EXPERIMENTAL ACUTE GLOMERULITIS*

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PLATES 20 TO 22

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It is believed by many that acute glomerular nephritis follows as a sequel to some infectious process, the cause of which is usually streptococci, often of the hemolytic type. Observations on the forms of infection associated with acute diffuse glomerular nephritis, and the varieties of bacteria producing these infections have been published elsewhere (1). In another paper (2) there was some discussion of the idea that the onset of the acute attack of glomerular nephritis was dependent, not exclusively upon the acute infection, but, in addition, upon an altered reaction of the body to the infection, an allergic state which might render the cells and capillaries of the kidney peculiarly vulnerable to injury.

The attempts of many investigators to produce glomerular nephritis in animals by the intravenous or subcutaneous injections of bacteria, or by the products of their growth or disintegration, have been only partially and irregularly successful. A series of experiments was therefore devised to determine whether an induced state of hypersensitiveness could be produced, that would increase or alter the response of the kidney cells to large doses of bacterial bodies when they were brought in concentrated form directly in contact with the renal circulation.

The experimental production of glomerular nephritis in animals has been recently reviewed by McNider (3), Leiter (4) and Fishberg (5). The majority of investigators have employed streptococci for this purpose. Ophüls (6) described occasional glomerular lesions after the intravenous injection of rabbits with

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streptococci; while Bell, Clawson and Hartzell (7) have noted scattered glomerular lesions in monkeys after the repeated injection of both hemolytic and non-hemolytic streptococci. Indefinite or entirely negative results have been obtained by LeCount and Jackson (8), by Faber and Murray (9), by Major (10) and by Leiter, all of whom injected streptococci intravenously. Leiter also employed intracardiac injections, to avoid the pulmonary filter, with negative results. Kinsella and Sherburn (11) infected the aortic valves of dogs with Streptococcus viridans, and found that those which died within 12 to 14 days showed no glomerular lesion, while those which lived longer than 14 days showed hyaline thrombi and hemorrhages within the glomerulus. Clawson (12) describes focal embolic nephritis following repeated intracardiac injections of Streptococcus viridans emulsified with agar. Asch (13) employed the method of injecting bacteria directly into the renal artery in an attempt to produce changes in the kidney. The same method has been used by Winternitz and Quimby (14), by Bloomfield (15), by Pappenheimer, Hyman and Zeman (16), Leiter, and Long and Finner (17). Winternitz and Quimby injected cultures of B. bronchisepticus into the renal artery of dogs and obtained a variety of inflammatory lesions, in some instances involving the glomeruli. Both Bloomfield and Leiter used dead cultures of Streptococcus viridans with negative results. Pappenheimer, Hyman and Zeman studied the acute changes occurring 24 hours after the injection of living and heat killed hemolytic streptococci into the renal artery of normal and sensitized rabbits. They describe lesions in the glomeruli which were more extensive in the sensitized than in the normal rabbits. Exclusive of the extensive work with diphtheria toxin, there is little evidence to show that the products of bacterial growth, or their "soluble toxins," will cause glomerular lesions in normal animals. Morse (18) used toxic filtrates of staphylococcus with negative results, and Stoddard and Woods (19) employed extracts of streptococcus, staphylococcus and tuberculin without effect. Duval and Hibbard (20) report the reproduction of glomerular nephritis in dogs and rabbits by the intravenous injection of filtered bacteriolyzate, obtained from the destruction of hemolytic streptococci injected into the peritoneal cavity of rabbits immunized against this organism. Reith, Warfield and Enzer (21) were unable to confirm these observations. Rich, Bumstead and Frohisher (22) have produced glomerular lesions, with hemorrhage, in rabbits by the intravenous injections of filtrates of cultures of Streptococcus viridans. Gray (23) describes lesions, which were not proliferative in character, in the glomeruli of rabbits, following the injection of filtrates from scarlatinai streptococci. Long and Finner produced extensive lesions in the kidneys of tuberculous swine by the infusion of tuberculin into the renal artery.*

