

THE ANTICOMPLEMENTARY EFFECT OF KIDNEY TISSUE  
ITS ASSOCIATION WITH AMMONIA PRODUCTION

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(Received for publication, July 1, 1959)

The research being reported here was undertaken in an investigation of the kidney's peculiar vulnerability to coliform bacterial infections. That group of organisms, although the commonest cause of human pyelonephritis, is rarely found solely responsible for disease of other organs. Tests of virulence in animals have shown that large numbers can be inoculated intravenously or intraperitoneally without causing death or localized infection (1), but some evidence has been obtained to indicate that host defense mechanisms are less effective in the kidney. For example, intravenously inoculated *Escherichia coli* are destroyed at a slower rate in the kidney than in other organs (2). Furthermore, whereas heavy inocula can be injected into most tissues without the development of localized infection, introduction of only 10 to 100 bacteria into the renal medulla usually results in acute suppuration (3).

The findings in the present studies show that kidney tissue interferes with the bactericidal action of blood serum for coliform bacilli by inactivating complement. This anticomplementary effect appears to be associated with the formation of ammonia by renal glutaminase.

*Materials and Methods*

*Tissue Suspensions.*—Normal animals were killed by severing the spinal cord in the neck, or by intravenous injection of air. The organs to be tested were excised, freed of surface fat and connective tissue, weighed, cut into small slices, and ground, either by means of a manually operated glass tissue grinder, or by a motor-driven cutting blade. The tissues were then suspended either in saline or a minimal medium (M.M.) described by Davis and Mingioli (4), in approximately 10 per cent concentration. Larger particles were removed from this by centrifugation for 5 minutes at 500 R.P.M., and the supernatant material was stored at 4°C. until needed.

*Serum Bactericidal Test.*—The 2206 strain of *E. coli* (1) was employed as test organism. M.M. was used as the diluent fluid to avoid the bactericidal effect of saline. The bactericidal activity of each mixture was determined by counting the surviving bacteria in a single tube; total volume in the tube at the beginning of the test was 1 ml. Each tube contained 0.2 ml. of 1:5 fresh human or rabbit serum, with 0.2 ml. of tissue homogenate when indicated, and volume was

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adjusted to 0.9 ml. with diluent. The bacterial inoculum was 0.1 ml. of fluid containing approximately 1500 living cells. After mixing, 0.1 ml. was withdrawn for counting, following which the tubes were placed in a 37°C. bath, and additional samples were removed at 30, 60, and 90 minutes. Each sample was spread on a previously dried nutrient agar plate and incubated overnight at 30°C., after which the number of colonies was counted.

*Measurement of Complement.*—In early phases of the work both rabbit and human sera were employed as sources of complement; all of the later experiments were done with human serum. Freshly separated sera were stored at  $-20^{\circ}\text{C}.$  until needed, when they were thawed in a 37°C. bath. For the measurement of hemolytic activity a 3 per cent suspension of sheep erythrocytes sensitized with 6 hemolytic units (H.U.) of rabbit hemolysin was used. Veronal buffer, pH 7.4, was the diluent. Determination of complement activity of each serum was carried out on the day of an experiment, and a dilution sufficient to supply  $4 \times 50$  per cent H.U. of complement was used to test for anticomplementary activity.

*Measurement of Anticomplementary Activity.*—The tissue suspension, 0.2 ml. in volume (or made up to that volume with diluent) was incubated with 0.2 ml. of serum dilution containing  $4 \times 50$  per cent H.U. of complement, in a 37°C. bath. After 15 minutes, 0.2 ml. of sensitized sheep cells was added and the incubation continued for an additional 40 minutes. The tubes were then centrifuged at 800 R.P.M. for 5 minutes, and the degree of hemolysis estimated by comparing the color of the supernatant fluid with that of a series of tubes containing 10, 20, 30, 40, 50, 60, 80, and 100 per cent of the same suspension of erythrocytes lysed with water.

