STUDIES IN IMMUNITY AND ANAPHYLAXIS.

THE PROTEINS OF THE KIDNEY AND LIVER.*

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The investigations of the past eight years, having for their object the demonstration of antisera for the nucleoproteins of various animal cells, have not given uniform results (1). For this reason, and also because the earlier work of one of us (Pearce) on this subject has been criticised, the present investigation was undertaken. The scope of the work has been somewhat broadened, in that an attempt has been made to produce antisera for globulins and albumins as well as for nucleoproteins. Further, in order to determine specificity, the same proteins have been used in experiments on anaphylaxis for the reason that by that method the question of relative specificity, or "special action," could more readily be determined. As most of the previous investigations were concerned with antisera for the liver and kidney, the work has been limited to the proteins of these organs.

METHODS.

The organs were rendered free of blood in situ by prolonged washing through the blood vessels. By placing a ligature about a cannula in the aorta at the level of the diaphragm and making an opening in the vena cava, a continuous flow of warm 0.85 per cent. salt solution was passed through the liver and both kidneys simultaneously. As the operation was done under ether anesthesia, the washing frequently was well under way before the death of the animal took place. Nine to twelve liters of salt solution were necessary to blanch the organs completely. Bile and urine were also removed to some extent by severing the bile ducts and ureters. The organs were hastily re-

* Aided by a grant from the Rockefeller Institute for Medical Research, New York. Received for publication, May 13, 1911.
moved, and the fat and coarser connective tissue having been dissected away, were passed quickly through a hashing machine. The resulting mass, after the addition of infusorial earth and sand, was pressed in a Buchner press. Two volumes of 0.85 per cent. salt solution and a little chloroform were added to the combined press juice and tissue pulp and the mixture was placed in a cold storage compartment at a temperature varying from $-1^\circ$ to $+2^\circ$ C. After eighteen to twenty-four hours, the coarser particles having been removed by straining through gauze, the residue was centrifuged to remove suspended particles. From the fluid thus obtained, the nucleoproteins were precipitated by acetic acid, which after eighteen to twenty-four hours rest in cold storage were removed by centrifugalization. The nucleoproteins were first repeatedly washed with 0.85 per cent. salt solution and then dissolved in it after the addition of sodium carbonate. This process of precipitation, centrifuging, washing, and solution was repeated two or three times, and the final precipitate was dissolved in 0.5 per cent. sodium bicarbonate. If not used immediately it was kept at a temperature close to freezing point. From the fluid remaining after the first precipitation of the nucleoproteins by acetic acid, the globulins and albumins were obtained after neutralization with sodium bicarbonate. Ammonium sulphate was added to half saturation, and after eighteen to twenty-four hours in cold storage the precipitate (globulins) was filtered off and dissolved in distilled water to which a little sodium bicarbonate had been added. This process was repeated once and the final solution allowed to dialyze in running water for from four to six days. It was then made alkaline with sodium bicarbonate, filtered, and kept in cold storage until used.

From the filtrate of the half saturated solution the albumin portion was obtained by complete saturation with ammonium sulphate. The precipitate was dissolved in distilled water and allowed to dialyze in running water for from four to six days. It was then filtered and placed in cold storage.

The separation of the globulin and albumin precipitates was hastened by suction filtration and by the use of the centrifuge. Except during the relatively short periods of filtration, the solutions were kept at a temperature close to the freezing point. As fresh preparations were made for each series of injections, the period elapsing between the completion of the preparation of the product and the time of injection was, with one exception, never more than twenty-four hours, and usually less than one hour. This one exception occurred in the liver series in which the same lots of nucleoprotein, globulin, and albumin were used for the fourth and fifth injections of each of these substances. The presence of nucleoprotein in the preparation known by that name was determined by demonstrating the presence of the purin bases by Kossel's method, which was considered a more satisfactory criterion than the phosphorus content.

The various fractions were injected into rabbits intraperitoneally, at intervals of five to seven days. A total of five injections was given, after which the animals were bled at the expiration of a period of at least seven days after the final injection. It seemed best, in order to keep the preparation in as natural a condition as possible, to estimate the amount injected as the equivalent of so many grams of moist liver or kidney substance. Thus, for example, when the total globulin yield of 120 grams of liver was injected into four
rabbits in equal quantities, we have considered each as receiving the globulin equivalent of 30 grams of liver substance. On this basis, all animals, in both kidney and liver series, received, at the first injection, nucleoprotein, globulin, or albumin equivalent to 20 grams of moist tissue with a gradual increase to an equivalent of 40 to 50 grams at the final injection.

