Experimental Production of Diffuse Proliferative Glomerulonephritis Utilizing Arteriovenous Fistula Stress with Bacteremia

By C. Walton Lillehei, M.D., J. R. R. Bobb, M.D., D. Wargo, B.S., M. B. Visscher, M.D., J. M. Shaffer, M.D., and E. T. Bell, M.D.

By combining the effects of systemic cardiovascular stress produced by large arteriovenous fistulas with bacteremia, a new method has been found by which acute diffuse proliferative glomerulonephritis may be produced experimentally.

The observation has been reported from this laboratory\(^1,2\) that dogs with large arteriovenous fistula loads spontaneously acquired endocarditis and that some also developed acute diffuse proliferative glomerulonephritis, in the period of six weeks to five months following the construction of their arteriovenous shunts. On the basis of these initial observations, an experimental method has been developed\(^3,4\) for the production of endocarditis and diffuse proliferative glomerulonephritis in dogs. This method utilizes the cardiovascular stress of bilateral arteriovenous fistulas in the lower extremities plus a bacteremia produced by intravenous injections of relatively small numbers of bacteria. By use of this method, the incidence of endocarditis has been increased to 100 per cent. Proliferative glomerulonephritis has been observed to occur in approximately one-third of these animals with large arteriovenous fistula loads and with endocarditis occurring either spontaneously or induced by bacterial injection. The pathogenesis of the endocarditis and of the glomerulonephritis in these animals is believed to depend upon the fact that a cardiovascular stress such as large arteriovenous fistulas causes certain physiologic alterations to occur within these dogs which result in a significant and specific increase in the susceptibility of the endothelial surfaces of the heart and kidneys to bacteria or to the products of bacterial infection. Moreover, the relatively small numbers of bacteria necessary to produce these lesions in the presence of large arteriovenous fistulas serves further to emphasize this profound effect of a systemic stress in promoting endothelial susceptibility. Thus, it would appear that systemic stress also plays an important role both in the localization and in the development of endocarditis and glomerulonephritis.

It is the purpose of this report to describe the occurrence and pathology of these glomerular lesions in greater detail. We believe that these observations are of significance, since they present a new method and a new approach for reproducing in the kidneys of a readily available experimental animal, such as the dog, a disease the pathogenesis of which in the human is still controversial and a disease the treatment of which is still largely nonspecific.

**Plan of Experiments**

Below are listed six groups of dogs which have been used in these experiments. The incidence of glomerulonephritis in each of these groups has been determined by microscopic study of kidney sections made from each of the 81 animals. Although the incidence of glomerulonephritis is the primary subject of this report,
the occurrence of endocarditis in these same animals has been noted for completeness. A more detailed report upon the pathogenesis of endocarditis has been published previously.4 The six groups of animals included in this study of the incidence of glomerulonephritis are as follows:

Group 1. Twelve Dogs with Large Arteriovenous Fistula Loads Developing “Spontaneous” Endocarditis.

Group 2. Eleven Dogs with Large Arteriovenous Fistula Loads Receiving Intravenous Injections of Beta Hemolytic Streptococci. (Group D, “Strain I.F.”)

Group 3. Eight Dogs with Large Arteriovenous Fistula Loads subdivided into: (a) Six Dogs Receiving Intravenous Injections of an Alpha Hemolytic Streptococcus. (b) Two Dogs Receiving a Coagulase Negative Staphylococcus.


Group 5. Ten Normal Dogs (Controls) Receiving Intravenous Injections of the Same Bacteria Used in the Experiments of Groups 2, 3, and 4.

Group 6. Thirty-six Dogs from the Same Animal Colony Which Have Been Used in a Wide Variety of Other Experiments.

BACTERIOLOGIC DATA

In the animals of group 1, all but a few of the earliest dogs studied had repeated blood culture studies done. The organisms in all of these animals with positive blood cultures were acquired fortuitously as has been previously described.1–2 The organisms recovered from the blood of these animals were identified as to species.

In the animals of groups 2, 3, 4, and 5, a temporary bacteremia was produced by the intentional intravenous injection of one of the three bacterial strains described below:

“Strain I.F.,” which has been identified as a Lancefield group D strain of beta hemolytic streptococcus. This organism, originally chosen at random from a hospital laboratory for use in these experiments, had been isolated from a patient* with cholangitis and hepatic abscesses due to a malignant obstruction of the common bile duct. The colony count of a 24 hour broth culture of this organism ranged from 1,290,000 to 2,450,000,000 organisms per ml. with a mean of 1,500,000,000 per ml. We have previously reported4 from this laboratory our experiences in the production of endocarditis using “Strain I.F.”