For purposes of comparison here, 1 anticomplementary unit is defined as the dry weight in milligrams of material required to inactivate 1 H.U. of complement. That is to say, the weight necessary to reduce  $4 \times 50$  per cent H.U. to  $1 \times 50$  per cent H.U. is listed as 3 anticomplementary units.

*Measurement of Ammonia Production.*—The tissue suspension, the diluent, and 0.05 M glutamine, each in 0.5 ml. volume, were incubated at 37°C. for 30 minutes. Samples of 0.2 ml. were removed from the mixture before and after the period of incubation, and the ammonia content determined by the method of Seligson and Seligson (5).

#### EXPERIMENTAL

*Effect of Tissue Homogenates on the Bactericidal Activity of Normal Serum.*—Tissue suspensions were prepared aseptically from various organs of mice, rats, and rabbits. These were tested for effect on the bactericidal activity of normal human or rabbit serum against the test strain of *E. coli*. It was found that liver, spleen, lung, and muscle had little or no influence on the rapidity and completeness of the destruction of bacteria by serum. Kidney tissue, on the other hand, acted in some way to prevent the occurrence of this destruction. Fig. 1 shows the results of an experiment of this type, in which kidney and liver homogenates from a mouse are compared. It is seen that the bacteria were killed almost as rapidly in the presence of the liver tissue as in the tube containing only serum. In the presence of kidney tissue there was no evidence of a bactericidal action, and by the end of 90 minutes multiplication of the organisms was taking place.

*Anticomplementary Activity of Kidney Tissue.*—In view of the fact that the bactericidal activity of serum depends on both complement and a heat-stable fraction usually identified with antibody, the next experiments were planned to test whether kidney tissue possessed an unusual degree of anticomplementary

activity. This was done by incorporating the tissue homogenates in the usual system involving lysis of sensitized erythrocytes in the presence of complement. Kidney tissue was found to be 5 to 15 times more anticomplementary than the others examined. This is illustrated in Table I, in which the relative effects of kidney, spleen, liver, lung, and heart muscle from a rabbit are compared. It is shown there that  $4 \times 50$  per cent H.U. of complement was reduced to  $1 \times 50$  per cent H.U. in the presence of 0.02 ml., or 0.06 mg. of kidney tissue.

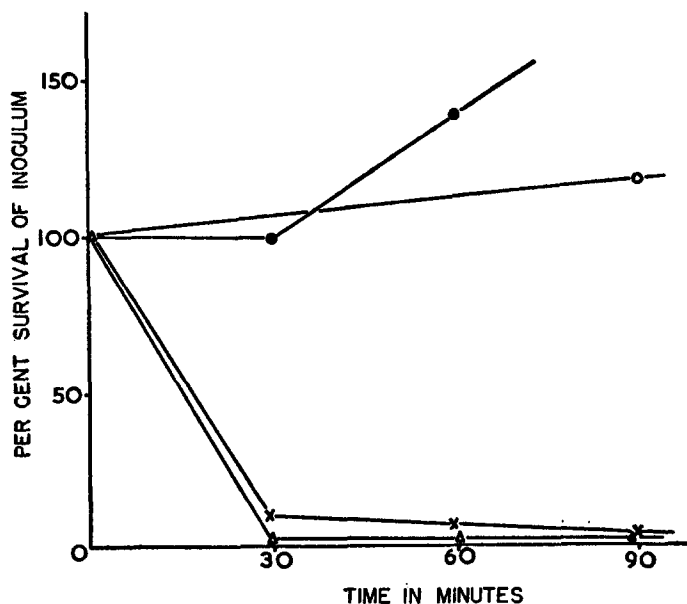


FIG. 1. The bactericidal action of rabbit serum for *E. coli* 2206, and its inhibition by mouse kidney homogenate.

-△-△-, rabbit serum, diluted 1:5. -●-●-, rabbit serum, diluted 1:5, plus kidney homogenate. -X-X-, rabbit serum, dilute 1:5, plus liver homogenate. -O-O-, diluent only.

This, then, would be equivalent to 50 anticomplementary units per mg. of kidney tissue. The other homogenates, by this method of calculation, exhibited less than 6 units anticomplementary activity per mg.