RESULTS.

Tests in Vitro.—The results obtained by testing the activity of the sera in vitro are given in tables I and II. Much stress has been laid on the presence of agglutinins and precipitins in nucleoprotein sera as evidence of special or specific action. Although such substances may be present, they do not, if we are to follow the rules of bacterial immunization, indicate cytotoxic activity, any more than a fluid agglutinating the typhoid bacillus is to be considered as bactericidal. The tests were made with eighteen sera representing three rabbits of each of the six types of immunization; only one of each type, however, is given in the tables.

The manner of making the tests did not differ materially from that generally employed, except in some minor details.

In testing for agglutinins for organic cells, the washed organs were ground with a hashing machine, suspended in two volumes of 0.85 per cent. sodium chloride solution, and centrifuged at high speed (about 2,000 revolutions per minute) until three distinct strata could be seen. It was found that the middle stratum was most satisfactory. To one cubic centimeter of such a suspension, 0.1 c.c. of the antiserum was added, the mixture shaken and allowed to stand at room temperature. Agglutination of the red blood corpuscles was determined by adding 0.1 c.c. of the serum to 1 c.c. of a 5 per cent. suspension of unwashed dog's blood. This unwashed blood was used in order to approximate the conditions existing in the body in the injection experiments to be described later. Hemolysis was determined in the tubes used for hemagglutination. In the precipitation tests, 1 c.c. of the various solutions of globulins, nucleoproteins, and albumins was mixed with 0.1 c.c. of immune serum. This means that the serum was used in a dilution of 1 to 11. This dilution was followed throughout, but other dilutions were used with selected sera, as will be mentioned later.

The various solutions of nucleoproteins, globulins, and albumins, used in the precipitation tests, were similar in every way to those used for injection. Their reaction to litmus was neutral or slightly alkaline—as nearly neutral as the method of preparation would allow—but never acid.

Sera were used within forty-eight hours after withdrawal from
the rabbits, except that the same specific hemolytic serum was used for all the experiments. The results were usually controlled by independent readings by at least two persons.

As will be seen by reference to the accompanying tables (tables I and II), the results were controlled by a specific hemolytic serum, a normal rabbit serum, and by salt solution. The hemolytic serum used was prepared by making five injections, at intervals of five days, of five cubic centimeters of unwashed defibrinated dog's blood into the peritoneum of the rabbit. The normal rabbit serum was always drawn at the same time as the sera to be tested. The time of reading was as near one half hour, one hour, two hours, and four hours as convenience would permit. It was found that the results at the end of twenty-four hours were usually identical with those after four hours, and for this reason the later readings are not included in the charts.

Table I, which presents examples of the experiments with the different types of kidney sera, shows that the sera exert a rapid agglutinating effect on the kidney cell suspension and no effect on the liver cell suspension. That this is not specific is shown by the fact that the anti-liver sera as well as normal rabbit serum produce exactly the same effect. It appears to be a matter of agglutinability of cells rather than of the agglutinating action of the sera. The same general statement is true of the agglutination of red blood corpuscles. Two other sera in each of these three groups were tested with similar results as to agglutination, except that one antiglobulin serum failed to agglutinate kidney cells.

The sera are very slightly hemolytic except when incubated, under which circumstances one serum of each group showed moderate hemolyzing activity after two to four hours.

With regard to precipitation, it is seen that no serum has a precipitating action on dog serum analogous to that of the hemolytic immune serum. This held with the other sera tested, except in the case of one anti-albumin serum.

The anti-nucleoprotein serum presented in the table shows a late precipitating action on all the albumin solutions. This is characteristic of this type of serum, though the action was more rapid in one
of the sera not presented. The anti-globulin serum is entirely inactive, while the anti-albumin serum is highly active throughout. The only suggestion of relative specificity was seen in the case of one anti-globulin serum and one anti-albumin serum which precipitated the kidney solutions somewhat sooner than the liver solutions. Attempts to show a more definite specificity by the use of higher dilutions (1:100, 1:500, 1:1000) were fruitless, the sera not precipitating in these dilutions. The hemolytic serum does not act more powerfully on one group of protein than on the other.

In considering table II, in which the experiments with anti-liver sera are presented, it should be said that the statements made in the discussion of table I hold true as regards the agglutinating action of the sera on organ cells; that is, kidney cells and not liver cells are agglutinated. These sera are much less active agglutinators of the red blood corpuscles, however, than the anti-kidney sera, and are practically free from hemolytic activity.