The alpha hemolytic streptococcus (Streptococcus viridans) used in these experiments was isolated from the blood of a patient† with bacterial endocarditis and a congenital aortic-pulmonary septal defect. The colony count of a 24 hour broth culture of this organism ranged from 13,000,000 to 178,000,000 organisms per ml. with a mean of 83,000,000 per ml.

The source of the coagulase negative staphylococcus used in these experiments cannot be traced. The mean colony count for this organism on a 24 hour broth culture was 1,500,000,000 organisms per ml.

All of the bacterial injections in this series of dogs were made intravenously using sterile syringes and a preparation of the skin indicated below. An injection of 0.05 ml. or 0.005 ml. of a 24 hour broth culture was made by diluting 1.0 ml. of the 24 hour broth culture to 10 ml. or to 100 ml. with normal saline, and then injecting 0.5 ml. of the resulting dilution. Blood cultures were made at frequent intervals by withdrawing 10 ml. of blood from the jugular vein into a sterile syringe after preparation of the skin using the following routine: shaving off the hair, soap and water, and then tincture of Zephran (1:1000). One-half of this blood was placed in broth and the other half into 3.5 per cent sodium citrate solution for use in making pour plates for colony counts. The number of organisms per ml. of blood was roughly quantitated for all positive cultures. The beta hemolytic streptococcus (group D, “Strain I. F.”) used in these experiments was tested for sensitivity to penicillin, Aureomycin, and streptomycin, and was found to be inhibited in vitro by the following concentrations of antibiotics:

* I. F., U. H. #817510
† G. M., U. H. #816233
penicillin 1.9 units per ml.
Aureomycin 7.8 μg per ml.
streptomycin >62.5 μg per ml.
penicillin plus Aureomycin 1.9 units or μg. per ml. of each drug
penicillin plus streptomycin 3.9 units or μg. per ml. of each drug

**METHOD OF STUDY**

All of the animals used in these studies were adult dogs of medium size of either sex, previously dewormed, immunized against distemper, and acclimated to the animal colony for four weeks before use. In the later experiments (groups 2 thru 5) the animals were also dipped at regular intervals in a parasiticid. All dogs were fed a daily diet consisting of dog biscuits supplemented with fresh horsemeat and crude cod liver oil.

The technic used in the construction of the arteriovenous shunts has been described in earlier publications. Penicillin in oil (300,000 units per day) was given to each animal for one week prior to each surgical procedure.

Daily rectal temperatures and pulse rate determinations were taken during the preoperative control period and after the construction of the arteriovenous shunts. Daily auscultation of the fistulas was added to this routine in the postoperative interval.

In all dogs with arteriovenous fistulas, a negative blood culture was obtained before bacterial injections were begun. This precaution is essential because an occasional animal with bilateral arteriovenous shunts will be found to have acquired a bacteremia spontaneously during even a short postoperative interval.

A more detailed description of the experimental conditions pertaining to each of the six groups of dogs studied for the occurrence of glomerulonephritis follows:

**Group 1. Dogs with Large Arteriovenous Fistula Loads Developing "Spontaneous" Endocarditis**

The occurrence of endocarditis in dogs with large arteriovenous fistula loads without the necessity for intentional injection of bacteria has been referred to above. The reader is referred to earlier publications from this laboratory for details as to the size, location, and duration of the arteriovenous fistulas necessary for the observance of this endocarditis as well as for a more complete description of the experimental conditions for this group of animals. The dogs in this group were of two age groups, (1) young adult dogs of 2 to 5 years of age, (2) old dogs estimated to be 10 or more years of age.