The hemolytic test of complement activity was employed in all subsequent experiments, because of its short time requirement and simplicity.

*Anticomplementary Activity of Kidneys of other Animal Species.*—Extensive comparisons of the anticomplementary activities of several tissues from the same animal were made in the case of mice, rats, and rabbits. In addition, however, kidney tissue was obtained from a pig, a calf, an ox, and from one human being (at autopsy). Homogenates of these organs were prepared in the usual way, and all were found to possess strong anticomplementary activity.

*Comparison of Renal Cortical and Medullary Tissue.*—A fresh rabbit kidney was sliced with a razor blade, and pieces of cortical and medullary tissue were separated. Homogenates of the two kinds of tissue were then tested, and were found to have approximately the same anticomplementary effect, according to the wet weights of tissues used. This indicates that the material which inactivates complement is associated with renal tubules, and not localized in one zone of the kidney.

*Effect of Urinary Obstruction on Anticomplementary Activity.*—Kidney infections occur more frequently in the presence of urinary obstruction. An experiment was therefore carried out to determine what effect urinary obstruction might have on the anticomplementary activity.

TABLE I  
*The Anticomplementary Effect of Homogenates from Various Rabbit Organs*

Tube No.	1	2	3	4	5	6
Homogenate, ml.....	0.16	0.08	0.04	0.02	0.01	0.005
Veronal buffer, ml.....	0.04	0.12	0.16	0.18	0.19	0.195
4 × 50 per cent H.U. serum, ml.....	0.2	0.2	0.2	0.2	0.2	0.2
Sensitized cells, ml.....	0.2	0.2	0.2	0.2	0.2	0.2
Per cent hemolysis with						
Kidney.....	0	0	10	50	70	90
Spleen.....	70	90	100	100	100	100
Liver.....	60	90	100	100	100	100
Lung.....	60	90	100	100	100	100
Heart.....	80	100	100	100	100	100

Each homogenate had a salt-free dry weight of 3 mg./ml.

The left ureter of a rat was ligated. Six days later both kidneys were removed, and about 0.2 ml. of fluid was aspirated from the hydronephrotic sac of the left kidney. The two kidneys were ground separately in the same quantity of M.M., and tested for anticomplementary activity, together with the aspirated fluid.

The result of this experiment indicated that anticomplementary activity was no greater in the obstructed than in the normal organ, and that the fluid from the hydronephrotic sac had no detectable influence on the assay.

*Effect of Dialysis.*—The anticomplementary activity of a rabbit kidney homogenate was tested, before and after dialysis against saline.

2 ml. of homogenate was dialysed against 300 ml. of saline at 4°C. overnight. The anticomplementary action of the dialysed homogenate was then compared with that of an equal quantity of the original material, and there was no appreciable difference.

Under these conditions the anticomplementary agent did not pass through a semipermeable membrane.

*Effect of Centrifugation.*—To learn whether the anticomplementary agent was associated with tissue particles the effect of centrifugation at moderate speed was tested.

A sample of rat kidney homogenate in saline was centrifuged at 6000 R.P.M. for 1 hour at 4°C. The opalescent supernatant fluid was removed, and the sediment resuspended in fresh diluent. Only a trace was demonstrable in the supernatant fluid, whereas the resuspended sediment had about the same activity as the original homogenate.

It appears, then, that in crude homogenates of the type used in this study, most of the anticomplementary activity is associated with tissue particles which can be sedimented by centrifugation of moderate force.

*Effect of Ultrasonic Vibrations.*—In attempting to obtain a more homogeneous suspension of the anticomplementary material as a preliminary to fractionation, kidney tissue suspensions were subjected to ultrasonic vibrations. This was found to cause rapid reduction in anticomplementary activity. By the end of 2 minutes of treatment approximately half of the activity had been lost, and after 5 minutes it was too slight to be determined. The accompanying decrease in turbidity of the tissue suspension indicated a parallel between anticomplementary activity and existence of particulate material.

