Reduction of Serum Complement Following *in vivo* Tissue Antigen-Antibody Reactions. (20962)

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In a previous study(1) serum complement activity was found to be reduced following the interaction of *circulating* antibody and *injected* antigen in the rabbit. Two types of evidence pointed to the requirement of circulating antibody for the reduction of complement. First, 2 injections of antigens about 90 minutes apart into an immunized rabbit apparently bound all of the circulating antibody so that a third injection of antigen failed to produce a decrease of complement(1). Second, when circulating antibody was allowed to disappear without further injection of antigen, subsequent injections of antigen did not bring about a decrease of complement(1). However, these experiments did not eliminate the possibility that *tissue* antigen-antibody reactions, where either antigen and antibody or both were associated with the tissues, could also cause the depression of complement *in vivo*. There have been reports(2-4) of the lowering of complement in connection with cytotoxic or allergic manifestations *in vivo* which probably involved tissue antigen-antibody reactions. Therefore, it was of interest to ascertain the effect on complement of the injection of rabbit anti-rat kidney serum into rats. Such injections are known to produce a rapid reaction between tissue antigens in the kidney, liver and lungs and corresponding antibodies in the antikidney serum(5). This reaction results in a renal disease which simulates the nephrotic syndome as it is observed in infants and children(6).

Materials and methods. Animals. Sprague-Dawley rats of both sexes weighing about 130-160 g were employed. They were fed a diet of Friskies and water. *Preparation of antisera.* Antikidney serum was prepared in rabbits by intraperitoneal injections for several weeks of a 20% homogenate in saline of perfused whole rat kidney(6). It was stored at −20°C. In the course of the work the possibility arose that complement was being fixed as a result of reactions between rat plasma and antibodies to rat plasma in the rabbit antiserum. Therefore, aliquots of antikidney serum were absorbed with rat plasma to remove corresponding antibodies. In order to try to define the role of the kidney antigen-antibody system sera were also absorbed with rat kidney. 0.3 ml of a 50% rat kidney homogenate was lyophilized in a narrow tube. 0.3 ml of rat plasma was pipetted into a test tube and lyophilized. For absorption 0.25 ml of antikidney serum was placed in a tube containing either lyophilized kidney or plasma and the dried kidney or plasma resuspended or redissolved in the serum. After 1 hour's incubation at 37°C it was considered that the plasma antibodies had been removed and the contents of the tube were called "absorbed antiserum." Tests described below indicated that the plasma antibodies had been removed completely by this procedure. There was no suitable method for determining *in vitro* whether the kidney antibodies had been removed. Antibodies to rat red cells were removed by absorption of the antikidney serum with an equal volume of washed rat red cells. *Estimation of antibody.* Antibody to rat plasma was estimated by the *ring reaction.* Rabbit antikidney serum was placed in a capillary tube and overlaid with an equal volume of rat plasma. The appearance of an interphasic precipitate indicated a positive reaction. However, a much more sensitive method for detecting this antibody was provided by the *hemagglutination reaction* as modified in one of our laboratories (A.B.S.)(7) from Boyden(8). Red blood cells were treated with tannic acid after which they adsorbed rat plasma on their surface. These tannic acid-plasma cells were then agglutinated by antibodies to rat plasma. By this method rat plasma antibodies have been found consistently in the rabbit anti rat kidney serums. *Assay of complement.* Comple-

ment was assayed by the method of Ecker, Hiatt, and Barr(9) in terms of 50% hemolytic units, that is, the number of ml of serum required to lyse 50% of a standard suspension of sensitized sheep red blood cells. *Therefore, the higher the figure the lower the complement concentration of serum and vice versa. Other procedures.* Rats were bled by cardiac puncture. About 0.25 ml of blood was taken each time. Nephrectomies were performed by lumbar approach under ether anesthesia. Nephrectomized animals were fasted and thirsted after the operation. The severity of the renal disease was estimated by quantitative determination of proteinuria, by the presence of anasarca, changes in blood chemistry and histological changes(6). In each experiment the rats were first bled by cardiac puncture. Whereas in previous work(6) the serum was given in divided dosage, in the present study it was given in one dose immediately after the initial bleeding. Thirty minutes and 48 hours after the injection of the serum the animal was bled again. The serum was separated, quick-frozen in CO_2-alcohol and placed in a deep freeze at −20°C until the day of assay. The sera from each experiment were assayed on the same day.

