A PHYSIOLOGICAL STUDY OF EXPERIMENTAL NEPHRITIS DUE TO BACTERIAL POISONS AND CYTOTOXIC SERA.*

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In an earlier communication 1 we have described the vascular reactions characteristic of those forms of nephritis in the dog caused by the administration of various powerful renal irritants. Therein were discussed forms of nephritis in which either tubular or vascular changes predominated; the nephritides due to uranium nitrate, potassium chromate, and corrosive sublimate represented the first and those caused by arsenic and cantharidin, the second type. It was found, however, that although either a tubular or vascular lesion might be the predominating characteristic of a nephritis due to a given poison, it was impossible to conclude that a nephritis was purely vascular or purely tubular; and moreover it was shown that a tubular nephritis might pass into a stage closely resembling, if not identical with, the vascular type. This inability to demonstrate experimentally purely vascular or epithelial types of the affection is in accord with our knowledge that although in one form of nephritis in man the most striking lesion may be in the glomeruli, in another it may be in the tubular epithelium, and in neither is the evidence of kidney injury limited to the most apparent lesion. Experimental nephritis, however, cannot be brought absolutely into relation with the nephritides of man, because in the latter the powerful chemical agents used to produce the experimental lesions are seldom concerned etiologically, and the lesions which these cause, in so far at

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least as the metallic salts are concerned, are of a type seldom seen in man. We have therefore, in the present investigation, attempted the study of forms of experimental nephritis more nearly analogous in etiology and anatomical changes to those occurring in man. For this purpose diphtheria toxin has been employed as representing the etiological factor in a typical variety of nephritis of bacterial origin. Mallein and tuberculin have also been used as additional bacterial poisons. Certain cytotoxic sera, on account of the peculiar action on the kidney caused by their nephrotoxic and hemolytic properties, have likewise been utilized.

METHODS.

The methods employed are similar to those used in the earlier study and are, with slight modifications, based on the procedures described by Schlayer and Hedinger. Briefly stated these include a simultaneous kymographic record of the blood pressure, the volume of the kidney and the flow of urine. The blood pressure was taken from the left femoral artery with a mercury manometer; changes in the kidney volume were determined by the use of a gutta-percha capsule connected with a bellows recorder; and the flow of urine, obtained from a cannula in the bladder, was registered by an electrical drop-recorder. For testing the power of contraction of the renal vessels, adrenalin (Parke, Davis and Co.) in doses of one drop of the 1 to 1000 solution was used; and for testing the power of dilatation and diuresis, caffein or sodium chloride solution or both were employed. The caffein was used in the proportion of two cubic centimeters of a one per cent. solution, and the sodium chloride in the proportion of five cubic centimeters of a five per cent. solution per kilo. of body weight. Dog urine was used in doses of three cubic centimeters for the purpose of rapidly lowering the blood pressure and of testing the efficiency of the oncometer. All the fluids were introduced through a cannula in the right femoral vein, due regard being taken of their temperature and speed of injection.

All experiments were performed under complete ether anesthesia.

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Spontaneous nephritis and other accidental lesions of the kidney were eliminated by a preliminary period of observation. Alterations in the physiology of the kidney were eventually correlated with the anatomical lesions and the changes in the urine.

**THE EFFECT OF DIPHTHERIA TOXIN.**

Schlayer and Hedinger found that, in the rabbit, diphtheria toxin produced, as far as the vascular reactions were concerned, a type of nephritis lying midway between the typically tubular and the typically vascular. In the early stages the lesion was apparently tubular and in its later stages certainly vascular in character. The toxin which they used (Höchster Farbwerken 1½ fach normal Toxin) was injected subcutaneously as well as intravenously in doses of 0.25 to 1 cubic centimeter, and it caused albumin to appear within a few hours and casts within twenty-four hours; death occurred suddenly much as in cantharidin poisoning. Up to nine hours after injection the vascular reactions remained unaltered; the power of the kidney vessels to dilate and contract was unchanged and diuresis scarcely affected, although at the end of nine hours the urine contained a large amount of albumin, an occasional hyaline cast and a few erythrocytes. At twelve hours, though the vessels still reacted to stimuli, diuresis was lessened and the blood pressure indicated the beginning of general poisoning. At twenty-four hours diuresis and all vascular responses either failed or were minimal, and the kidney showed no pulsation. The last condition is obviously that of an overwhelming intoxication, and Schlayer and Hedinger produced it purposely in order to obtain the maximum effect on the kidney.

The object of our experiments was to produce at the outset, if possible, less severe lesions, the gradually increasing severity of which might be followed accurately. The toxin used was kindly supplied by Dr. W. H. Park, of the Research Laboratories of the New York City Board of Health. Its toxicity was approximately 1/800; that is, 1/800 cubic centimeter was sufficient to kill a 250 gram guinea-pig in four to five days; the exact lethal dose, however, was not determined.