The absence of precipitating action on dog serum, as shown in the table, was not uniform, as it occurred in a slight degree in one of three sera of each type. The hemolytic serum appears to be more active in this series than in the anti-kidney series, probably because it is somewhat older. As in the kidney series, higher dilutions of the anti-liver sera were tried, but no degree of specificity was observed.

It seems safe to conclude that the specific organic precipitating power of any of these sera is at best slight and relative only. Moreover, a similar power has been demonstrated in the anti-globulin and anti-albumin sera rather than in the anti-nucleoprotein sera. Absolutely no organ specificity is demonstrable as regards the agglutination experiments, since agglutination appears to depend on agglutinability of the cells rather than on specific properties of the sera. Hemolytic activity is absent in all the sera except after incubation, when it puts in a delayed appearance of slight degree.

Tests in Vivo.—The effect of the sera on living animals was determined by intravenous and intraperitoneal injections into dogs. All injections were in the proportion of two cubic centimeters of serum per kilo of body weight. The urine of all the animals was
carefully studied, as were sections from the liver and kidneys. Dogs suffering from spontaneous nephritis, or other conditions causing albuminuria, were excluded. As experience has led us to view with suspicion findings based on the examination of urine passed in the ordinary animal cages, we have kept all animals in metabolism cages, fed on a dog biscuit diet, and have used only female dogs, in order that the presence of coagulable protein in a "cage" urine might be controlled by the examination of a specimen obtained by catheterization. This precaution is most important and has transformed several apparently positive results into negative ones. Foreign coagulable protein in the urine as the results of vomiting, diarrhea, bloody vaginal discharge, and oozing from a wound have in each instance been detected by comparison with a catheterized specimen, whereas without the latter the result would have been considered positive or doubtful. Oozing of serum or blood from the wound necessary for intravenous injection has been prevented by utilizing a small vein of the lower leg, where a tightly fitting gauze dressing and bandage can be applied. All injections were made under ether anesthesia. The urine was examined by the heat and acetic acid test and with acetic acid alone for a control; to doubtful urines the ferrocyanid test was also applied.

Kidney Nucleoprotein Serum.—Six dogs received serum from four different rabbits. In two, the injection was intraperitoneal, and in the others, it was intravenous. Vomiting occurred immediately in the animals injected intraperitoneally, but not in those injected intravenously. It was transient in character and the animals, with the exception of two which developed diarrhea, remained in excellent condition. All were chloroformed at the end of from five to ten days. In one, a flocculent precipitate occurred in the "cage" urine on the first day, increasing to one eighth gram, by Esbach's method, on the third day, but on each of these days the catheterized specimen showed no coagulable protein. The source of this could be ascribed to oozing from the operation wound, the dog having torn away the bandage each night. In a second animal, the occurrence of a slight flocculent precipitate in the "cage" urine, not confirmed by the catheterized specimen, was traced to a previously unobserved mild diarrhea. In a third animal, the urine was negative for two days, but after a second injection of serum, a fine flocculent precipitate appeared on the third day, but was not detected on the three succeeding days. The urine of the remaining three animals was free from albumin.¹

¹ Statements concerning the presence of traces of coagulable protein, in the dog's urine must always be viewed with caution. In normal dogs, it is customary
Kidney Globulin Serum.—Six dogs were injected with this serum, four intravenously and two intraperitoneally. The urine in two of the former was free from albumin for five and seven days respectively; in a third, coagulable protein appeared on the day following injection and persisted until the third day when the animal was chloroformed. The amount of albumin for each day by Esbach's method equalled one fourth gram. The catheterized urine on the third day was strongly ammoniacal; and at autopsy a hemorrhagic cystitis was found. The interpretation of the albuminuria, therefore, was doubtful. The "cage" urine of a fourth animal, receiving two injections, the second of which was after an interval of four days, contained coagulable protein intermittently; but repeated catheterization showed none in the bladder urine. The coagulable protein in the "cage" urine was found to be due to contamination by diarrheal stools. Of the animals receiving the serum intraperitoneally, one developed albuminuria on the day following the injection. This persisted, equaling from one fourth to three eighths gram by Esbach's method, for four days, when the animals were chloroformed. No adventitious cause for the presence of albumin could be discovered. Upon histological examination, the kidneys of this animal show moderate granular degeneration of the tubular epithelium with an occasional granular cast in the collecting tubules. The second animal which received the same treatment showed no albumin for a period of eight days.