The influence of the dog's age upon endocarditis has been discussed previously. Group 2. *Dogs with Large Arteriovenous Fistulas Receiving Intravenous Injections of Beta Hemolytic Streptococcus (Group D)*

All of the 11 animals in this group were 2 to 5 years of age and had bilateral arteriovenous fistulas made side to side from 10 mm. to 32 mm. in length between the arteries and veins of both lower extremities in the positions indicated in table 3. The shunts were constructed in stages usually a week or more apart. A recovery period of at least two weeks was allowed in all animals following the last surgical procedure for complete healing of the operative wounds and for the compensatory changes of the arteriovenous fistulas to take place before beginning the daily intravenous injections of beta hemolytic streptococcus (group D, "Strain I.F."). The dose of bacteria used was from 0.005 to 0.5 ml. of a 24 hour broth culture per day from 2 to 19 days.

**Group 3. Dogs with Large Arteriovenous Fistulas Receiving Intravenous Injections of an Alpha Hemolytic Streptococcus or Coagulate Negative Staphylococcus**

The eight dogs in this group were exactly similar to those of group 2 except for the fact that following a similar recovery period after their last shunt operation, six animals received intravenously 0.005 to 0.5 ml. of a 24 hour broth culture of an alpha hemolytic streptococcus per day for 7 to 22 consecutive days (group 3a). Two other animals in this group received 0.5 ml. per day for 10 to 55 days of a 24 hour broth culture of a coagulate negative staphylococcus (group 3b).

**Group 4. Dogs with a Small Arteriovenous Fistula Load Receiving Intravenous Injections of a Beta Hemolytic Streptococcus. (Group D, "Strain I.F.")**

The four dogs in this group all had a single short (10 to 11 mm.) femoral arteriovenous shunt. The postoperative period before bacterial injections were begun was intentionally varied from 4 to 30 days in order to assay the relative importance of varying durations of a small increase in cardiovascular stress in localizing the pathologic involvement to the heart valves and kidney glomeruli. Also, it was expected that the animals with a very short interval between construction of their arteriovenous shunt and the intravenous injection of bacteria might acquire an infection at their fistula site, and the consequent resulting continuous bacteremia plus a definite but small increase in cardiovascular stress (from the

* Mean colony count = 1,500,000,000 organisms per ml.
† Mean colony count = 83,000,000 organisms per ml.
‡ Mean colony count = 1,500,000,000 organisms per ml.
single femoral arteriovenous fistula) would provide a
test of the relative importance of these factors in
localizing bacterial involvement to the valve leaflets
and kidneys. Each of the dogs in this group was
given a daily intravenous dose of 0.5 ml of beta
hemolytic streptococci* (group D, "Strain I.F.")
for seven consecutive days.

**Group 5. A Group of Normal Control Animals**

These control dogs were similar in all respects to
groups 2 through 4 except for the absence of arterio-
venous fistulas. These normal dogs received intra-
venous injection in the amounts indicated below of
one or more of the same three strains of bacteria used
for the experiments involving groups 2, 3, and 4.
(a) Beta hemolytic streptococcus (group D,
"Strain I.F.") in intravenous doses varying from 0.5
to 50.0 ml per day of a broth culture* for periods
varying from 7 to 15 consecutive days.
(b) Coagulase negative staphylococcus in in-
travenous doses varying from 0.5 to 1.0 ml of a 24 hour
broth culture* per day for from 16 to 48 successive
days.
(c) Alpha hemolytic streptococcus in intravenous
doses of 0.5 ml of a 24 hour broth culture† for 7 to
42 consecutive days.

**Group 6. Dogs from the Animal Colony**

Dogs from the same animal colony, which had
been used in a wide variety of other experiments,
were subjected to a careful autopsy, and their hearts
and kidneys studied grossly and microscopically
to determine the incidence of glomerulonephritis and/or
endocarditis in our animal colony.

**Pathology**

Each of the animals reported upon in this
paper had a complete autopsy performed at
the time of death. Blocks were cut from both
kidneys as well as from other organs and fixed
immediately in 10 per cent formalin. The para-
fine tissue sections have been stained routinely
with hematoxylin and eosin. Azocarmine stains
also have been utilized to demonstrate changes in
the connective tissue elements. The term
proliferative glomerulonephritis is used to de-
scribe a proliferative reaction of the glomerular
endothelium with resulting obstruction of the
capillaries. The glomerular lesions occurring in
these animals have been graded 1 plus to 3
plus on the following basis:

Glomerulonephritis grade 1 plus consists of a

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* Mean colony count = 1,500,000,000 organisms
per ml.
† Mean colony count = 83,000,000 organisms per
ml.

