chemistry, Interscience Publishers, N. Y., 1949.
15. Brunswick, O., *Z. Wiss. Mikr.*, 1922, v39, 316.
16. Deane, H. W., Morse, A., *Anat. Rec.*, 1948, v100, 127.
17. Lison, L., *Histochimie et Cytochimie Animals*, Gauthier-Villars, Paris, 1953.
18. Gomori, G., *Microscopic Histochemistry*, Univ. of Chicago Press, 1952.
19. Pearse, A. G. E., *Histochemistry: Theoretical and Applied*, Little, Brown and Co., Boston, 1954.
20. ———, *Ciba Foundation Colloquia on Endocrinology*, 1952, v4, 1.

Received April 9, 1958. P.S.E.B.M., 1958, v98.

Anaphylaxis in the Mouse Produced with Soluble Complexes of Antigen and Antibody.* (24106)

SEIKICHI TOKUDA AND RUSSELL S. WEISER (Introduced by E. J. Ordal)
Department of Microbiology, University of Washington School of Medicine, Seattle

The concept recently expressed by Germuth(1) and Germuth and McKinnon(2) that lesions of hypersensitivity diseases may result from local accumulation of soluble complexes of antigen (Ag) and antibody (Ab) derived from the circulating blood is a highly significant contribution toward an understanding of the mechanisms of immunologic tissue injury. The proposition that soluble Ag-Ab complexes initiate development of allergic lesions is indirectly supported by many earlier observations, principally concerned with temporal relationships of development of lesions to blood clearance of Ag and appearance of circulating Ab. Production of anaphylaxis in the guinea pig with soluble complexes of Ag and Ab(2) provides strong support to this proposition. Of the observations important to development of the concept were those of Hawn and Janeway(3) and Germuth, Pace and Tippett(4) that acute lesions of "serum sickness" in rabbits develop while Ag is still present in circulating blood, and heal only after free circulating Ab appears, and those of Talmage, Dixon, Bukantz and Dammin(5) that a rapid "immune phase" of Ag elimination by Ab always precedes appearance of free Ab in the circulation. These findings are consistent with the report of von Pirquet and Schick(6) that clinical symptoms of serum sickness in humans commonly developed a week or more before appearance of any precipitating Ab in blood, and often subsided before Ab disappeared. Proportions of various types of antibodies present in soluble complexes of Ag and Ab reported to occur in actively sensitized rabbit following large doses of bovine serum albumin (BSA)(2,5) are not known. Therefore, it is not possible to predict the respective roles that precipitating and non-precipitating Ab may play in development of allergic reactions through the agency of their soluble complexes with Ag. However, recent observations that soluble complexes of BSA and anti-BSA rabbit Ab, presumed to be composed largely of complexes of precipitating Ab and Ag, produce anaphylaxis in guinea pigs(2) and in mice(7) indicate that precipitating Ab is important in pathogenesis of allergic reactions by this mechanism. The observation of Kabat and Benacerraf(8) that non-precipitating Ab is equally as effective as precipitating Ab for producing passive anaphylaxis in the guinea pig favors the prediction that complexes of non-precipitating Ab and Ag would likewise produce anaphylaxis. That non-precipitating Ab can cause serum sickness in humans appears to have been well established by Karelitz(9). The concept that a non-precipitating Ab may be important in diseases such as rheumatic fever has been tested by Weiser (unpublished), Kuhns and McCarty(10), Quinn(11) and Rejholec(12). Rejholec (12) observed that rheumatic fever subjects show a greater capacity to form incomplete Ab against brucella vaccine than do non-rheumatic subjects.

Materials and methods. Unless otherwise

* This investigation supported in part by Washington State Heart Assn.

TABLE I. Anaphylaxis in Mouse Produced with Soluble BSA Anti-BSA Complexes Formed in Various Regions of Ag Excess.

Challenge preparation	Vol of challenge dose, ml	Ab N in challenge dose, mg	Deaths*	% mortality	Symptoms of survivors† Severe	Mild
Complex 8x Ag excess	.4	.462	28/34	82	6	
Idem 8x	.75	"	27/31	87	4	
" 20x	.4	"	17/21	81	4	
" 40x	.4	"	15/21	72	6	
" 60x	.4	"	12/30	40	14	4
" 180x	.75	"	5/32	16	20	7
" 180x (converted from 8x Ag excess)	.75	"	0/ 6	0	5	1
Ag 200x 5 min. before challenge with complex 8x Ag excess	.4	"	6/ 6	100		
Controls						
Complex 8x Ag excess + normal rabbit serum containing 44 mg protein	.4	.462	25/27	93	2	
Ag 200x	.75		0/ 7	0	no shock	
Anti-BSA serum	.75	1.30	0/20	0	*Idem*	
Ag 8x + normal rabbit serum	.4		0/ 8	0	"	
Supernatant fluid from zone of equivalence	.6		0/12	0	"	
BGG (2.5 mg) in anti-BSA serum	.4	.462	0/ 8	0	"	
Anti-BSA serum + EL (75 mg)	.5	"	0/12	0	"	

* Denominator represents total No. of animals; numerator, the No. that died of shock. Death occurred 15 to 35 min.