Kidney Albumin Serum.—Four animals were injected, of which three received the serum intravenously. One showed no albumin; the "cage" urine of the other two contained coagulable protein, which, however, was not present in the catheterized urine, and was made out to be due to a slightly bloody vaginal discharge. One of the two received two injections at two day intervals. The fourth animal, injected in the peritoneal cavity, developed albuminuria after twenty-four hours, which persisted until the eighth day, when the animal was chloroformed. By Esbach's test, the albumin varied from one fourth to one half gram. No cause other than kidney injury could be found for this albuminuria. Upon histological examination, the convoluted tubules show granular and fatty degeneration with much desquamation and, in the collecting tubules, an occasional fine granular cast.

The results in the above experiments are based on single injections, and in at least one animal of each series, on a double injection of the serum after an interval of two or four days. Still another experiment made for the purpose of comparing the action of the three different sera on the same animal, yielded negative results. Nucleoprotein, globulin, and albumin sera were successively injected intravenously at intervals of four days, and the animal was chloroformed six days after the last injection without producing albuminuria.

Liver Nucleoprotein Serum.—Seven animals were injected, four intravenously to obtain a slight turbidity on adding acetic acid or potassium ferrocyanid, which after standing for twenty-four hours settles to the bottom of the tube as a faint fluffy cloud. This turbidity is due, in all probability, to proteins of the mucin and nucleo-albumin type derived from the collecting tubules, which are normal constituents of dog's urine. It has therefore been disregarded, and we have considered as evidence of kidney injury only a flocculent precipitate or a turbidity produced by acetic acid which was greatly increased by the application of heat.
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and three intraperitoneally. Two of the first and three of the latter group showed no change in the urine, but one of the second group developed a severe attack of vomiting shortly after injection and died on the second day from acute intestinal obstruction due to intussusception. The two remaining animals of the series developed albuminuria on the day following injection. In one, the albuminuria on the second day equalled one fourth gram by Esbach's test and then began to diminish; in the other it equalled seven eighths gram on the fourth day, when the animal was employed for oncometric studies. Both animals were injected intravenously and had transient vomiting shortly after receiving the serum. No cause for the albuminuria, other than kidney injury could be found. The kidney of both these animals showed moderate granular and vacuolar degeneration with occasional casts.

Liver Globulin Serum.—In this series alone was a lethal effect observed. A dog under ether anesthesia receiving serum intravenously, in the proportion of 1 c.c. to 600 grams of body weight, died within five minutes. To eliminate the possibility of death from ether, the same serum was injected intraperitoneally in the same dose into two other animals. Both vomited excessively, showed evidence of collapse, and died after three and four hours respectively. At autopsy the spleen in each was intensely congested, and in one, a few small hemorrhages were found in the gastric mucosa. The blood in the vessels showed in each evidence of laking, and the urine gave a slight, but definite, flocculent precipitate by the heat and acetic acid test. The effects are suggestive of a powerfully hemolytic serum, but as all the serum had been used for injection, no in vitro tests could be made. The sera of three other rabbits in this series failed to produce this acute effect, and there was no renal disturbance during periods of five to eight days after either single or double injections given either intravenously or into the peritoneal cavity.

Liver Albumin Serum.—Seven dogs were injected. Vomiting was observed in each of two animals receiving an intraperitoneal injection but not after intravenous injection in the other five animals. The urine of six showed no albumin; the seventh showed albumin after twenty-four hours, which persisted until the third day when the animal was used for oncometric studies. The albumin reached three fourths gram by Esbach's test, and the urinary sediment contained a few fatty epithelial cells but no definite casts. Section of the kidney showed extreme granular and fatty degeneration but no casts.

To supplement the single and double injections, as in the kidney series, one animal was given intravenously, in succession, the three types of liver sera. The globulin serum followed by the albumin serum produced no changes in the urine; on the day following the administration of the nucleoprotein serum, which was given last, a hemorrhagic diarrhea developed and the animal died on the third day. At autopsy an extensive hemorrhagic colitis was found. The catheterized urine of the second and third days showed only a faint turbidity by the heat and acetic acid test.

DISCUSSION.