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diffuse involvement of all or virtually all of
the glomeruli visible in the stained sections by
a definite increase in the number and size of
endothelial cells lining the capillaries. Micro-
scopically, under low magnification, the glom-
eruli appear densely cellular and few or no
erythrocytes are to be seen.

Grade 3 plus consists of a severe involve-
ment of all the glomeruli characterized by a
proliferation of endothelial cells to such a de-
gree that all the capillaries are partially or
completely obstructed.

Grade 2 plus was applied to the glomerular
lesions intermediate in severity between these
two categories.

It is emphasized that in several dogs classi-
fied as negative, there was seen a definite endo-
thelial cell proliferation in some glomeruli, but
the majority of the glomeruli were not involved.
It is considered quite likely that those animals
with lesser degrees of involvement represent
an early stage of the same pathologic process.

**Results**

The over-all incidence of glomerulonephritis in
relationship to these varying experimental
conditions outlined above is summarized in
table 1. It is significant that all of the 11 ani-
mals developing glomerulonephritis in our ex-
periments were dogs with large arteriovenous
fistula loads (groups 1, 2, and 3a). The inci-
dence of glomerulonephritis in each of these
three groups was rather constant, varying only
from 33 to 50 per cent. With two excep-
tions (dogs 1042 and 1318, table 4) all of the
dogs with glomerulonephritis had endocarditis.
All of the 11 dogs developing glomerulonephri-
tis had a lesion classified as acute diffuse pro-
liferative glomerulonephritis, characterized by
an intracapillary proliferation of the glomerular
endothelium with resulting obstruction of the
capillaries. Seven (64 per cent) of the dogs were
classified as 1 plus, indicating a definite wide-
spread partial capillary obstruction by endothe-
lial cell hypertrophy and hyperplasia. Figures
3 and 4 are low and higher power photo mi-
crographs indicating the histologic appearance
of the kidneys classified as 1 plus glomeru-
lonephritis. These changes may be compared
with the appearance of normal canine kidneys.
under similar magnification (figs. 1 and 2). Two dogs (18 per cent) had a more severe degree of capillary obstruction and were classified as 2 plus glomerulonephritis. Two animals (18 per cent) had a severe and widespread degree of capillary obstruction classified 3 plus. Figures 5 and 6A and B indicate the histologic features of the kidneys so classified. The cells of the tubules showed no noteworthy changes although the lumens of the tubules contained

were caused by an alpha hemolytic streptococcus, two by a coagulase positive staphylococcus and one dog had no cultures.*

The diagnosis of glomerulonephritis in these animals was frequently suggested before death by the observation of gross hematuria. At autopsy, the kidneys showed grossly only swelling.

Among the 12 dogs with large arteriovenous fistula loads developing endocarditis without

* Lancefield group D, "Strain I.F."

Table 1.—Incidence of Glomerulonephritis in Relation to Cardiovascular Stress

<table>
<thead>
<tr>
<th>Summary</th>
<th>Acute Diffuse Proliferative Glomerulonephritis</th>
<th>Etiologic Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of Cardiovascular Stress</td>
<td>No. Dogs</td>
<td>Incidence</td>
</tr>
<tr>
<td>Group 1: Large Arteriovenous Fistula Loads with Endocarditis occurring spontaneously</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Group 2: Bilateral Arteriovenous Fistulas with I.V. injection of Beta Hemolytic Strep.*</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Group 3a: Bilateral Arteriovenous Fistulas with I.V. injection of Strep. Viridans</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Group 3b: Bilateral Arteriovenous Fistulas with I.V. injection of Coag. Neg. Staph...</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Group 4: Single Short Femoral A.V.F. with I.V. injection of Beta Hemolytic Strep.*</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Group 5: None (Normal Dogs with I.V. injection of Beta Hemolytic Strep.,* Strep. Viridans, or Coag. Neg. Staph.)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Group 6: None (Dogs from our animal colony used in other experiments)</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

*At autopsy a blood culture taken from the inferior vena cava disclosed a mixed growth of alpha hemolytic streptococcus, Aerobacter aerogenes, and proteus in this animal (dog 6).
that although *Aerobacter aerogenes* was the organism most frequently responsible for the endocarditis in this particular group of animals, there were no instances of this organism causing glomerulonephritis. In this group of dogs, the time interval from the construction of the arteriovenous shunts until death of the animal

![Fig. 1. Normal (dog) kidney, H & E stain, photomicrograph × 150.](image1)

![Fig. 2. A normal (dog) glomerulus, H & E stain, photomicrograph × 450.](image2)

in those dogs developing acute glomerulonephritis varied from a minimum of 14 days (dog 177) to a maximum of about two and one-half months (dog 1737).

