in the filtrate. The latter, which Vedder believes to be an albumin, gave rise to the hemolytic sensitizer for human cells while no antibodies were obtained by immunization with the acetic acid precipitate.

Bennett and Schmidt[2] carried out experiments with the CO_2-globulin isolated from a solution of the constituents of ox red cells after removal of stroma by centrifuging and filtration. Rabbits were immunized with this protein and a specific sensitizer and an agglutinin for the homologous red cells were obtained. Since the experiments were carried out with the red cells of only one species and since the experiments of Vedder indicate that possibly in the red cells of other species a protein other than the CO_2-globulin may be the antigen concerned in the production of hemolysis, it appeared to us desirable to carry out experiments with the globulins obtained from the red cells of several other species.

Antigens were prepared from the red cells of the sheep, the pig and man in accordance with the method described by Bennett

[1] Ford, W. W., and Halsey, J. T., *Jour. of Medical Research*, 1904, xi, 403.

[2] Bennett, C. B., and Schmidt, C. L. A., *Jour. of Immunology*, 1919, iv, 29.

[3] Muir, R., "Studies on Immunity," London, 1909, p. 129.

[4] Vedder, E. B., *Jour. of Immunology*, 1919, iv, 141.

and Schmidt. Rabbits were immunized by intraperitoneal injections of these respective antigens at definite intervals of time and tests for the presence of hemolytic sensitizer and of agglutinin in the serum of the injected animals were carried out as in the experiment with the globulin from ox cells. The data follows:

Rabbit No. 44 was given a total of 75 c.c. of a suspension of CO_2-globulin prepared from sheep cells. This corresponds to 35 c.c. of whole sheep blood and contained approximately 20 mgs. of nitrogen. The limit of the hemolytic titer was found to be 0.4 c.c. of 1 : 6,000. The serum showed marked agglutinative properties.

Rabbit No. 45 received the same amount of sheep-cell globulin as No. 44. The limit of the hemolytic titer was 0.2 c.c. of 1 : 6,000 and the sheep cells were markedly agglutinated by this serum.

Rabbit No. 40 was given a total dosage of 175 c.c. of CO_2-globulin prepared from pig cells, an amount which corresponds to 80 c.c. of whole blood. Its content of nitrogen was 60 mgs. The limit of the hemolytic titer was found to be 0.1 c.c. of 1 : 250 and this was also the limit of agglutination.

Rabbit No. 42 received the same amount of pig-cell globulin as No. 40. The limit of the hemolytic titer was found to be 0.1 c.c. of 1 : 250 and the highest dilution of serum which agglutinated the pig cells was 0.3 c.c. of 1 : 1,250.

Rabbit No. 36 was given a total dosage of 210 c.c. of human red-cell globulin. This corresponds to 50 c.c. of whole blood and contained 23 mgs. of nitrogen. The hemolytic titer of the serum was found to be 0.2 c.c of 1 : 50 and a suspension of red cells were agglutinated in a serum dilution of 1 : 250.

Rabbit No. 37 received the same dosage of CO_2-globulin from human red cells as animal No. 36. The hemolytic titer of the serum was found to be 0.1 c.c. of 1 : 10 and the highest dilution of serum which agglutinated red cells was 0.1 c.c. of 1 : 1,250.

Rabbit No. 38 received the same dosage of human CO_2-globulin as the two previous animals. The hemolytic titer was found to be 0.4 c.c. of 1 : 50 and the limit of agglutination was 0.1 c.c. of 1 : 1,250. It was not found possible to raise the

titer of the last three sera. However it is frequently found that only comparatively low titer sera are obtained when rabbits are immunized with human red cells.

These experiments definitely indicate that immunization with the CO_2-globulin prepared from the water-soluble portion of the red cells of the sheep, the pig and man leads to the appearance, in the blood stream of the treated animal, of a specific hemolytic sensitizer and of an agglutinin. It appears possible, although our experiments are incomplete on this subject, that the globulin from the water-soluble portion of the red cell is closely related to one of the proteins contained in the stroma, since by immunization with either of these antigens, the same antibody is obtained.

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The separation of the hexone bases from a protein hydrolysate by electrolysis.[1]

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In a previous communication describing a method for the preparation of glutamic acid, the need of developing cheaper methods for the production of amino acids in quantity was pointed out. This is especially true with respect to the amino acids arginin, histidin and lysin. The cost of reagents and the labor required for the preparation of these amino acids prohibits experimental work in which large quantities of these substances are required.

Some years ago Ikeda and Suzuki[2] described a method for separating certain fractions of the products of protein hydrolysis. Their method has apparently not come into general use and experimental data are not available. On passing direct current through a solution of the protein cleavage products, which is placed in the center of a three-compartment cell, the amino acids are separated into three fractions consisting of (*a*) the amino

[1] Aided by a grant from the Research Board of the University.

[2] Ikeda, K., and Suzuki, S., U. S. Patent No. 1015891, Jan. 30, 1912.