From the physiological reactions presented, it is evident that aside from the lethal effect of one globulin serum, the only evidence
of toxicity is presented by the somewhat frequent gastro-intestinal disturbances, vomiting, and diarrhea produced, and an occasional mild albuminuria. As albuminuria develops as often with liver as with kidney sera, the several disturbances appear to indicate an effect on the organs of elimination rather than a special action on the organs for which the sera are supposed to have a special affinity. The theory that the cell nucleoprotein is the essential substance in the production of a cytotoxic serum cannot be supported in view of the fact that a serum prepared by the injection of nucleoprotein is no more toxic than sera prepared from the globulins and albumins of the same organs.

Furthermore, the histological examination of the livers and kidneys of the injected animals gives no support to the view of "specific" or "special" action. The kidneys of animals receiving either liver or kidney sera showed the same general condition of moderate granular and vacuolar degeneration of the epithelium. Glomerular lesions were entirely absent except in animals killed by the peculiarly toxic liver-globulin serum. Abundant cast formation is never seen in sections, but a few casts were once found in the kidney nucleoprotein series, twice in the kidney globulin, and twice in the kidney albumin series. On the other hand, casts occurred seven times in the liver series. A comparison of the histological changes in the kidneys of animals receiving the various types of sera shows but little variation. The same moderate degenerative lesions are produced by liver and kidney sera alike, and represent apparently a common effect arising in the process of elimination through the kidney.

Moreover, the liver failed to show a relation between the type of serum injected and the histological changes present. Those cells of the liver which were not normal showed more or less granular and vacuolar degeneration, but this was not more frequent after the injection of liver serum than after kidney serum. Focal necroses were never seen.

ANAPHYLAXIS.

The failure to obtain by the methods of immunization antisera having special action led to the use of the methods of anaphylaxis.
The specificity of the anaphylaxis reaction in so far as the proteins of different animals are concerned is now a generally accepted fact, but organ specificity is by no means certain. Kraus, Doerr and Sohma (2), Andrejew (3), Uhlenhuth (4), and Pfeiffer and Mita (5) have clearly demonstrated the specificity of the anaphylaxis reaction for the proteins of the crystalline lens. Such anaphylaxis is a crossed reaction for the lenses of different species but not for serum, which fails to interact with the lens extract either as a sensitizer or as an intoxicating body. Pfeiffer and Mita, Thomsen (6), and Doerr and Moldovan (7) claim to have shown, by both active and passive anaphylaxis, a qualitative difference in reaction to erythrocyte proteins and serum proteins. Pfeiffer and Mita extend this observation to the differentiation of white and yolk of egg. Ranzi (8) has made an extensive study using the liver, kidney, spleen, and ovary, and has reached the conclusion that these give a species reaction but no evidence of organ specificity. Pfeiffer (9) objects to Ranzi's experiments on the ground that the organs used were unwashed and therefore could not be expected to show specificity either as sensitizer or intoxicator because of the natural content of serum protein. He did not repeat the experiments with all the organs that Ranzi used but confined his work to the crossed reactions with extracts of the kidney, spermatozoa, erythrocytes, and ox serum. He estimates, according to his elaborate formula, that the animals sensitized to a given organ extract respond more markedly to that extract than to the extracts of other organs, and he therefore claims a definite organ specificity. He admits, however, that the animals sensitized to kidney react very considerably to the proteins of spermatozoa, but he thinks that this is due to the close embryonic origin of kidney and testicle. In spite of his conclusions, an examination of his charts shows that the so-called specificity is not absolute but merely relative.

METHODS.

Our experiments differ from those of previous investigators in that instead of working with organ extracts we used the same protein fractions (nucleoprotein, globulin, and albumin) as in the immunization experiments. The protein solutions were of such concentration that one cubic centimeter contained the nucleoprotein, globulin, or albumin of one gram of organ substance, and when not neutral they were very faintly alkaline. As Ranzi has shown that larger injections are necessary for sensitization to organ extracts than to serum, the equivalent of two grams of organ substance was used. The intoxicating dose of the various proteins was likewise the equivalent of two grams of organ substance. The sensitizing dose of dog serum, used as a control, was 0.05 cubic centimeter, and the intoxicating dose two cubic centimeters. As controls, amounts of all protein fractions and of dog serum equal
# Table I.


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<th>Anti-blood serum</th>
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to the intoxicating dose were injected intravenously without effect into normal guinea pigs.

RESULTS.