The study of the effect of this toxin was made on ten dogs. Early in the work it was found that doses of 0.5 to 1 cubic centimeter caused death in dogs weighing from nine to eleven kilos, in from nineteen to twenty-seven hours. The animals were in such a weakened condition from the general effect of the toxin, that physiological studies were impossible. The powerful action of the toxin was shown by the occurrence of excessive vomiting, bloody diarrhea, muscular weakness, and, occasionally, convulsions. At autopsy a
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general hemorrhagic condition, involving the serous and mucous membranes, was present; acute splenic tumor, and cloudy swelling of the heart, liver, and kidney were constant lesions; and occasionally hemorrhages in the kidney were found. The urine after ten to twelve hours contained traces of albumin and, later, red corpuscles; anuria was not infrequent.

A striking feature of the action of diphtheria toxin and one that has been emphasized by Schlayer and Hedinger in their work on rabbits, is, after a preceding period of apparently perfect health, the sudden onset of symptoms. The latent period in our experiments varied from five to twelve hours and was followed abruptly by severe symptoms of prostration, and death after a comparatively short period. That the toxin produced very slight changes in the vascular system almost immediately which can be demonstrated by physiological methods, is shown in the following experiments in which large amounts of toxin were injected intravenously.

\[
\text{TABLE I.}
\]

\[
\text{Normal Dog.}
\]

Dog, weight 8,940 grams, ether anesthesia.

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood pressure in millimeters mercury</th>
<th>Kidney volume* in millimeters</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.15</td>
<td>142</td>
<td>0</td>
</tr>
<tr>
<td>3.16</td>
<td>142</td>
<td>0</td>
</tr>
<tr>
<td>3.18</td>
<td>136</td>
<td>−1</td>
</tr>
<tr>
<td>3.23</td>
<td>128</td>
<td>−3</td>
</tr>
<tr>
<td>3.28</td>
<td>138</td>
<td>−11</td>
</tr>
<tr>
<td>3.35</td>
<td>136</td>
<td>−4</td>
</tr>
<tr>
<td>3.45</td>
<td>134</td>
<td>−5</td>
</tr>
</tbody>
</table>

* The horizontal level of the writing lever at the beginning of the experiment is taken as normal kidney volume for the conditions obtaining. Increase or decrease in volume, as the case may be, is indicated in millimeters preceded by a plus or minus sign. The change in level was measured on the tracing with the base line as a constant factor.

This evidence of slight immediate effect of the toxin, coupled with the observations on the severe conditions developing after twelve to fifteen hours, led to the use of small doses which would prolong the period preceding marked vascular disturbance. We have in this way been able to delay the appearance of the late lesion for twenty-
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four hours, but never for forty-eight hours. To bring about this
delay, doses as small as 0.0015 to 0.003 cubic centimeter of toxin
per kilo. of body weight have been necessary. The early stage, which
is essentially a tubular nephritis, is illustrated in the following ex-
periment.

TABLE II.

Early Diphtheria Nephritis.

Dog, weight 8,000 grams, ether anesthesia. Received 0.003 cubic centimeter
diphtheria toxin per kilo. subcutaneously twenty-two hours before test began.
Urine showed trace of albumin.

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood pressure in millimeters mercury</th>
<th>Kidney volume in millimeters</th>
<th>Urine drops in 5 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial reading .</td>
<td>3.15</td>
<td>102</td>
<td>0</td>
</tr>
<tr>
<td>Adrenalin .</td>
<td>3.20</td>
<td>144</td>
<td>-25</td>
</tr>
<tr>
<td>Caffein .</td>
<td>3.21-3.26</td>
<td>94</td>
<td>-5</td>
</tr>
<tr>
<td>Salt solution .</td>
<td>3.30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dog urine .</td>
<td>3.39-3.44</td>
<td>130</td>
<td>+21</td>
</tr>
<tr>
<td>Dog urine .</td>
<td>3.45</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dog urine .</td>
<td>3.50-3.55</td>
<td>130</td>
<td>+19</td>
</tr>
<tr>
<td>Dog urine .</td>
<td>3.56</td>
<td>78</td>
<td>-60</td>
</tr>
</tbody>
</table>

This experiment, except for a somewhat unusual increase of blood
pressure after the injection of caffein, shows results typical of the
reactions in early tubular nephritis and varying but slightly from
those of the normal kidney. Similar results were obtained at the
end of twenty-four hours in other dogs receiving approximately the
same dose, but when the dose was raised to 0.025 cubic centimeter
per kilo. the vascular reactions were, after the same period, less
marked and diuresis less active. Even with smaller doses it was
impossible to carry the animals through the second twenty-four
hours without evidence of serious vascular disturbance. This is
shown in the following experiment.

