16787

Nature of the Heterogenetic Hapten Reacting with Hemagglutinins in Horse Serum Sickness.

J. TOMCSIK AND H. SCHWARZWEISS.

From the Hygienic Institute, University of Basel.

An increase in the sheep's blood hemagglutinins in horse serum sickness has been described by both Hanganutziu and Deicher. Paul and Bunnel have described a similar phenomenon in infectious mononucleosis. It has subsequently been reported in numerous publications that the hemagglutinins in serum sickness and in infectious mononucleosis correspond to two different heterophilic antibodies (*e.g.*, Davidsohn *et al.*,[1-3] Stuart *et al.*[4-6] Schiff[7] even referred to a serum sickness antibody and gave the name serum sickness antigen (S.S.A.) to the receptor which reacts with the hemagglutinin in serum sickness. For the sake of simplicity, this expression will be retained in the present publication, although it should be noted that the part played by this antigen in the genesis of serum sickness has been in no way confirmed.

According to our conception, it is justifiable to include in the group of heterogenetic antigens, in addition to the Forssman antigen, both S.S.A. and the erythrocyte-antigen reacting with the antibodies in infectious mononucleosis (M.A.) as separate antigens. However, this conception does not appear to be a general one. For example, Boyd[8] writes: "The Forssman antigen is probably not a definite chemical entity, but a serological conception, a collective term covering substances which, injected into rabbits produce sheep hemolysins. The original Forssman antigen was a concept somewhat more narrow." In contrast to this conception, we consider it more correct to retain the original precise definition of the Forssman antigen and to recognize the other heterogenetic antigens mentioned as different substances. However, this conception presupposes a mosaic-like structure of certain cell antigens, the individual antigens being separable from one another even when they are components of a large molecule. The purpose of this work was to separate the S.S.A. from the other heterogenetic antigens described above, even when they occur in the same cell, and to elucidate its nature.

The technics for carrying out the hemagglutination and the inhibition reactions were described in previous papers.[9,10] When the nature of the hemagglutination is not more precisely defined, sheep's blood agglutination is to be understood. By degree of activity, we understand the reciprocal value of the highest dilution of antigen, calculated in terms of dry substance, which is capable of completely suppressing the hemagglutinating power of two antigen units.

For the purpose of selecting a human serum to serve in the subsequent course of the work as a serological indicator for the study of the S.S.A., 26 human sera were examined. The shortest interval of time between injection of the horse serum and removal of a sample of blood was 7 days. In 16 cases, the serum was obtained from more or less severe cases of serum sickness with exanthema. In 17 cases, the agglutination titer was more than

1 Davidsohn, J., *J. Immunol.*, 1929, **16**, 259.

2 Davidsohn, J., *J. Immunol.*, 1930, **18**, 31.

3 Davidsohn, J., *J. Infect. Dis.*, 1933, **53**, 219.

4 Stuart, C. A., Tallman, J., and Brintzenhoff, E., *J. Immunol.*, 1935, **28**, 85.

5 Stuart, C. A., Griffin, A. M., Wheeler, K. M., and Battey, S., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 212.

6 Stuart, C. A., Fulton, Mc. D., Ash, R. P., and Gregory, K. K., *J. Infect. Dis.*, 1936, **59**, 65.

7 Schiff, F., *J. Immunol.*, 1937, **33**, 305.

8 Boyd, W. C., Fundamentals of Immunology, Interscience Publishers, 1947, 2nd ed., p. 141.

9 Tomesik, J., and Schwarzweiss, H., *Schweiz. Z. Path. und Bakt.*, 1947, **10**, 407.

10 Schwarzweiss, H., and Tomesik, J., PROC. SOC. EXP. BIOL. AND MED., accompanying paper.

TABLE I.
Inhibition of Sheep Agglutinins in Serum Sickness, Infectious Mononucleosis, and Forssman Serum by Various Agents.

Antigens	Inhibition titer of the antigens toward hemagglutinins in		
	Serum sickness	Inf. mononucl.	Forssman serum
Sheep erythrocyte stroma	6,000	24,000	32,000
Beef " "	64,000	64,000	0
Horse " "	4,000	8,000	0
" "	120*	320*	0
" serum	4*	0	0
Rabbit erythrocyte stroma	250	0	0
" "	500*	0	0
" " hemolyzed	trace	0	0
" spleen and kidney	16,000	250	0
" liver	2,000	250	0
" muscle	2,000	0	0
Guinea pig kidney	4,000	0	1,200
Alcoholic extr. of g. pig kidney	4,000	0	16,000
Several peptones	0	0	0

0 = no inhibition in a dilution 1 : 62.5, calculated in terms of dry weight of stroma or of various organs, or in 10% suspension of erythrocytes.
* = the dilutions were calculated exceptionally in volumes and not in weight of the dry substance.