Results. Correlation of complement level and renal disease. Table I shows that the injection of normal rabbit serum into rats was not followed by a reduction of complement. On the other hand, the injection of antikidney serum practically always was followed by depression of complement whether renal disease ensued or not. The failure to produce nephrosis with the injection of antikidney serum was noted (Rats No. 3, 4, 7, 9, 10, 11) and probably due to an insufficient concentration of kidney antibodies in the serum. However, renal disease was not produced in the absence of a reduction of complement.

TABLE I. Serum Complement Levels and Incidence of Renal Disease in Rats after Injection of Control Rabbit Serum, and Anti-Rat Kidney Serum.

Rat #	Serum #	C′ at time 0	C′ at time 30 min.	C′ at time 48 hr	Renal disease
1	Normal	.9	.85	.80	0
2		.5	.5	.5	0
3	502 AK	.7	1.9	.5	0
4		1.2	4.1	.7	0
5	506 AK	1.7	4.0	2.0	+
6		1.1	4.0	1.7	+
7	493 AK	1.2	1.4	1.2	0
8		.5	3.0	3.0	+
9		.4	.45	.45	0
10	498 AK	1.7	1.7	1.6	0
11		1.6	3.2	3.1	0
12		1.6	3.4		+

C′—ml serum diluted 1:25 required for 50% lysis; AK—anti-rat kidney serum.

TABLE II. Level of Complement after Injection into Rats of Plain Anti-Kidney Serum (AK) or of Anti-Kidney Serum Absorbed with Rat Plasma (AK-RP).

Serum #	C′ titer at time 0	C′ titer at time 30 min.	C′ titer at time 48 hr
517 AK	.4	3.0	1.8
	.6	3.0	—
	.5	3.4	—
517 AK-RP	.5	3.5	.5
	.5	2.8	.8

Complement level following the injection of antikidney serum absorbed with rat plasma or rat red cells. The data in Table I showed that complement was reduced after inoculation of antikidney serum. However, this could have been due to antigen-antibody reactions not involving kidney antigen and antibody. Therefore, the antikidney sera were absorbed with lyophilized rat plasma. Such absorption resulted in reduction of the hemagglutination titer against plasma-coated red cells from 1/163,000 for the unabsorbed serum to 0 for serum absorbed with rat plasma and 1/20,000 for serum absorbed with rat kidney. Since this method will detect fractions of a microgram of antibody protein(7) a negative reaction is significant. The presence of rat plasma antigens in rat kidney preparations of the type used for immunization of rabbits was shown by the great reduction in antiplasma titer caused by absorption of the antikidney serum with lyophilized rat kidney.

Table II presents the results of injections of these absorbed sera into normal rats. It is evident that complement activity was decreased to about the same extent whether the antikidney serum was absorbed or unabsorbed with rat plasma. Complement was also reduced in rats injected with kidney

TABLE III. Reduction of Complement after Injection of Anti-Kidney Serum into Bilaterally Nephrectomized Rats.

Serum #	C' titer at time 0	C' titer at time 30 min.
516 AK	.4	2.5
"	1.0	3.0
None	.6	.6
"	.4	.4

antiserum from which rat red cell hemagglutinins were removed completely. *Reduction of complement after the injection of antikidney serum into normal and bilaterally nephrectomized rats.* Conclusive evidence of the participation of extra-renal tissues in the reduction of complement was sought by the injection of antikidney serum into nephrectomized rats (Table III). Complement was reduced in bilaterally nephrectomized rats given this serum whereas bilateral nephrectomy alone did not lead to a loss of complement. Complement was depressed in control rats given this serum and they developed severe renal disease.