There have been 13 additional dogs (not listed in table 2) which had arteriovenous fistula loads of magnitude comparable to the animals of group 1, and whose fistulas had existed for 14 days or more. These animals represent dogs that died of heart failure without endocarditis or were sacrificed deliberately before the development of endocarditis in order to determine the effect of arteriovenous fistulas upon adrenal gland weight. None of these 13 animals without endocarditis had glomerulonephritis.

![Fig. 3. Dog 1042. Acute diffuse proliferative glomerulonephritis grade 1 plus, note: the cellular proliferation in all of the visible glomeruli, H & E stain, photomicrograph × 150.](image3)
Fig. 4. Dog 1042. Closeup of a glomerulus (from fig. 3) with acute diffuse proliferative glomerulonephritis grade 1 plus. Note that the capillaries are partially blocked by the hypertrophy and hyperplasia of the endothelial cells, H & E stain, photomicrograph \( \times 450 \).

Fig. 5. Dog 114. Acute diffuse proliferative glomerulonephritis grade 3 plus. Note the large cellular glomeruli, H & E stain, photomicrograph \( \times 150 \).

Fig. 6. Dog 114. A glomerulus (from fig. 5) with acute diffuse proliferative glomerulonephritis grade 3 plus. A. Note the complete capillary obstruction. No erythrocytes are seen, H & E stain, photomicrograph \( \times 450 \). B. Note the capillaries are completely blocked by large endothelial cells. Azocarmine stain, photomicrograph \( \times 450 \).
Table 2.—Incidence of Glomerulonephritis in Dogs with Large Arteriovenous Fistula Loads Acquiring “Spontaneous” Endocarditis

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Age</th>
<th>Arteriovenous Fistulas</th>
<th>Acute Diffuse Proliferative Glomerulonephritis Grade†</th>
<th>Organism Acquired</th>
<th>Blood Culture (Maximum colonies per ml.)</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>177</td>
<td>Young</td>
<td>Iliac</td>
<td>2+</td>
<td>Coag. + Staph.</td>
<td>1320</td>
<td>Died, Glomerulonephritis &amp; Endocarditis</td>
<td>Abscess in laparotomy wound</td>
</tr>
<tr>
<td>114</td>
<td>Young</td>
<td>Iliac</td>
<td>3+</td>
<td>Strep. viridans</td>
<td>&gt;600</td>
<td>Died, Glomerulonephritis &amp; Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Glomerulonephritis &amp; Endocarditis</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Old</td>
<td>Iliac</td>
<td>1+</td>
<td>Not done</td>
<td>—</td>
<td>Died, Glomerulonephritis &amp; Endocarditis</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Old</td>
<td>Femoral</td>
<td>0</td>
<td>Aerobacter aerogenes</td>
<td>&gt;300</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>1737</td>
<td>Young</td>
<td>Iliac</td>
<td>1+</td>
<td>Coag. + Staph.</td>
<td>6500</td>
<td>Died, Glomerulonephritis &amp; Endocarditis</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Not done</td>
<td>—</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aorta-Cava</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Old</td>
<td>Iliac</td>
<td>0</td>
<td>Aerobacter aerogenes</td>
<td>45</td>
<td>Sacrificed,§ Endocarditis</td>
<td>Iliac A.V.F. Closed at autopsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Not done</td>
<td>—</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>451</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Not done</td>
<td>—</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iliac</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Aerobacter aerogenes</td>
<td>&gt;300</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Aerobacter aerogenes</td>
<td>1000</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Femoral</td>
<td></td>
<td></td>
<td></td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Young</td>
<td>Iliac</td>
<td>0</td>
<td>Beta Hemolytic Streptococcus</td>
<td>1000</td>
<td>Died, Endocarditis</td>
<td></td>
</tr>
</tbody>
</table>

* Animals of group 1 in text
† Graded on basis of 1+ to 3+
‡ Iliac A.V.F. partially closed prior to making aorta-cava shunt
§ Treated with Aureomycin
No. of dogs with endocarditis = 12; No. of dogs with acute diffuse proliferative glomerulonephritis = 4
In the 11 animals of group 2 with large arteriovenous fistulas and receiving intravenous as has been studied, between the number of bacteria injected or the duration of the endo-

**Table 3.**—Incidence of Glomerulonephritis with Bilateral Arteriovenous Fistulas and Bacterial Injection*  
Beta Hemolytic Streptococcus (Lancefield Group D, "Stain I. F.")