If this record be compared with that of table II it will be seen that
the power of the renal vessels to contract under the influence of
adrenalin is unaltered, but that the power to dilate under the in-
fluence of caffein and salt solution is lost; at the same time diuresis
is, after the administration of caffein, unaltered and after salt solu-
tion is somewhat less than in the earlier stage of nephritis. This
record exhibits apparently the transition from a renal lesion of the
tubular type to one of the severe vascular type. So rapidly does the
TABLE III.

Diphtheria Nephritis, after Forty-eight Hours.

Dog, weight 7,980 grams, ether anesthesia. Received 0.0026 cubic centimeter diphtheria toxin per kilo., subcutaneously, 48 hours before testing. Urine contained albumin and casts.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>3.45</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>3.47</td>
<td>122</td>
<td>-31</td>
</tr>
<tr>
<td>Caffein</td>
<td>3.54-3.59</td>
<td>88</td>
<td>+2</td>
</tr>
<tr>
<td>Salt solution</td>
<td>4.00</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>4.13-4.17</td>
<td>108</td>
<td>+3</td>
<td>45</td>
</tr>
</tbody>
</table>

latter come on that we have been able to secure only this one observation showing the transitional stage. The usual effect at eighteen to twenty-four hours after large doses, or at thirty-six to forty-eight hours after small doses, is given in the next experiment.

TABLE IV.

Severe Diphtheria Nephritis.

Dog, weight 5,430 grams, ether anesthesia. Received 0.09 cubic centimeter diphtheria toxin per kilo., twenty-two hours before the test. Fifteen hours after injection the dog vomited and developed diarrhea; at twenty-one hours he was much prostrated. At fifteen hours urine contained albumin. Anuria noted during the last seven hours.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>3.00</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>3.05</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>3.14</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Salt solution</td>
<td>3.15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3.18-3.28</td>
<td>36</td>
<td>+7</td>
<td>0</td>
</tr>
<tr>
<td>Caffein</td>
<td>3.40</td>
<td>20</td>
<td>+4</td>
</tr>
<tr>
<td>3.41</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3.45-3.57</td>
<td>28</td>
<td>+2</td>
<td>0</td>
</tr>
</tbody>
</table>

This experiment appears to illustrate the severe cardiac and vascular disturbances attending profound intoxication with diphtheria toxin; conditions which are the result of the general effect of the toxin and which render impossible conclusions concerning its late local or specific action on the kidney.

The histological study of sections from the kidneys of this series...
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shows the presence of both tubular and vascular lesions. The latter are the more striking in that in the severer lesions almost every glomerular tuft is congested and surrounded by a greatly distended capsular space filled with serum and fibrin. The tubules generally are filled with densely packed coagulated material and their epithelium is granular and vacuolated, while that of the convoluted tubules is frequently completely disintegrated. Hyaline and granular casts are fairly abundant. In the case of the milder lesions the evidence of epithelial injury is more striking than that of vascular injury, while the kidneys of those animals which received very small doses of toxin show no exudative lesions whatever.

Both the anatomical and physiological studies indicate, therefore, that during the early period after the administration of diphtheria toxin, the condition in the kidney is analogous to that described by Schlager and Hedinger as a tubular nephritis; yet the severity of the disturbance of the later period, as shown by physiological methods, gives the lesion a prominent place in that group of nephritides in which vascular injury is the most prominent feature.

THE EFFECT OF TUBERCULIN AND MALLEIN.

We used these products in the hope of obtaining, by the use of bacterial poisons, vascular lesions of less severity than those caused by diphtheria toxin. In this we were disappointed, as the lesions were essentially those of a mild tubular nephritis. Both products, in concentrated form, were obtained through the kindness of Dr. Reichel of the Pennsylvania State Live Stock Board Laboratory. The tuberculin had been prepared from bovine tubere bacilli. When injected subcutaneously and intraperitoneally in doses varying from three to twenty cubic centimeters and repeated sometimes on two or three successive days, these substances failed to produce any general clinical effect. The urine of all animals, however, showed traces of albumin which was sometimes in large enough quantity to yield a slight flocculent precipitate. Physiological studies of the kidney failed to show results differing from those shown by an early tubular nephritis. Somewhat exaggerated vascular reactions and free diuresis occurred such as are seen in the early slight nephritis due to uranium and chromium salts. Sometimes the vari-
RATION was so slight as to suggest merely an exaggeration of the reactions of the normal kidney; but the presence of albumin in the urine and the histological evidence of extensive granular and vacuolar degeneration of the tubular epithelium pointed conclusively to a tubular nephritis of slight severity. These effects, when contrasted with the severe vascular disturbance caused by diphtheria toxin, are of interest as demonstrating the occurrence of a renal lesion of bacterial origin essentially epithelial in character.