*Blackman, Brown and Rake (Bull. Johns Hopkins Hosp., 1931, 48, 74) report the results of convincing experiments, which show that both acute and subacute nephritis, with edema of the tissues, can be produced in rabbits by repeated intravenous injections of an autolysate prepared from Type I pneumococcus. Both the glomeruli and the tubular epithelium were affected.
Though it has, apparently, been possible at times to obtain scattered glomerular lesions resembling the focal or embolic form of glomerular nephritis in various animals by several methods of experimentation, one must conclude that the histological changes characteristic of the acute diffuse glomerular nephritis in man, or even of an extensive focal glomerular nephritis, have not yet been satisfactorily or regularly produced in animals.

Efforts were first made to produce chronic infections in the paranasal sinuses of goats with hemolytic streptococci with the idea of reproducing conditions that would be analogous to those in man. Probably for mechanical reasons, an infection of the sinuses could not be obtained. A number of preliminary experiments were next made with rabbits. Filtrates of hemolytic streptococci in doses of 10 cc. per kilo were injected intracardially into normal rabbits; and filtrates of hemolytic streptococci, of "Dick Toxin" and of lysates, obtained according to the technic of Duval and Hibbard, were injected into the left renal artery of normal rabbits in doses of from 1.5 cc. to 8.0 cc., the average dose being 3 cc. In sixteen such experiments an examination of the kidneys from 24 hours to 4 days after the injection showed no essential differences between the right and left organs.

After these preliminary experiments it was decided to employ bacterial bodies instead of soluble "toxins," and to study the effect of bacterial bodies upon the kidneys of normal rabbits and of rabbits in which there existed a local streptococcus infection. It has been shown by Zinsser and Grinnell (24), Dochez and Sherman (25), Dochez and Stevens (26), Derick and Swift (30) and by one of us (2) that the skin of rabbits, in which a local streptococcus infection has been produced, acquires the property of reacting to an intradermal injection of the filtrates of hemolytic or green streptococci. The reaction is characterized by an area of erythema which is often edematous. This phenomenon is usually ascribed to the presence of an allergic state. Realizing perfectly that we cannot define this change accurately, and that the rabbits, in the experiments to be reported, were locally infected as well as skin hypersensitive, we will, for convenience sake, term the animals prepared in this manner as "hypersensitive" or "sensitized."
Methods

Two strains of \( \beta \) hemolytic streptococci were used in these experiments. One (SD2) was obtained from the acutely inflamed tonsils of a case of acute nephritis. It produced, by the method previously described (2), a filtrate that was neutralized consistently by convalescent scarlatinial serum in skin tests, and did not produce skin reactions in doses of 0.5 cc. in normal rabbits. The lethal dose of this strain for mice was 0.5 cc. of a 24 hour beef infusion broth culture. The second strain was obtained through the kindness of Dr. Shwartzman, and the filtrate differed from filtrates of many other strains, with which we have worked, in that it caused quite frequently mild or marked skin reactions in normal rabbits. The animals were prepared or “sensitized” by the intradermal injection of 0.2 to 0.5 cc. of a heavy suspension of living \( \beta \) hemolytic streptococci obtained from washing the 24 hour growth from the surface of blood agar slants. At the same time, skin tests were usually made by the intracutaneous injection of 0.5 cc. of filtrate of the same strain of streptococcus, and of 0.2 cc. of heat killed culture. 5 to 10 days after the primary inoculation, a second skin test was performed, and if this proved positive the animals were used for operation on that day. In a few instances a repetition of the procedure was necessary before satisfactory skin reactions were obtained. The normal rabbits, which were used for comparison, were not subjected to preliminary skin tests for fear that this procedure might alter their reaction to the injections of vaccine made into the renal artery. Cultures of the two strains of hemolytic streptococci, killed by heating at 60°C. for 1 hour, were used for the injections into the renal artery. Vaccines were selected for this purpose because they lacked the power of growth and invasiveness, and because as particulate matter they might be retained in the glomerular capillaries longer than soluble “toxin.” With the idea of subjecting the kidney to the possibility of considerable damage, 3.0 to 4.0 cc. of a suspension (usually one 24 hour blood agar slant in 2.0 cc. of saline) of vaccine was injected directly into the left renal artery under aseptic precautions. The animals were anesthetized by ether. The artery was exposed rapidly by blunt dissection without manipulation of the kidney. A 27.5 gauge needle, bent at a right angle, was inserted in the artery. The injection fluid replaced the arterial flow and the kidney became very pale in color. Within a few seconds after the release of the artery and the cessation of the injection, the normal color returned to the kidney. The operation usually required but a few minutes. The method is summarized in detail in Protocol I.