1:48, *i.e.*, 0.1 cc of the serum diluted 1:16 still completely agglutinated 0.2 cc of a 0.75% suspension of sheep red blood corpuscles. The type of hemagglutinin was determined by examining all the sera with various dilutions of the following antigens: 1. Sheep blood stroma. 2. Cattle blood stroma. 3. Guinea pig kidney. 4. Rabbit erythrocytes. The hemagglutinins were considered to be characteristic for horse serum sickness when they were adsorbed by all 4 antigens. Of 9 adult sera, which showed a high agglutinin titer, 7 sera were found to be typical for horse serum sickness on the basis of their adsorption, while of 8 sera obtained from children only 2 were found to be typical for horse serum sickness. The serum HD 85, which was selected as serological indicator for the subsequent work, agglutinated sheep red blood corpuscles at a dilution of 1:240, cattle blood corpuscles at 1:120, and hemolysed both types of blood corpuscles at a dilution of 1:120. The serological results summarized in Table I are the average values of several determinations.

In assessing the results shown in Table I it is to be noted that the difference between a negative and a positive agglutination was very easy to determine in the case of the infectious mononucleosis and the Forssman sera, whereas the transition from a negative to a positive reaction after adsorption of the serum sickness serum took place stepwise, with the result that the titer of the antigen with the latter serum exhibited fluctuations of more than 50% in different readings.

As can be seen from Table I, the beef stroma exhibited the highest serological activity of all antigens examined, both with mononucleosis and with serum sickness serum. Since boiling the aqueous stroma suspension is not detrimental to the serological activity, it may be concluded that the B.T. (beef thermostable substance) described by Stuart, Griffin, Wheeler, and Battey[5] contains both M.A. and S.S.A. On the other hand, as already known, guinea pig kidney contains the Forssman antigen and S.S.A. Thus, if our conception described above is correct, it should be possible to isolate S.S.A. both from beef stroma and from guinea pig kidney. Since we were able, as previously reported,[10] to isolate the heterogenic mononucleosis antigen in a purified form from B.T. by fractional extraction with cold and boiling organic solvents, we also employed the same procedure for the isolation of S.S.A.

Table II shows that we were able by means of 100% boiling alcohol to extract S.S.A. from beef stroma with a considerable increase in its activity. Since, as shown in a previous study,[10] a considerable increase in the M.A.

TABLE II.

Inhibition of Sheep Agglutinins in Infectious Mononucleosis and Serum Sickness Serum by Beef Stroma Extracted First with Acetone and Ethyl Alcohol at Room Temperature and Later with Boiling Ethyl Alcohol.

Extracted with	Fraction	Inhibition titer of the extracts toward hemagglutinins in: Inf. mononucl.	Serum sickness
Acetone, room temperature	0- 4 days	0	0
Alcohol, " " 100%	0- 4 "	125	16,000
Alcohol, boiling, 99.9%	0- 4 hours	16,000	192,000
" " "	4- 8 "	48,000	256,000
" " "	8-12 "	48,000	256,000
Alcohol, boiling, 80%	0- 4 hours	1,100,000	6,000
" " "	4- 8 "	500,000	48,000
" " "	8-12 "	256,000	32,000

activity can be detected only in the fractions obtained with 80% boiling ethyl alcohol, it was possible to separate the two heterogenetic antigens from B.T. by means of a simple procedure based on their difference in solubility. After repeated application of this procedure, two fractions were obtained from beef stroma which exhibited the following activities:

Fraction A: S.S.A. activity 512,000, M.A. activity 8,000.

Fraction B: S.S.A. activity 16,000, M.A. activity 1,000,000.

Preparation of pure S.S.A. from rabbit stroma was not attempted as its activity was too small. It is all the more difficult to give an explanation of the small serological activity of this stroma in comparison with the intact or boiled rabbit erythrocyte suspension since the hemolysed erythrocytes were completely inactive; thus, the active substance of the stroma could not be detected outside the stroma.

The next question was whether the S.S.A. could be separated from the Forssman antigen of guinea pig kidney. 10 g dried and finely powdered guinea pig kidney were extracted twice, for 9 hr each time, with 250 cc 97% alcohol in a Soxhlet apparatus. Afterwards the residue was further extracted for varying lengths of time, up to 3 hours, with boiling 97% alcohol. Both antigens were detectable in the cold alcoholic extract, but when the already thoroughly extracted residue was further extracted with boiling alcohol, only S.S.A. with an activity of 1:1,000 could be detected. In contrast to beef stroma, it was thus possible to isolate the S.S.A. from guinea pig kidney, but without elevation of the serological activity.