† Severe and mild shock.

Ag = antigen; Ab = antibody; BSA = bovine serum albumin; Ab N = antibody nitrogen; EL = egg white lysozyme. An equivalent dose of Ag = 0.0424 mg AgN.

stated, materials and methods were similar to those employed in previous investigations(7). Soluble complexes of BSA† and anti-BSA were produced in various regions of Ag excess ranging from values of 8 "equivalent doses" of Ag to 180 equivalent doses of Ag. An equivalent dose of Ag is that amount of Ag present in a precipitate at equivalence for any given amount of Ab. The antisera were heat-inactivated at 56°C for 30 minutes immediately before use. Each preparation was made by adding heat-inactivated anti-BSA rabbit serum to a solution of BSA in physiological saline buffered at pH 7.0 with M/100 Sorenson's phosphate buffer. This phosphate buffered saline (PBS) was used as a diluent. The mixtures were incubated at 37°C for 20 minutes and centrifuged to remove any small amount of particulate matter present. In certain experiments soluble Ag-Ab complexes were prepared as described by adding concentrated solutions of Ag to precipitates formed in the zone of equivalence. Bovine gamma globulin (BGG)† and crystalline egg white lysozyme (EL)† were used in certain control preparations. The various challenge and control preparations were injected into the tail vein. Mice were distributed uniformly among various groups with respect to age, weight and sex. They were from 6 to 10 weeks old and ranged in weight from 23 to 30 g.

Results. In the first experiment groups of mice were challenged with soluble BSA anti-BSA complexes formed in various regions of

† Lots of bovine gamma globulin and crystalline egg white lysozyme were obtained from Armour and Co., Chicago, Ill. as was crystalline bovine serum albumin.

TABLE II. Anaphylaxis in Mouse Produced with Soluble BSA Anti-BSA Complexes Derived from BSA Anti-BSA Precipitates.

Challenge preparation	Vol of challenge dose, ml	Ab N in challenge dose, mg	Deaths	% mortality	Symptoms of survivors (severe)
Complex dissolved in 20x Ag excess	.6	.462	9/18	50	9
Idem, 180x	.6	.462	12/18	67	6

Ag excess. The results are presented in Table I. All control preparations devoid of Ag-Ab complexes were completely innocuous. The complexes formed in the regions of 60x and 180x Ag excess produced a significantly lower mortality than was observed with complexes formed in the regions of 8x, 20x and 40x Ag excess. Although there was a general trend of increasingly lower mortality over the range from 20x Ag excess to 180x Ag excess, the first significant reduction occurred at the level of 60x Ag excess. Despite the fact that differences in extent of shock observed among survivors may be of questionable significance it is notable that the only survivors which showed mild shock were in the groups that received the complexes formed in the regions of 60x and 180x Ag excess. It was observed that the anaphylactigenicity of the complex which was initially allowed to form in 8x Ag excess and then converted to 180x Ag excess by addition of Ag was similar to that observed for the complex initially formed in 180x Ag excess. This suggests that the large soluble complexes initially formed in regions of low Ag excess are readily reversible to simpler complexes in regions of extreme Ag excess. As expected, the intravenous injection of 200 equivalent doses of Ag 5 minutes prior to challenge with the complex formed in 8x Ag excess afforded no protection against shock.

Since it seemed possible that the nature of the soluble Ag-Ab complexes initially formed in the region of Ag excess might differ from soluble Ag-Ab complexes derived from precipitates formed in the equivalence zone, an experiment was next conducted with soluble complexes prepared by solubilizing such precipitates with excess Ag. An equivalent dose of BSA was added to anti-BSA rabbit serum, mixed, incubated for 1 hour at 37°C and the precipitate washed 3 times in PBS at 4°C. The precipitate was dissolved by agitation and incubation for 1 hour at 45°C in a concentrated solution of Ag containing 20 equivalent doses of Ag. The minute amount of precipitate remaining was removed by centrifugation and to one-half of the supernatant was added a concentrated solution of Ag to give a final solution containing 180 equivalent doses of Ag. An equal volume of PBS was then added to the 20x Ag solution and both solutions of the complexes were incubated at 45°C for 1 hour. They were stored in the refrigerator for 3 hours and warmed to 37°C immediately before injection into mice.