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sex</th>
<th>Arteriovenous Fistulas</th>
<th>Location</th>
<th>Length (mm.)</th>
<th>Days Duration at Start of Bacterial Injection</th>
<th>Dose† (24 hour broth culture) ml./day I. V.</th>
<th>No. Days Injected</th>
<th>Result</th>
<th>Acute Diffuse Proliferative Glomerulonephritis Grade‡</th>
<th>Comment</th>
<th>Days Duration of Life from First Bacterial Injection until Death.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1194</td>
<td>F</td>
<td>Iliac</td>
<td>Iliac</td>
<td>21</td>
<td>35</td>
<td>0.005</td>
<td>7</td>
<td>Sac.</td>
<td>1+</td>
<td>Endocarditis</td>
<td>27</td>
</tr>
<tr>
<td>1339</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>17</td>
<td>16</td>
<td>0.005</td>
<td>19</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
<td>65</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>Iliac</td>
<td>Iliac</td>
<td>20</td>
<td>133</td>
<td>0.05</td>
<td>2</td>
<td>Died</td>
<td>3+</td>
<td>&quot;</td>
<td>42</td>
</tr>
<tr>
<td>1293</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>18</td>
<td>22</td>
<td>0.05</td>
<td>7</td>
<td>Sac.</td>
<td>0</td>
<td>&quot;</td>
<td>64</td>
</tr>
<tr>
<td>56</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>23</td>
<td>137</td>
<td>0.05</td>
<td>7</td>
<td>Died</td>
<td>0</td>
<td>&quot;</td>
<td>61</td>
</tr>
<tr>
<td>1460</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>26</td>
<td>16</td>
<td>0.5</td>
<td>6</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
<td>18</td>
</tr>
<tr>
<td>779</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>24</td>
<td>17</td>
<td>0.5</td>
<td>7</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
<td>25</td>
</tr>
<tr>
<td>70</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>29</td>
<td>286</td>
<td>0.5</td>
<td>7</td>
<td>Died§</td>
<td>1+</td>
<td>&quot;</td>
<td>15</td>
</tr>
<tr>
<td>100-x</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>30</td>
<td>278</td>
<td>0.5</td>
<td>7</td>
<td>Died§</td>
<td>2+</td>
<td>&quot;</td>
<td>25</td>
</tr>
<tr>
<td>1886</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>25</td>
<td>23</td>
<td>0.5</td>
<td>7</td>
<td>Died‖</td>
<td>0</td>
<td>&quot;</td>
<td>48</td>
</tr>
<tr>
<td>718</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>32</td>
<td>17</td>
<td>0.5</td>
<td>10</td>
<td>Died</td>
<td>0</td>
<td>&quot;</td>
<td>17</td>
</tr>
</tbody>
</table>

*Animals of group 2 in text.
†Mean colony count on a 24 hour broth culture = 1,500,000,000 organisms per ml.
‡Graded on a basis of 1+ to 3+.
§The endocarditis was treated with penicillin and Aureomycin in therapeutic doses before death.
‖The endocarditis was treated with penicillin, Aureomycin, streptomycin, in therapeutic doses, and both arteriovenous fistulas were excised 23 days before death.

No. of animals with arteriovenous fistulas injected = 11; No. of animals with diffuse proliferative glomerulonephritis = 4.

injections of beta hemolytic streptococcus (group D, "Strain I.F.") there were four (36 per cent) that developed acute proliferative glomerulonephritis. Inspection of table 3 does not suggest any particular relationship, as far carditis and the occurrence of glomerulonephritis. However, it should be noted that two (dogs 70, 100-x) of the three animals receiving large doses of antibiotics as treatment for their endocarditis developed glomerulonephritis. A photo-
graph of the heart valve lesions associated with the glomerulonephritis in dog 100-x has been published. Likewise, both of the two animals (dogs 1194, 60) with bilateral iliac arteriovenous fistulas had profound septicemias. However, a similar septicemia, as has been previously reported, occurred as well in those animals of this group that developed endocarditis without glomeru-