*Effect of Temperature.*—Intact kidney tissue stored at -20°C. showed no detectable loss of anticomplementary activity over a period of several months. Kidney homogenates stored at 4°C. gradually lost activity over periods of several weeks. There was no appreciable deterioration after heating at 52°C. for 30 minutes; however when the material was heated at 60°C. for the same time there resulted a marked increase in turbidity, and almost complete loss of anticomplementary activity.

*Rate of Complement Inactivation at Different Temperatures.*—It was thought that a determination of the effect of temperature on the rate of complement inactivation would provide some evidence on the nature of the reaction.

An ox kidney homogenate and a sample of human serum were used in this experiment. Preliminary testing indicated that 1 ml. of the homogenate (dry weight 13.5 mg.) would reduce the complement titer of 4 ml. of the serum below a measurable level when incubated at 37°C. Accordingly these quantities were mixed together at 4°C., and 15 ml. veronal buffer added as diluent. This mixture was distributed in 16 chilled centrifuge tubes, which were then incubated for different times and at different temperatures, as indicated in Table II. At the end of the period of incubation each tube was chilled in an ice bath to 4°C., following which it was centrifuged at 3000 R.P.M. for 30 minutes at 0°C. The supernatant fluid was removed immediately and tested for complement content. The results are shown in the table.

Marked slowing in rate of complement inactivation was found to accompany decreasing temperatures. For example, inactivation of 8 units occurred within 4 minutes at 40°C., but only after 20 minutes at 30°C., and tubes incubated at 20°C. showed inactivation of only 3.5 units at the end of 150 minutes. The findings are consistent with a chemical reaction rather than a physical adsorption.

*Attempts to Isolate or Partially Purify the Anticomplementary Factor.*—Measures designed to separate the anticomplementary factor or factors from tissue particles usually resulted in marked loss of activity. Not only was it destroyed by ultrasonic vibrations, but also there was a considerable reduction after freeze-drying or after repeated freezing and thawing. It was possible, by the use of 6 M urea (6) to obtain an opalescent "solution" which, after dialysis against saline to remove the urea was found to have suffered little loss in anticomplementary activity. Several attempts were made to separate active fractions from the urea-treated material by precipitation with ammonium sulfate.

TABLE II  
*Effect of Temperature on the Rate of Inactivation of Complement by Ox Kidney Homogenate*

Time <i>min.</i>	Units complement inactivated at			
	40°C.	30°C.	20°C.	4°C.
2	2.5	—	—	—
4	8	2.5	—	—
10	10	3	1.5	—
20	10	8	1.5	—
40	10	9	1.7	—
90	—	10	2.5	—
150	—	—	3.5	1

Largest volume which could be tested was 0.4 ml. The original serum assayed at 125 units complement per ml., but was diluted 1:5 in the experiment; therefore maximum amount of complement available for inactivation was 10 units.

An ox kidney homogenate, to which urea had been added to 6 M, was treated with ammonium sulfate. One precipitate was removed at 30 per cent saturation, and a second at 35 per cent saturation. The two precipitates and the supernatant fluid, after dialysis against water, and addition of NaCl to 0.15 M, were tested for anticomplementary activity. As shown in Table III, none of the three fractions had much activity alone, but when recombined in the original proportions, on the basis of dry weights, there was restoration of the original activity.

These results suggest that the effect on complement involves participation of more than one factor in the tissue.

*The Component of Complement Inactivated by Kidney Tissue.*—To determine which of the four components of complement was affected by kidney tissue, tests were made, employing serum reagents known to be lacking in one or more of the four components.

Serum reagents lacking one or more components of complement were prepared according to standard methods (7). Each of these was assayed, to determine the maximum concentration which could be added to sensitized erythrocytes without causing hemolysis. This dilution of

reagent was then combined with an equal volume of serum inactivated by previous incubation with kidney extract, and the mixture tested for capacity to cause lysis of sensitized erythrocytes.