The data presented in Table II show that the complexes in both mixtures were essentially equivalent in their anaphylactigenicity. These results were in marked contrast to those of the first experiment in which it was observed that the complex formed in the region of 180x Ag excess and the complex converted to 180x Ag excess from 8x Ag excess were both markedly less anaphylactigenic than the complex formed in the region of 8x Ag excess.

Since it was thought that the use of unheated sera or physiological saline instead of PBS might alter the results, experiments were conducted using these materials. The results presented in Table III indicate that the anaphylactigenicity of the complexes was not lessened by the use of heated sera and physiological saline. They do not exclude the possibility that complexes formed in unheated sera are more anaphylactigenic than complexes formed in heated sera.

Discussion. Reasons to account for variations in the anaphylactigenicity of the various complexes are not readily apparent. There is suggestive evidence that large complexes derived from precipitates by solubilization with excess Ag have higher bond strength and are not as readily reversed to simpler complexes

TABLE III. Anaphylaxis in Mouse Produced with Soluble BSA Anti-BSA Complexes Formed in Various Regions of Ag Excess Using Unheated Antisera and Physiological Saline and PBS as the Diluents.

Diluent	Challenge preparation	Challenge dose, ml	Ab N in challenge dose, mg	Deaths	% mortality	Symptoms of survivors Severe	Mild
Saline	Complex 8x Ag excess	.5	.462	12/12	100		
"	*Idem*, 180x	"	"	1/12	8	8	3
PBS	" 8x	"	"	10/12	83	2	
"	" 180x	"	"	1/12	8	7	4

in extreme Ag excess as are soluble complexes initially formed in the region of low Ag excess(13,14,15). Perhaps it is only when complexes are allowed to form in extreme Ag excess that relative pure preparations of the simplest theoretical complex Ag-Ab-Ag are achieved and maintained.

There is limited information on the size of various soluble Ag-Ab complexes in protein anti-protein systems maintained in various regions of Ag excess and no definitive information on velocities of forward and backward reactions among these complexes which attend changes in Ag concentration. Singer and Campbell(16) observed that when a precipitate formed by BSA and salt-fractionated anti-BSA rabbit globulin was solubilized in low Ag excess the complexes were heterogeneous and very large. In contrast the complexes solubilized in regions of large Ag excess were smaller and rich in a complex of the apparent molecular composition Ag-Ab-Ag in linear array.

Additional factors which may influence rate of formation, size and composition of soluble complexes for any given Ag-Ab system include temperature(17), pH(18,19), salt concentration and ionic strength(14,18), and the presence of other macromolecular substances such as the various components of complement(20,21,22). Many of the problems concerned with determining the nature of such complexes have been discussed(14,17,23,24).

Despite the observation that the C'2 and C'3 components of complement are low in the mouse(25), it seems possible that in the present experiments mouse complement contributed to anaphylaxis by being fixed to and activated by the injected soluble Ag-Ab complexes. This possibility is especially attractive in view of the recent observations of Weigle and Maurer(21,22) that soluble complexes of BSA and anti-BSA rabbit Ab maintained in various regions of Ag excess fix decreasing amounts of complement with increasing Ag excess.

If complement is involved in the anaphylactigenic action of soluble Ag-Ab complexes its role may be 2-fold; that of precipitating with the complexes(20,21,22) and of being activated by them.

We wish to propose the hypothesis that anaphylactigenic activity of soluble BSA anti-BSA complexes for the mouse is dependent upon their capacity to react with complement.

Summary. 1) Soluble BSA anti-BSA complexes formed in regions of extreme antigen excess are less anaphylactigenic for the mouse than similar complexes formed in regions of low antigen excess. 2) The hypothesis is advanced that anaphylactigenic activity of these complexes for the mouse is dependent upon their capacity to react with complement.

1. Germuth, F. G., Jr., *J. Exp. Med.*, 1953, v97, 257.

2. Germuth, F. G., Jr., McKinnon, G. E., *Bull. Johns Hopkins Hosp.*, 1957, v101, 13.

3. Hawn, C. V., Janeway, C. A., *J. Exp. Med.*, 1947, v88, 571.

4. Germuth, F. G., Jr., Pace, M. G., Tippett, J. C., *ibid.*, 1955, v101, 135.

5. Talmage, D. W., Dixon, F. J., Bukantz, S. C., Dammin, G. J., *J. Immunol.*, 1955, v67, 243.

6. von Pirquet, C. F., Schick, B., *Serum Sickness*, 1905, Williams and Wilkins Co., 1951.