Since it was desired to study any acute changes that might occur in the kidney, the animals were killed from 24 hours to 8 days after injection. Later in the experiments a small number of animals were killed within a few minutes to 24 hours after injection.
Protocol I


March 19, 1930—At Site A—0.5 cc. killed culture Shwartzman strain intracutaneously. At Site B—0.5 cc. living culture Shwartzman strain (one 18 hr. blood agar slant in 1.2 cc. NaCl) intracutaneously. At Site C—0.5 cc. filtrate Shwartzman strain intracutaneously.

March 20, 1930—A = erythema 1.0 cm.; B = edema, erythema, hemorrhage, necrosis 8.0 cm. x 2.5 cm., C = faint erythematous blush.

March 21, 1930—A = papule 1.0 cm.; B = area 8.0 x 2.5 cm.; C = faint erythema.

March 22, 1930—A = same; B = area 8.0 x 3.0 cm. suppuration; C = negative.

March 24, 1930—At new site A'—0.5 cc. Shw. strain killed culture intracutaneously. At new site C'—0.5 cc. Shw. strain filtrate intracutaneously.

March 25, 1930—A = negative; B = 8.0 cm. x 4.0 cm.; C = negative. A' = papule and erythema 2.5 cm. x 2.5 cm.; B' = erythema 2.5 cm. x 3.5 cm.

March 25, 1930—Injection of 2.5 cc. Shwartzman strain killed culture in left renal artery. 9.43 a.m., ether started; 9.51, abdomen opened; 9.53, left kidney exposed; 9.54½, ligature in place; 9.56, injection started; 9.57½, injection complete, artery released; 9.57½, kidney white; 9.58, color returned; 10.04, ether stopped.

March 26, 1930—24 hours after operation rabbit killed. Right kidney normal; left kidney yellowish with pale mottling. Cut section pale yellowish, cortex thicker than right.

Microscopically.—right kidney normal. Left kidney—practically all glomeruli affected; hyaline thrombi, polymorphonuclear infiltration, pericapsular infiltration, many hyaline casts; collections of small round cells about large arteries. (See Fig. 1.)

Bacterial Stain.—Right kidney—no Gram-positive cocci. Left kidney—Gram-positive cocci plainly seen in a moderate number of glomeruli.

RESULTS

A total of 52 rabbits was used for these experiments, 23 of which had been infected or sensitized, and 29 of which were normal. Of the entire group 25, or 48 per cent, showed acute lesions in the left kidney, and since similar lesions could not be found in the right kidney, which served as a control, it may be concluded that the changes in the left kidney were caused by the vaccine injected into the left renal artery. A description will first be given of the lesions and then the incidence of these lesions will be compared in the infected or sensitized group with those in the normal group.

Although the changes in the glomerular tufts attracted particular
attention, both the tubules and the interstitial tissue were usually
affected to some extent. The process was therefore diffuse, inasmuch
as the different elements of the kidney structure were involved, but
since it rarely happened that all portions of the cortex shared equally
in these changes, the lesions often occurred in patches and were thus
focal in distribution.