The results are shown in Table IV. They indicate that kidney tissue exerts its anticomplementary action by means of an effect on the fourth component of complement. Activity could be restored by adding heated serum (lacking the first and second components), or by adding zymosan-treated serum (lack-

TABLE III  
*Anticomplementary Activity of Fractions of Ox Kidney Homogenate Obtained by Treatment with Ammonium Sulfate in Presence of 6 M Urea*

Material, or fraction	Anticomplementary activity	Recovery of total anticomplementary activity
	<i>units per mg.</i>	<i>per cent</i>
Original homogenate.....	15	100
Precipitated at 30 per cent saturation (fraction A).....	4	1.25
Precipitated at 35 per cent saturation (fraction B).....	7.5	37
Supernatant from above (fraction C).....	0	0
Fractions A, B, and C recombined.....	15	100

TABLE IV  
*The Component of Complement Inactivated by Kidney Tissue*

Reagent	Hemolysis
	<i>per cent</i>
Untreated human serum 1:10.....	100
Heated serum (lacking 1st and 2nd components) (H-S).....	0
Zymosan-treated serum (lacking 3rd component) (Z-S).....	0
Ammonia-treated serum (lacking 4th component) (N-S).....	0
Serum inactivated by kidney tissue (K-S).....	0
K-S + H-S.....	20
K-S + Z-S.....	60
K-S + N-S.....	0

ing the third component), but not by adding ammonia-treated serum (lacking the fourth component).

The only chemical agents known which selectively inactivate the fourth component of complement are ammonia and primary amines. This finding directed the course of further work toward testing the hypothesis that kidney tissue exerts its anticomplementary effect by the formation of ammonia.

*Sources of Ammonia in the Animal Body.*—There are two main sources of ammonia: that formed by bacterial action in the intestinal tract, and that produced by tubular epithelial cells of the kidney (8). Other tissues play a negligible role in its synthesis.

The production of ammonia by the kidney is subject to great variation, being one of the mechanisms for maintaining acid base equilibrium.

The principal mechanism by which the kidney forms ammonia is from the hydrolysis of glutamine (9). The enzyme mainly responsible is called glutaminase I (10). *In vitro* demonstration of its activity, *i.e.* demonstration of the liberation of ammonia from glutamine, is usually carried out with crude homogenates of kidney tissue, prepared in a manner similar to that used in the present work. This enzyme has not been isolated in pure form but its action is known to be inhibited by bromosulfalein and quinacrine, and to be enhanced in the presence of phosphate (11). The recent findings of Klingman and Handler (12) using the purest preparations so far obtained, show that the role of phosphate is not so much one of enhancement as of protecting the enzyme from the rapid destruction which it may undergo when incubated at 37°C. in the absence of multivalent anions.

The opinion has been expressed that up to 40 per cent of ammonia production by the kidney may be from the deamination of amino acids. Richterich and Goldstein have presented evidence against this concept, and suggest that only glutamine and glutamic acid are the immediate precursors of urinary ammonia (13).

Glutaminase I can be demonstrated in both the cortical and medullary zones of the kidney, but the quantity present in the inner part of the medulla seems adequate to account for all ammonia production by the kidney (13), a fact which agrees with micro-puncture experiments of Walker in which ammonia content of the tubular fluid was found to rise abruptly in the distal tubule and collecting ducts (14). Richterich and Goldstein suggest therefore that the glutaminase I elsewhere in the nephron may serve other functions, such as general amino acid metabolism.

*Effect of Glutaminase Inhibitors.*—An attempt was made to interfere with the anticomplementary effect of kidney tissue by using bromosulfalein and quinacrine, in concentrations which inhibit the liberation of ammonia by kidney tissue (11). Unfortunately both agents were anticomplementary in the concentrations necessary to inhibit glutaminase.

*Comparison of Anticomplementary Activity and Ammonia Production.*—An old preparation of ox kidney, which had suffered considerable deterioration as a result of long storage at 4°C., was compared with a freshly prepared homogenate of rabbit kidney, with respect to ability to liberate ammonia from glutamine and to inactivate complement. The results are shown in Table V. This table also shows a comparison of ammonia production and anticomplementary activity of a homogenate of rabbit kidney carried out in the course of a later experiment dealing with effect of phosphate. It will be seen that there is a good agreement between these two activities of kidney tissue under different conditions. This is compatible with the possibility that both are measures of the same function. The lack of agreement between the amount of ammonia produced and units of the anticomplementary activity in the two different experiments included in the table is probably attributable to variations in conditions of tests done at different times. The important correlation is that shown between the first two and the second two test systems.