Discussion. Several different types of evidence in the present and other studies(5,10) indicate that antikidney serum contains antibodies which react with other components of extra-renal tissues. These cross-reactions appear to be due to the possession by many tissues of common connective tissue antigens associated with basement membranes, reticulum, sarcolemma and neurilemma(10). There is some evidence that these antigens may be mucopolysaccharides(11). The decrease in complement which occurs upon injection of antikidney serum into bilaterally nephrectomized rats shows that the kidney antigens are not absolutely required for this reduction. In fact, no data have been obtained in the present work which definitely implicate the kidney antigen-antibody reaction in the reduction of complement. Indeed there is no definite information on the actual tissues participating, though from recent studies tissues containing common antigens must be involved. Antibodies to rat plasma, however, have been eliminated from participation in this reduction by absorption of the antikidney serum with plasma. Therefore, the presumption is strong that complement was reduced as a result of *tissue* or *cellular* antigen-antibody reactions, excluding red blood cell antigens. Further study will be required to exclude the participation of antigen-antibody reactions between humoral antigens other than those in plasma and corresponding antibodies in the antikidney serum.

The development of the nephrotic syndrome in the rat has always been accompanied by a marked but transient lowering of serum complement. In the human nephrotic syndrome complement was diminished during the active phase of the disease and persisted at low levels for many days or even weeks(12,13). However, even assuming that the lowering of complement in the human disease is associated with an antigen-antibody reaction the situation is not entirely analogous to that in the rat. In the animal the passively conferred kidney or tissue antibodies presumably combine rapidly with the tissue antigens with the reduction of complement. In the human disease the concentration of antibody may be maintained by continued active antibody formation and cause a prolonged antigen-antibody reaction with continued fixation of complement.

In view of these considerations further study is needed to define the role *if any* of complement in the pathogenesis of the experimentally produced nephrotic syndrome of rats as well as other cytotoxic or allergic manifestations of tissue antigen-antibody reactions. Obviously, the reduction of complement which accompanied tissue antigen-antibody reactions in the present study is not incompatible with the participation of complement in these manifestations.

Summary. Serum complement was reduced within 30 minutes after injection of rabbit anti-rat kidney serum into rats. Complement was decreased in amount following the injection of this antiserum into bilaterally nephrectomized rats. Complement also was decreased in rats given this antiserum after antibodies to rat plasma and rat red cells had been removed. Therefore, it was concluded that complement may be reduced *in vivo* as a result of extra-renal tissue antigen-antibody reactions. The significance of the lowering of complement in relation to the

development of the nephrotic syndrome remains to be determined.

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Effect of Coronary Perfusion of Prostigmine on Ventricular Fibrillation in the Hypothermic Dog.* (20963)

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(Introduced by C. A. Maaske.)
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Ventricular fibrillation is a frequent event during cardiac surgery with inflow occlusion in the experimental hypothermic dog. Bigelow *et al.*(1), Rosenhain and Penrod(2), and Swan *et al.*(3) have demonstrated that the incidence of ventricular fibrillation increases with body temperatures below 25°C with CO_2 retention and with cardiac manipulation. To minimize the first 2 of these factors in the present work, the body temperature was lowered to the range between 23° and 26°C and the animals were vigorously hyperventilated with pure oxygen sufficiently to maintain a blood pH of about 7.6. A suitable pharmacological agent was then sought to alter the fibrillatory threshold during cardiac manipulation. The drug which significantly altered this fibrillatory threshold was prostigmine.

Methods. Mongrel dogs were placed in an ice water bath after being deeply anesthetized with I.V. sodium pentobarbital to prevent shivering. Following tracheal intubation, the dog was hyperventilated with pure oxygen. When 29°C rectal temperature was obtained, the dog was removed from the bath; a further drop of 3 to 6 degrees of temperature occurred. Using aseptic technic a right anterolateral thorocotomy incision was made through the 5th intercostal space. After entering the right thorax the azygos vein was ligated, the pericardial sac opened widely and both cavae were occluded. Fifteen seconds later the ascending aorta was occluded with a non-crushing clamp about one inch distal to the coronary ostia. A control series of dogs received no medications; the experimental group was treated as follows: Some received prostigmine intravenously before occlusion of circulation. In others, either prostigmine or acetylcholine was injected into the root of the aorta until the heart rate was reduced to 10 to 25 beats per minute. At this point injection of prostigmine was stopped; however, since its action is so brief, acetylcholine required a slow continuous perfusion to maintain the slow heart rate. This method of administration into the clamped aorta is termed coronary perfusion. To produce cardiac slowing, the range of dosage for prostigmine proved to be one to 2 ml of 1-4000 solution. For acetylcholine from one to 3 mg

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