The earliest abnormalities that were observed were seen in the
left kidney of a normal rabbit, which was killed 4 hours after the in-
jection of killed culture into the left renal artery.

In this instance a few tubules were dilated and contained hyaline casts. Most
of the glomeruli appeared normal, but here and there a glomerulus was seen in
which isolated capillaries were filled with hyaline thrombi about which were
collected polymorphonuclear leucocytes. Other glomeruli showed an unusual
number of polymorphonuclear leucocytes within the capillaries. Similar changes
were observed in the left kidney of another normal rabbit killed 8 hours after the
injection of killed culture into the left renal artery.

Nine rabbits were killed 24 hours after the intraarterial injection,
of which five were normal and four were supposedly sensitized. The
left kidneys of two of the five normal rabbits and of two of the four
sensitized rabbits were abnormal.

The left kidney of three of these rabbits, two of which were normal and one of
which was sensitized, showed very slight changes. These consisted of isolated
collections of small round cells about the arcuate vessels, dilatation of occasional
tubules which contained hyaline casts, and the presence in isolated glomeruli of
hyaline thrombi and collections of polymorphonuclear leucocytes. The left
abnormal kidney from the second rabbit, which had been sensitized, showed
changes that were similar except that they were much more extensive. A con-
siderable number of glomeruli were involved. There were many in which the
glomerular capillaries showed hyaline thrombi, often containing fragmented nuclei.
Several glomeruli showed an increase of polymorphonuclear leucocytes in the
capillaries, and some showed a collection of a few polymorphonuclear leucocytes
directly about the capsule. Many tubules contained hyaline casts. (Fig. 1.)

Thirteen rabbits, eight of which were normal and five sensitized,
were killed 48 hours after intraarterial injection. Three of the eight
normal rabbits and four of the five sensitized rabbits showed lesions
in the left kidney.
The intensity and extent of the changes varied considerably. In the sections in which there was least change, there were the same scattered perivascular collections of mononuclear cells and dilated tubules containing casts, such as were seen in the 24 hour sections. There were a few hyaline thrombi in the glomerular capillaries, but many glomerular capillaries appeared empty or were unusually cellular. In some glomeruli there were localized collections of large cells of mononuclear type.

In the left kidney from two rabbits the changes were very extensive. There was edema with more or less diffuse infiltration of small mononuclear cells between the tubules. The tubular epithelium was swollen and hyaline casts were numerous. Practically all the glomeruli were involved in the process. The capillaries were often obliterated by hyaline thrombi and the tufts were frequently infiltrated with polymorphonuclear leucocytes. In most of the glomeruli the capillaries did not contain blood. Many of the tufts appeared to consist of necrotic material infiltrated with leucocytes and containing nuclear fragments. There was frequently a pericapsular infiltration of mononuclear leucocytes, but hemorrhages were not seen in the glomerular capsules. A crescentic arrangement of fibrin or necrotic material containing nuclear fragments or elongated cells was very occasionally observed in the glomerular spaces. (Figs. 2 and 3.)

Seventeen rabbits, 9 of which were normal, and 8 of which were sensitized, were killed 3 to 5 days after the intraarterial injection. Fifteen of the total 17 rabbits were killed on the 4th day. More or less extensive lesions were found in the left kidney of all of the 8 sensitized rabbits and very slight lesions in 3 of the 9 normal rabbits.