*Anticomplementary Effect of Renal Glutaminase in Soluble Form.*—In 1954 Otey *et al.* (15) reported a method of treatment of kidney tissue which released glutaminase in such form that it was not sedimented by high speed centrifugation. The procedure involved treatment of kidney homogenate with butanol at 2°C., washing with acetone and ether, and extraction with water.

This procedure was carried out on a homogenate of ox kidney, and a yellowish fluid was obtained, which had a dry weight of 12 mg. per ml., after centrifugation at 25,000 g for 1 hour.

TABLE V  
*Comparison of Anticomplementary Activity and Ammonia Formation by Different Kidney Homogenates*

State of homogenate	Diluent	NH <sub>3</sub> production	Anticomplementary activity
		$\mu\text{g. N/mg.}$	<i>units per mg.</i>
Ox kidney, after 8 wks. at 4°C.	Phosphate buffer containing glutamine	0.25	3
Rabbit kidney, freshly prepared	“ “	1.1	13
Rabbit kidney, after dialysis against saline	Saline, containing glutamine	0.6	25
Rabbit kidney, after dialysis against saline	Phosphate buffer containing glutamine	1.0	37

TABLE VI  
*Effect of Phosphate on Anticomplementary Activity of Kidney Homogenate*

Electrolyte solution	Volume dialysed kidney homogenate, ml.				
	0.2	0.15	0.1	0.07	0.05
Saline.....	50*	60	100	100	100
Phosphate buffer.....	0	0	40	50	60

\* Figures represent per cent hemolysis.

Sodium chloride was added, to a concentration of 0.15 M. The fluid was tested for capacity to inactivate complement, employing diminishing amounts of serum. In the control system the 50 per cent endpoint was obtained in the tube containing 0.05 ml. of the serum dilution, while the system containing soluble kidney extract required 0.1 ml. of the same dilution of complement.

The difference, while not great, is at least consistent with the possibility that renal glutaminase is anticomplementary. Undoubtedly most of the original activity had been lost during the chemical manipulations.

*Phosphate "Activation" of Anticomplementary Effect.*—In view of the fact that a distinctive characteristic of renal glutaminase is enhanced activity in

the presence of phosphate, experiments were planned to determine whether the anticomplementary effect of kidney tissue also varies with the presence of this ion.

In order to remove phosphate from the kidney homogenate and from the serum to be used as source of complement, 4 ml. samples of each were dialysed overnight against 4 liters of saline at 4°C., with constant stirring. The anticomplementary action of the homogenate was then tested in two different salt solutions, one a 0.15 M phosphate buffer containing 0.005 M MgSO<sub>4</sub>; the other 0.15 M NaCl with the same concentration of MgSO<sub>4</sub> added. Each of these salt solutions contained, in addition, 1 mg. glutamine per ml. (Titration of complement in the two solutions showed no appreciable difference, so that results obtained in the tests are not attributable to effects of pH or ionic strengths in the suspending fluids).

This experiment, with minor variations, was carried out four times, and the results were similar: anticomplementary activity of kidney tissue being greater in the presence of phosphate ions. The result of one experiment is shown in Table VI. In the NaCl solution neutralization of 3 × 50 per cent H.U. required 0.2 ml. of diluted kidney extract, whereas in the phosphate buffer this was obtained with only 0.07 ml. of the same extract.

*Effect of Glutamine.*—The foregoing experiment was repeated, comparing the anticomplementary effect of dialysed kidney homogenate in the same two salt solutions, with and without addition of glutamine. In addition to the enhancement by phosphate there was some additional enhancement from the glutamine. In one experiment the anticomplementary activity of a kidney homogenate was 20 units in saline, and 33 units in saline with glutamine. With phosphate buffer as diluent the two comparable values were 38 and 58 units. Considering the probability that all of the enzyme substrate and the phosphate would not have been removed by simple dialysis these differences give support to the thesis that the glutaminase system is responsible for the anticomplementary effect of kidney tissue.