A study of table III shows that the globulins and albumins are good sensitizers and especially so for the toxic dose of protein of an homologous organ. This is shown more definitely in the liver than in the kidney series. The liver nucleoprotein fraction sensitizes to the toxic dose of liver proteins more markedly than to the kidney proteins and appears to be fully as active a sensitizer as the globulins and albumins. On the other hand, a study of the sensitizing action of the kidney nucleoprotein shows an entirely different condition, this fraction being almost inert.

The same general relation holds in reference to the toxic action of the several fractions; the globulins and albumins being distinctly more toxic than the nucleoproteins and showing also a definite predilection for the homologous organ fractions. Just as the liver nucleoprotein is more active and slightly more specific than the kidney nucleoprotein as a sensitizing agent, so it is as an intoxicating agent.

A study of the sum of the reactions shows a relative organ specificity. Eight of the nine animals sensitized to liver fractions were distinctly anaphylactic when injected with liver fractions, whereas of the nine injected with kidney protein, only two were distinctly anaphylactic, the others showing only moderate or slight symptoms. In the same way, five of the nine animals sensitized to kidney fractions were distinctly anaphylactic to kidney proteins, and a similar group of nine failed to react when injected with liver proteins. In contrast with this slight organ specificity is the fact that in no sense can any degree of protein specificity be shown.

The crossed reactions with dog serum fail to indicate specificity. When the serum is used as a sensitizer, distinct anaphylaxis was produced by the subsequent injection of organ protein fractions with one exception (liver globulin), which probably was due to some individual idiosyncrasy in the animal. As a toxic agent, the serum is active almost uniformly, the only exception being in the animals sensitized to kidney nucleoprotein, a fraction notably defi-
cient in sensitizing power throughout the entire series. Animals sensitized to dog serum react no more strongly to toxic doses of serum than do those sensitized to the various organ protein fractions.

CONCLUSIONS.

1. The sera of rabbits injected repeatedly with the nucleoproteins, globulins, and albumins of the liver and kidney of the dog give no evidence in vitro or in vivo experiments of organ affinity. The precipitin test offers no proof of the specificity of these sera for the proteins employed as antigens.

2. The anaphylaxis reaction applied to the same proteins indicates a slight relative organ affinity but no specificity as far as the respective protein fractions are concerned. The relative organ affinity resides, rather, in the globulin and albumin fractions than in the nucleoprotein fraction. Dog serum used both as a sensitizing and an intoxicating agent gives rise to very active cross reactions with organ proteins, thus failing to support the theory of organ or of protein specificity.

3. These results do not support the view put forward that nucleoproteins play an important part in the course of production of cytotoxic immune sera.

TABLE III.

Anaphylaxis.

<table>
<thead>
<tr>
<th>Toxic Doses of</th>
<th>Kidney albumin</th>
<th>Kidney globulin</th>
<th>Kidney nucleoprotein</th>
<th>Liver albumin</th>
<th>Liver globulin</th>
<th>Liver nucleoprotein</th>
<th>Dog serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitized to</td>
<td>M.A.</td>
<td>A.</td>
<td>M.A.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
</tr>
<tr>
<td>Liver albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver globulin</td>
<td>S.A.</td>
<td>N.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
</tr>
<tr>
<td>Liver nucleoprotein</td>
<td>M.A.</td>
<td>S.A.</td>
<td>A.</td>
<td>S.A.</td>
<td>S.A.</td>
<td>A.</td>
<td></td>
</tr>
<tr>
<td>Kidney albumin</td>
<td>A.</td>
<td>A.</td>
<td>M.A.</td>
<td>N.</td>
<td>S.A.</td>
<td>S.A.</td>
<td>S.A.</td>
</tr>
<tr>
<td>Kidney globulin</td>
<td>A.</td>
<td>A.</td>
<td>N.</td>
<td>S.A.</td>
<td>S.A.</td>
<td>S.A.</td>
<td>S.A.</td>
</tr>
<tr>
<td>Kidney nucleoprotein</td>
<td>N.</td>
<td>N.</td>
<td>N.</td>
<td>S.A.</td>
<td>N.</td>
<td>M.A.</td>
<td>S.A.</td>
</tr>
<tr>
<td>Dog serum</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
<td>M.A.</td>
<td>A.</td>
<td>A.</td>
<td>A.</td>
</tr>
</tbody>
</table>

S.A. = slight symptoms, such as slight dyspnea, rubbing of nose, and general twitching.

M.A. = somewhat more marked symptoms, the twitching changing to slight general convulsions.

A. = very marked dyspnea, cyanosis, general convulsions, and coma, often followed by death.
BIBLIOGRAPHY.