The changes varied again in this group from scattered lesions to the most extensive and diffuse alterations which occurred in two of the sensitized rabbits. In the kidneys that were least involved the perivascular round cell infiltration was moderate in extent, and there was little change in the tubular epithelium. 10 to 20 per cent of the glomeruli were usually involved. The process appeared much less acute than that studied 48 hours after infection. Occasionally hyaline thrombi were seen but these were rare. More often a collection of large cells was seen in one-half or one part of a glomerulus, filling that part of the capsular space, increasing the size of the glomerulus and sometimes surrounding a hyaline-like center. Other glomeruli were much enlarged, filled the capsular space and were apparently composed of large cells with pale nuclei. It was frequently impossible to distinguish the capillaries. Occasionally there were crescentic bodies about these abnormal glomeruli. Collections of small round cells were sometimes seen about the capsules. (Fig. 4.) The sections from the kidneys showing the extensive lesions were somewhat similar to those examined 48 hours after injection. In one kidney there were definite thrombi in small arteries with infarctions of the cortex. Edema and diffuse infiltration of small round cells was marked. Practically all glomeruli were affected. Capillary thrombosis, necrosis and diffuse
infiltration of the glomeruli were common. Many glomeruli showed the presence of the large cells already described. This gave the appearance of proliferation of the glomerular endothelium or lining cells. Crescentic formations composed of necrotic material or blood, infiltrated with large cells, were frequently seen, and pericapsular infiltration especially by mononuclear cells was marked. (Figs. 5, 6 and 7.)

Twelve rabbits were killed 8 days after injection. Six of these were sensitized and 6 were normal. The kidneys from the normal rabbits showed no abnormal changes. Three of the sensitized rabbits showed left kidneys that were abnormal.

In one of these the changes were extensive and diffuse. Many tubules were dilated and contained hyaline casts, some tubules appeared atrophied. The infiltration of small round cells was diffuse and very marked in places. There was no definite increase in interstitial connective tissue. Almost no normal glomeruli could be found. Many were large and appeared as though composed of large cells with pale nuclei which looked much like the cells of the convoluted tubules but were smaller. Other glomeruli were similar but smaller and the capsule was thickened and cellular. Still others were infiltrated with mononuclear cells, a few so densely that the glomerulus could scarcely be identified as such. Occasionally the infiltration of cells was in crescentic form. There were no hemorrhages. (Figs. 8 and 9.)

The impression that one gains from a study of these sections is of a pathological process which may be detected within 4 to 8 hours after the intraarterial injection of killed streptococci, which progresses rapidly, reaching an acute florid stage within 4 to 5 days, and appears as if undergoing a healing or reparative process by the 8th day. The glomeruli show hyaline thrombi within the capillaries, with necrosis, infiltration of the tufts with polymorphonuclear leucocytes and later small round cells; pericapsular infiltration; the formation of crescents, and an increase in large cells in the later stages of the process. The interstitial tissue shows focal or diffuse infiltration by mononuclear cells and edema. The tubules often show swelling of the epithelium and hyaline casts are numerous, but red blood cells were very rarely seen within the lumen of the tubules.

A comparison of the incidence of the lesions that have been described in the infected, or sensitized group, of rabbits with the normal group, shows that glomerulitis occurs much more frequently in the former. Of the 23 infected or sensitized animals the histological changes of
glomerulitis were present in 17, or 73.9 per cent; of the 29 normal animals they were present in 8, or 27.5 per cent. Further analyses of these changes, according to the strain of streptococcus that was employed, and the severity of the lesions that were produced, are shown in Tables I and II. In the experiments with Strain SD2, the filtrates from which did not cause skin reactions in normal rabbits, the incidence of lesions in the normal rabbits was low: 18 per cent; whereas in the experiments with Strain Shw., the filtrates of which did often produce skin reactions in normal rabbits, the incidence of renal lesions was comparatively high: 57 per cent. It was also noted that the total incidence of lesions with the Shw. strain is much higher: 76 per cent + than it is with the SD2 strain: 28 per cent +.