After these investigations of its heterogenetic occurrence, S.S.A. was finally investigated in the original antigen, *i.e.*, in horse serum. 750 cc horse serum with a S.S.A. activity of 1:2 were coagulated at 100° and the coagulated mass of albumin separated from the liquid portion on a Buchner filter. Both fractions were dried in a vacuum drying oven. The suspension of coagulated albumin was still inactive against serum HD 85 in a dilution of 1:50, while the dried residual substance obtained from the filtrate showed an activity of 1:1,200. By fractional extraction with alcohol, however, no increase in activity could be achieved. Both the cold and the hot alcoholic extracts had a serological activity of about 1:1,500.

Summary. 1. From beef stroma pretreated at room temperature with acetone and alcohol, a fraction was isolated with boiling 100% alcohol which, in a dilution of 1:500,000, combines with the sheep blood agglutinin of human serum produced during serum sickness. Using the terminology of Schiff, this fraction corresponds to the heterogenetic serum sickness antigen; it could be separated to a large extent from the heterogenetic mononucleosis antigen which also occurs in beef stroma.

2. The so-called serum sickness antigen also

occurs heterogenetically in guinea pig kidney and it could also be isolated from the Forrsman antigen although without increase in activity.

3. Horse serum is a poorer source for isolation of the so-called serum sickness antigen. The latter could be isolated from the serum, after removal of the albumin bodies by coagulation with heat, with an activity of 1:1,500.

16788 P

Dimethyl Ether of *d*-Tubocurarine Iodide as an Adjunct to Anesthesia.

V. K. STOELTING, J. P. GRAF, AND Z. VIEIRA. (Introduced by K. K. Chen.)

From the Department of Anesthesiology, Indiana University School of Medicine, Indianapolis.

The capacity of curare to block efferent nervous impulses at the myoneural junction has long been known. Bennett[1] administered curare clinically to minimize the trauma to patients undergoing convulsive shock therapy. Subsequently, Griffith and Johnson,[2] and Cullen[3] reported the use of curare as an adjuvant for relaxing the abdominal muscles during inhalation anesthesia.

The dosage of *d*-tubocurarine chloride administered to patients during anesthesia rarely produces toxic cerebral or cardiovascular manifestations. The drug frequently produces moderate or severe respiratory depression. For this reason, other natural and synthetic compounds have been investigated. One of the more promising preparations is dimethyl ether of *d*-tubocurarine iodide.[4,5] This compound has a chemical formula $C_{40}H_{48}O_6N_2I_2 \cdot 3H_2O$. It will be referred to as M-curare. In animal experiments, Chen and Swanson[6] demonstrated that M-curare effectively decreases muscle tone without producing respiratory depression.

Experimental. We have administered M-curare to anesthetized patients of both sexes, ranging in age from 10 to 84 years of age. The degree of relaxation reported was based on the evaluation of the surgeon.

TABLE I.

Anesthesia	No. of patients	Range of initial dosage in mg		
		Min.	Max.	Avg
Cyclopropane	16	1	4	2
Ether	49	1	5	2.5
Nitrous oxide	35	1	6.5	3

The drug was dissolved in a distilled water and the curare adjusted to .5 mg per cc.* A preservative was added. The pH varied from 4.0 to 5.0. The solutions were stored at room temperature and used over a period of several weeks. Different lots of the drug were administered by the intravenous route. The initial dose consisted of 1 mg or more of the drug.

Data presentede in the table show the amount of M-curare required to produce adequate relaxation. The dosage recorded was given in one or more injections within a period of 10 minutes. Adequate relaxation was not noted in any patients who received less than 1 mg of M-curare.

It will be noted that an average of 2 mg of M-curare produced satisfactory relaxation in 16 patients receiving cyclopropane anesthesia. An average of 2.25 mg was required to produce adequate relaxation with ether

1 Bennett, A. E., *Am. J. Med. Science*, 1941, **202**, 101.

2 Griffith, H. R., and Johnson, G. E., *Anesthesiology*, 1942, **3**, 418.

3 Cullen, S. C., *Surgery*, 1943, **14**, 261.

4 King, H., *J. Chem. Soc.*, London, 1935, (pt. 2), 1381-1389.

5 Collier, H. O., and Paris, S. K., *Nature*, 1948, **161**, 817.

6 Chen, K. K., and Swanson, E. E., personal communication of unpublished data.

* Supplied by Eli Lilly Research Laboratories, Indianapolis, Ind.