7. Tokuda, S., Weiser, R. S., *Science*, 1958, v127, 1237.

8. Kabat, E. A., Benacerraf, B., *J. Immunol.*, 1949, v62, 517.

9. Karelitz, S., *Ann. N. Y. Acad. Sci.*, 1949, v52, 705.

10. Kuhns, W. J., McCarty, M., *J. Clin. Invest.*, 1954, v33, 759.

11. Quinn, R. W., *J. Immunol.*, 1956, v76, 246.
12. Rejholec, V., *Ann. Rheum. Dis.*, 1957, v16, 23.
13. Kleinschmidt, W. J., Boyer, P. D., *J. Immunol.*, 1952, v69, 257.
14. Johnson, P., Ottewill, R. H., *Faraday Soc. Discussions* #18, 1954, 327.
15. Weigle, W. O., Maurer, P. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 371.
16. Singer, S. J., Campbell, D. H., *J. Am. Chem. Soc.*, 1952, v74, 1794.
17. ———, *ibid.*, 1955, v77, 3499.
18. Marrack, J. R., Grant, R. A., *Brit. J. Exp. Path.*, 1953, v34, 263.
19. Singer, S. J., Campbell, D. H., *J. Am. Chem. Soc.*, 1955, v77, 3504.
20. Morton, J. I., Deutsch, H. F., *Arch. Biochem. and Biophys.*, 1956, v64, 26.
21. Weigle, W. O., Maurer, P. H., *J. Immuol.*, 1957, v79, 211.
22. ———, *ibid.*, 1957, v79, 223.
23. Becker, E. L., *J. Immunol.*, 1953, v70, 372.
24. Goldberg, R. J., Williams, J. W., *Faraday Soc. Discussions* #13, 1953, 224.
25. Muschel, L. H., Muto, T., *Science*, 1956, v123, 62.

Received April 10, 1958. P.S.E.B.M., 1958, v98.

Development of Interarterial Intercoronary Anastomoses by Arteriovenous Fistula Between Pulmonary Artery and Left Atrium.* (24107)

STACEY B. DAY,† VINCENT L. GOTT, C. WALTON LILLEHEI AND OWEN H. WANGENSTEEN
Departments of Surgery and Physiology, University of Minnesota, Minneapolis

Observations made on man in open heart surgery have suggested the likelihood of existence of rich intercoronary networks in patients with congenital cyanotic heart disease (Tetralogy of Fallot). The possibility that inter-coronary anastomoses developed in response to cyanosis suggested the following study.

Method. Selected mongrel dogs 15-25 kg in weight were used. Weak or sick animals were rejected. All animals were anaesthetized with 3% sodium nembutal (30 mg/kg body wt.) and placed on a cam type respirator utilizing compressed air. Clean but not sterile technic was employed. A left-sided thoracotomy was performed and the 4th rib was removed. The pericardium was opened parallel to the phrenic nerve so as to display the main trunk of the pulmonary artery and the left auricle. A side to side arteriovenous anastomosis 10-17 mm in length was made in each animal between the pulmonary artery distal to the semilunar valves and the left atrium. The thoracic cavity was closed in layers and 500,000 units crystalline penicillin was given IM for 2 days. Systemic blood for sampling of oxygen content and saturation was obtained by direct cut-down upon the femoral artery. Determinations were by the method of Van Slyke and Neill(1). All animals tolerated the procedure well. Postoperative care consisted of routine kennel rations and a mandatory exercise period of 15 minutes each day for every animal. At varying periods (2 days to 14 weeks) after operation the dogs were sacrificed and the pattern of coronary arteries was studied by corrosion digestion preparations(2). An earlier study of the architecture of coronary arteries in 50 normal dog hearts, using the same injection-corrosion method, served as an adequate control.

Observations. Results are summarized in Tables I and II. With the exception of one animal that died 2 days after operation and in which the shunt was 16 mm in circumference, no dog appeared in acute respiratory distress. Subsequent experiments utilized a fistula no larger than 10-12 mm in circumference. In these animals, including several not reported at this time, there has been no postoperative death. One animal showed signs of dyspnea on exertion. The exercise

* Supported by U.S.P.H.S. Training Grant in Cardiac Surgery.
† Sponsored by The Aga Khan.
Technical assistance by James A. Vick.