The difference in the figures between the two groups of animals is sufficiently great to be of real significance, and though it will be necessary to repeat these experiments, and possibly to modify them in one way or another, before one can draw definite conclusions, the

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**TABLE I**

Comparison of the Incidence of Glomerulitis in Normal and “Sensitized” Rabbits

<table>
<thead>
<tr>
<th>Strain</th>
<th>Normal</th>
<th>“Sensitized”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per cent</td>
</tr>
<tr>
<td>SD2</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Shw.</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total..</td>
<td>29</td>
<td>21</td>
</tr>
</tbody>
</table>

**TABLE II**

Severity of Glomerulitis in Normal and “Sensitized” Rabbits

<table>
<thead>
<tr>
<th>Extent of lesion</th>
<th>Normal</th>
<th>“Sensitized”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pos.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>++</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>+++</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>++++</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

The difference in the figures between the two groups of animals is sufficiently great to be of real significance, and though it will be necessary to repeat these experiments, and possibly to modify them in one way or another, before one can draw definite conclusions, the
observations show that, when killed hemolytic streptococci are injected into the renal artery of rabbits, acute exudative lesions in the glomeruli and interstitial tissues are much more prone to occur in infected or sensitized animals than in normal animals.

There are other factors, however, which might have some bearing on this differentiation, and which, therefore, had to be taken into account. During the injection of the renal artery there is, of necessity, a momentary cessation of arterial blood flow to the kidney; and it is conceivable that variations in the time of possible renal anoxemia, and of the interference with the blood flow might have some influence in predisposing to the renal lesion.

### TABLE III

*Relation of Duration of Occlusion of Renal Artery before Intraarterial Injection to Occurrence of Glomerulitis*

<table>
<thead>
<tr>
<th>Duration of arterial occlusion(min.)</th>
<th>Total animals</th>
<th>Glomerulitis present</th>
<th>Glomerulitis absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>18</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>1-2</td>
<td>11</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2-8</td>
<td>23</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>

In Table III, figures are given which show the relation of positive and negative results to the duration of the occlusion of the renal artery. There is no indication that this factor is of importance in determining the occurrence of renal lesions. When the figures are further divided among the infected or sensitized and the normal rabbits, they are quite scattered and show even less evidence that the arterial occlusion has been a factor in the incidence figures. Moreover, the actual occurrence of severe lesions, after very short arterial occlusion, and the absence of lesions, after a very long occlusion lasting 6 to 9 minutes, leads us to disregard this factor.

A study was next made of sections of the kidneys by bacterial stain, to determine how long after the injections the presence of streptococci could be demonstrated in the kidney; and whether the
persistence of these dead bacteria was related to the presence of glomerular lesions.

In four rabbits, 2 normal and 2 sensitized, dying 5 minutes after injection, numerous Gram-positive streptococci were found in the majority of glomerular capillaries. Two rabbits killed 4 hours after an injection of 1.0 cc. of vaccine showed an increased number of polymorphonuclear leucocytes in the glomerular tufts with moderate numbers of Gram-positive streptococci in the glomerular capillaries.

Into the renal artery of two normal rabbits were injected 3.0 cc. of vaccine from Strain Shw. One rabbit killed 8 hours later showed slight acute lesions in many glomeruli, which contained Gram-positive cocci. The other, killed 16 hours after injection, showed no abnormalities in the kidney and bacteria could not be found in stained sections. The result of the examinations of the kidneys from the remaining rabbits killed from 24 hours to 8 days after the injection are given in Table IV.

It can be seen from Table IV that, though demonstrable streptococci were by no means constantly present in the kidneys which showed lesions, they were found considerably more often in these kidneys than in the normal kidneys. In this respect there was no essential difference between the kidneys of normal and of sensitized rabbits. When streptococci were found in the abnormal kidneys they were more numerous when the lesions were diffuse than when they were scattered or focal. Although a constant relationship between the presence of glomerulitis and the presence of streptococci in the lesions could not be demonstrated, it must be inferred that such

<table>
<thead>
<tr>
<th>Rabbit killed; time after injection</th>
<th>Total</th>
<th>Glomerulitis present</th>
<th>Glomerulitis absent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cocci +</td>
<td>Cocci 0</td>
</tr>
<tr>
<td>24 hrs.</td>
<td>9</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>48 &quot;</td>
<td>13</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4 days</td>
<td>17</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>8 &quot;</td>
<td>13</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total...</td>
<td>52</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>
a relationship does exist; but it is evident that streptococci may remain in the glomerular capillaries for many hours without giving rise to definite alterations, and the observations show that extensive changes may progress without the demonstrable presence of streptococci.