#### DISCUSSION

The findings reported here establish that kidney tissue is capable of interfering with the *in vitro* destruction of Gram-negative bacilli by serum. The demonstration of a strong anticomplementary effect seems to provide an acceptable explanation for the mechanism of this interference. The studies of glutaminase activity are consistent with the hypothesis that the mechanism of the anticomplementary effect is associated with the formation of ammonia. Whether these *in vitro* studies can be regarded as shedding light on events which lead to the establishment of infection in the living animal must await further work.

The possibility that kidney tissue exerts its anticomplementary effect by formation of ammonia was suggested by the finding that the fourth component of complement was the one affected. The following findings had already

indicated that an enzymatic reaction was involved: (*a*) the active principle was found to be heat-labile and non-dialysable; (*b*) it was associated with tissue particles, and attempts at fractionation yielded evidence that more than one factor was participating; (*c*) the speed of the reaction was greatly affected by temperature. The enzyme principally or wholly responsible for ammonia formation, glutaminase I, has not yet been isolated, and is characterized somewhat vaguely in terms of specific inhibitors and "activation" by phosphate. Certain technical problems rendered difficult a comparison of all known properties of glutaminase I with the anticomplementary action of kidney tissue; for example, the finding that the inhibitors of glutaminase were also inhibitors of complement. However, it was possible to show a reasonably close parallel between quantity of ammonia liberated and extent of anticomplementary effect. The strongest evidence linking glutaminase with anticomplementary action came from the experiments testing for enhancement of action in the presence of phosphate and glutamine. Because of the fact that a test of anticomplementary activity requires the presence of serum, it was difficult to provide optimal conditions also for a determination of phosphate and glutamine requirement; nevertheless evidence of enhanced anticomplementary effect by glutamine and phosphate was found consistently. The differences obtained are probably as great as could be expected, in view of the difficulty of removing all phosphate and enzyme substrate from the necessary raw materials.

Regarding the relevance of these observations to infection in the living animal, it may be said first that the work was undertaken because a number of pieces of evidence indicated that there must be some flaw in host defenses in the kidney. The finding of an anticomplementary mechanism offers a plausible explanation for the susceptibility to coliform bacterial infection. The bactericidal power of serum for these organisms is a highly effective process, and the efficiency with which animals dispose of them following experimental inoculation suggests that a similar action is taking place within the body. The evidence that the medulla of the kidney is the principal site of ammonia formation seems to fit with previous findings that this zone of the kidney is more favorable to the initiation of coliform bacterial infection than is the cortex (3). The role of complement in defense against bacteria which are not subject to serum bactericidal action is less obvious, although there is good reason to believe that complement has an opsonic function (16). Conceivably, therefore, an environment in which complement is inactivated might be a more favorable one for infection by both Gram-negative and Gram-positive bacteria.

The fact that renal glutaminase activity can be caused to vary widely (9, 17, 18) suggests a possible means, not only to obtain further information on the problem under consideration, but also to improve methods of treating human pyelonephritis.

## SUMMARY

In studying the problem of the peculiar susceptibility of the kidney to coliform bacterial infection it was found that kidney tissue, unlike that of other organs, interferes with the ability of normal serum to destroy these organisms.

The effect was attributable to strong anticomplementary activity, 5 to 15 times greater than that of other organs. Inactivation of complement by kidney tissue was found to have characteristics of a chemical reaction, the active principle being heat-labile, non-dialysable, and difficult to separate from tissue particles. Attempts to purify it or to obtain it in a soluble form usually resulted in great loss in activity.

The component of complement affected was the fourth; *i.e.*, that which is characterized by susceptibility to injury by ammonia. Similarities were found to exist between the conditions of ammonia formation and complement inactivation by kidney homogenates, the most notable being enhancement by phosphate and glutamine.

The possibility is suggested that these findings may help to explain the vulnerability of the kidney to certain infections, especially those due to bacteria which are destroyed by the combined action of complement and antibody.

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