**THE EFFECT OF NEPHROTOXIC IMMUNE SERUM.**

The lesion caused in the dog's kidney by the so-called nephrotoxic immune serum\(^a\) consists of a form of renal injury more closely analogous etiologically to certain forms of nephritis in man than those produced by other experimental procedures, and it is therefore highly worth study by physiological methods. A serum prepared by the repeated injection into the rabbit of dog kidney cells, freed from blood, has been utilized for this purpose. Four dogs received serum from three treated rabbits, in doses of two cubic centimeters per kilo. of body weight. Physiological tests were made on the second, third and fourth days after the injection, and at a time when the urine of the respective animals contained abundant casts and an amount of coagulable protein varying from three fourths to three and one half grams by the Esbach method. The tests gave results characteristic of tubular nephritis; that is, exaggerated vascular reactions and excessive diuresis.

Histologically the kidneys of these animals showed lesions of the tubular epithelium with fairly abundant cast formation, and occasionally a slight accumulation of serum in the glomerular capsule. Physiological study, therefore, supports the conception based on histological examination, that this lesion consists essentially of a tubular nephritis. The slight changes in the glomerular capsule, however, point to some vascular injury, presumably of such a nature or of so slight a degree as not to be recognized by physiological methods.

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THE EFFECT OF HEMOLYTIC IMMUNE SERUM.

The most characteristic lesion in the kidney of an animal receiving a hemolytic serum is, aside from evidence of the destruction of the red corpuscles and of epithelial degeneration, the occurrence of an exudate, composed chiefly of serum, in the glomerular capsule. As this effect is evidence of injury to the capillary loops of the glomerular tufts, the lesion would appear particularly suitable for the study of vascular reactions. A hemolytic immune serum was prepared by repeatedly injecting rabbits with the red blood corpuscles of the dog, and the serum so obtained was then injected intraperitoneally into dogs, in amounts sufficient to cause hemoglobinuria. The physiological tests were performed at two periods: (a) the height of the elimination of hemoglobin; and (b) shortly after it had disappeared from the urine.

The results of these tests, contrary to the expectation based on the anatomical evidence of extensive glomerular injury, were not such as to indicate the existence of vascular insufficiency, but were those indicative of the occurrence of a so called tubular nephritis. Exaggerated reactions to caffeine and salt solution, and marked diuresis instead of the expected decreased dilatation and diminished diuresis were obtained. The results indicate that injury to the vessels of the kidney sufficient to permit exudation is not necessarily, physiologically speaking, that leading to reactions of vascular nephritis; but unlike the lesions caused by arsenic and diphtheria toxin, cannot be recognized by the physiological procedures in this investigation. The injury produced by arsenic is apparently one interfering with the mechanism which causes the vessels to contract and dilate, while the lesion following hemoglobinuria is, apparently, one affecting only the endothelial membrane which controls the passage of fluid. It is evident, therefore, that we may have, as the lesion indicates, anatomical evidence of vascular injury which cannot be recognized by the physiological methods employed for the purpose by Schlayer and Hedinger; while, on the other hand, we may have, in arsenical nephritis, physiological evidence of serious vascular nephritis in the absence of demonstrable evidence of anatomical changes.
CONCLUSIONS.

The physiological study of experimental nephritis caused by poisons of bacterial origin demonstrates that the poisons may produce types of nephritis in which either vascular or tubular changes predominate. Diphtheria toxin produces a nephritis which in its late stage is of the vascular type, but in its early stage is distinctly tubular. Tuberculin and mallein uniformly cause lesions of the tubular type, which do not pass into the vascular type.

Nephrotoxic and hemolytic immune sera cause changes in the kidneys which by physiological methods of observation present no evidence of vascular injury, but which are anatomically characterized by exudative glomerular lesions of moderate severity. This discrepancy between the results of anatomical and physiological study indicates that a lesion of the membrane controlling the passage of fluids may occur without alteration in the power of the vessels to contract and dilate. This fact is shown clearly by the lesion caused by hemolytic immune serum, which is in sharp contrast to the lesion caused by diphtheria toxin, since the latter substance not only alters the permeability of the membranes but also influences markedly the power of the vessels to contract and dilate. It is necessary therefore if the term "vascular" is used in its broadest sense, to recognize three types of vascular nephritis: (1) one in which little or no anatomical evidence of vascular injury is found, but in which physiological methods show profound vascular changes, as in arsenical nephritis; (2) one in which anatomical evidence of vascular (exudative) injury is prominent, but in which the physiological tests are negative, as in nephritis caused by a hemolytic immune serum; and (3) one in which both anatomical and physiological changes are prominent, as in diphtheria toxin nephritis.