It was finally necessary to determine whether particulate matter, of approximately the same size as the minute clumps of streptococci in the vaccines, could produce changes when injected into the renal artery of rabbits. For this purpose bismuth oxychloride was selected, since the material was insoluble in physiological salt solution, and since the particles were of approximately the same size as the minute clumps of streptococci in the heated suspensions (Hill (27)).

A preparation, consisting of 5.4 per cent bismuth oxychloride and of 7 per cent acacia in 0.9 per cent NaCl, was used for injection. This suspension was approximately a hundred times as dense as the suspensions of killed streptococci used for injection. 3 cc. of the bismuth suspension was injected into the left renal artery of each of 10 normal rabbits. Four rabbits were killed 48 hours after injection, 13 were killed 4 days and 3 were killed 8 days after injection. In several instances gross infarctions were found in the kidneys, and in a few kidneys a large irregular cell simulating a foreign body giant cell was occasionally found in a glomerulus.

Though particles of bismuth, often in large quantities, could be demonstrated in the glomeruli of eight of the kidneys of these ten rabbits, there were no exudative lesions either in the glomeruli or in the interstitial tissue; and it therefore seemed obvious that a glomerulitis, such as occurred after the injection of killed hemolytic streptococci, could not be produced by particulate matter such as bismuth oxychloride.

**DISCUSSION**

The experiments described show conclusively that an acute glomerulitis, often simulating very closely certain forms of glomerular nephritis in man, may be produced in rabbits by the injection of a suspension of killed hemolytic streptococci directly into the renal artery. Since only one kidney was affected, the opposite organ appearing normal, an adequate control was available for each experiment.

As the pathological changes, therefore, were limited to one kidney,
the process cannot be considered as nephritis, comparable to nephritis in man. The matters, therefore, which were of most concern, were the character of the histological lesions and their mode of origin. It seems clear that the changes in the glomeruli were often associated with the presence of hyaline thrombi in the capillaries and consisted principally of necrosis and exudation. A more careful study of these sections is being made, by special stains, to determine whether or not proliferation of the endothelial cells also occurs. The glomerulitis was often focal in distribution, but at times became so widespread that it involved practically every glomerulus. The histological picture, in many respects, resembled therefore the form of glomerular nephritis that is seen in bacterial endocarditis rather than that encountered after scarlet fever; but unlike the former, the process was not confined to the glomeruli for it affected in addition the tubular epithelium and interstitial tissue. From evidence obtained through a study of sections stained for bacteria, it appears probable that the retention of clumps of dead streptococci in the glomerular capillaries is the direct and immediate cause of these changes. Plugging of the glomerular capillaries by particulate matter alone, however, does not produce these alterations; for in the experiments with bismuth oxychloride no such lesions were found, even though, several days after injection, large amounts of this material were seen in the glomerular capillaries.

It has not been possible to determine all the factors that are responsible for the occurrence of glomerulitis in some animals and its absence in others. The condition of the renal circulation at the time of intraarterial injection, and immediately afterwards, may very well have some bearing on this question; but it has not been possible, as yet, to obtain any definite information as to what this may be. The persistence of bacterial bodies in the glomerular loops seems to be important; and, in combination with this, the only other factor which appears to bear any relation to the variations in the occurrence of the glomerulitis, is the presence of a localized streptococcus infection in the skin of the rabbit, which renders the skin sensitive to the filtrates and dead bodies of the strains of hemolytic streptococci employed. It seems possible that under such circumstances the kidney may be rendered more susceptible than the kidney of normal rabbits to the action of the disintegrated products of dead streptococci, where, for
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some unknown reason, they remain entangled in the glomerular capillaries. It is conceivable that the retention of streptococci in the glomeruli may be accounted for by some such phenomenon as that described by Rich and Brown (28) and by Rich (29), who later demonstrated that, independently of an allergic reaction, pneumococci may be clumped, agglutinated and held in the skin when injected intracutaneously into immune rabbits, or injected in combination with immune serum into the skin of normal rabbits. As an analogy to a possible allergic reaction in the kidney, it is important to note that Derick and Swift (30) have described a specific eye reaction to green streptococci in a certain proportion of rabbits rendered sensitive to green streptococci. Julianelle (31) has obtained similar eye reactions in rabbits, which could be elicited by applying to the scarified cornea of rabbits, made allergic to pneumococci by intracutaneous injections, the nucleoprotein or other solution of pneumococcus, from which the acid precipitable and heat coagulable proteins had been removed.

CONCLUSIONS

1. Both focal and diffuse glomerulitis has been produced in rabbits by the injection directly into the left renal artery of suspensions of heat killed hemolytic streptococci.

2. Similar lesions in the glomeruli could not be obtained by the injection of suspensions of bismuth oxychloride into the left renal artery of normal rabbits.

3. The acute glomerulitis occurred in only about one-half of the rabbits employed for the experiments.

4. Glomerulitis was observed much more frequently in rabbits in which an acute localized streptococcus infection had been produced by the intracutaneous injection of living hemolytic streptococci, than in normal rabbits. The occurrence of acute glomerulitis was usually associated with a well marked skin reaction to the filtrates of hemolytic streptococci.

BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 20

**Fig. 1.** Rabbit 383. "Sensitized" to Strain Shw. β hemolytic streptococci. 2.5 cc. heated killed Shw. into left renal artery. Killed 24 hours after intraarterial injection. High power; left kidney showing acute changes in glomerulus with pericapsular infiltration.
ACUTE GLOMERULITIS

Fig. 2. Rabbit 218. Normal. 3.0 cc. of heat killed β hemolytic streptococci strain Shw. in left renal artery. Killed 48 hours later. Low power; left kidney showing changes in tubules, interstitial tissue and glomeruli.

Fig. 3. Same as Fig. 2. High power of glomerulus showing hyaline thrombi and pericapsular infiltration by mononuclear cells.

Plate 21

Fig. 4. Rabbit 302. "Sensitized" to β hemolytic streptococci Strain SD2. 3.0 cc. heat killed β hemolytic streptococci SD2 in left renal artery. Killed 4 days later. Low power; left kidney showing focal infiltration by small round cells and changes in glomeruli.

Fig. 5. Rabbit 275. "Sensitized" to β hemolytic streptococci Strain SD2. 3.0 cc. heat killed β hemolytic streptococci Strain SD2 injected into left renal artery. Killed 4 days later. Low power; left kidney showing diffuse changes in tubules, interstitial tissue and glomeruli.

Fig. 6. Same as Fig. 5. High power; glomerulus showing hyaline thrombi with necrosis and pericapsular infiltration of small round cells.

Plate 22

Fig. 7. Same as Fig. 5. High power; glomerulus showing crescentic formation.

Fig. 8. Rabbit 304. "Sensitized" to β hemolytic streptococci Strain SD2. 3.0 cc. heated killed β hemolytic streptococci SD2 injected into left renal artery. Killed 8 days later. Low power; left kidney showing extensive diffuse infiltration of interstitial tissue, changes in tubules and glomeruli.

Fig. 9. Same as Fig. 8. High power view of glomerulus.
(Lukens and Longcope: Acute glomerulitis)
(Lukens and Longcope: Acute glomerulitis)