<p>| Table 4.—Incidence of Glomerulonephritis with Bilateral Arteriovenous Fistulas and Bacterial Injection* |
|--------------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sex</th>
<th>Arteriovenous Fistulas</th>
<th>Location</th>
<th>Length (mm.)</th>
<th>Days Duration at Start of Bacterial Injections</th>
<th>Organism</th>
<th>Dose (24 hr. Broth Culture) ml./day, I.V.</th>
<th>No. of Days</th>
<th>Result</th>
<th>Acute Diffuse Proliferative Glomerulonephritis Grade</th>
<th>Comment</th>
<th>Days Duration of Life from First Bacterial Infection until Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1277</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>14</td>
<td>17</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.005</td>
<td>7</td>
<td>Sac.</td>
<td>0</td>
<td>No Endocarditis</td>
<td>32</td>
</tr>
<tr>
<td>1318</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>13</td>
<td>20</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.005</td>
<td>7</td>
<td>Sac.</td>
<td>1+</td>
<td>No Endocarditis</td>
<td>68</td>
</tr>
<tr>
<td>1361</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>17</td>
<td>15</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.05</td>
<td>7</td>
<td>Sac.</td>
<td>0</td>
<td>No Endocarditis</td>
<td>63</td>
</tr>
<tr>
<td>1042</td>
<td>F</td>
<td>Iliac</td>
<td>Iliac</td>
<td>15</td>
<td>16</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.05</td>
<td>7</td>
<td>Sac.</td>
<td>1+</td>
<td>No Endocarditis</td>
<td>29</td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>Iliac</td>
<td>Iliac</td>
<td>20</td>
<td>138</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.5</td>
<td>7</td>
<td>Died</td>
<td>0</td>
<td>Endocarditis</td>
<td>16</td>
</tr>
<tr>
<td>81</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>20</td>
<td>22</td>
<td>Alpha Hem. Strept.‡</td>
<td>0.5</td>
<td>22</td>
<td>Died</td>
<td>1+</td>
<td>&quot;</td>
<td>56</td>
</tr>
<tr>
<td>745</td>
<td>M</td>
<td>Iliac</td>
<td>Femoral</td>
<td>20</td>
<td>18</td>
<td>Coag. Neg.§</td>
<td>0.5</td>
<td>10</td>
<td>Died</td>
<td>0</td>
<td>&quot;</td>
<td>28</td>
</tr>
<tr>
<td>98</td>
<td>F</td>
<td>Iliac</td>
<td>Femoral</td>
<td>23</td>
<td>23</td>
<td>Coag. Neg.§</td>
<td>0.5</td>
<td>55</td>
<td>Sac.</td>
<td>0</td>
<td>No Endocarditis</td>
<td>145</td>
</tr>
</tbody>
</table>

* Animals of groups 3a and 3b in text.
† Graded on basis of 1+ to 3+.
‡ Mean colony count on a 24 hour broth culture = 83,000,000 organisms per ml.
§ Mean colony count on a 24 hour broth culture = 1,500,000,000 organisms per ml.
No. of dogs with arteriovenous fistulas injected = 8; No. of dogs with diffuse proliferative glomerulonephritis = 3.

Arteriovenous fistulas had both glomerulonephritis and endocarditis. The significance of this observation, if any, will have to be determined by future experiments. Table 6 contains the results of the blood cultures for the four dogs of this group developing glomerulonephritis. It will be noted on inspection of these results that all of these animals developing glomerulonephritis. The incidence of endocarditis in this group has been 100 per cent.

Of the six dogs with large arteriovenous fistula loads receiving varying doses of an alpha hemolytic streptococcus (group 3a) intravenously, three (50 per cent) developed glomerulonephritis and two developed endocarditis (table 4). However, two of the dogs with glo-
merulonephritis (dogs 1318, 1042) did not have endocarditis, but dog 1042 did have an infected vegetation at the arteriovenous fistula site. The results of the blood cultures taken on these three dogs with glomerulonephritis (dogs 81, 1042 and 1318) are summarized in table 6.

The results in this group of animals have been discussed in more detail in a previous publication in relationship to endocarditis. The zero incidence of glomerulonephritis and endocarditis in these animals with only a small increase in cardiovascular stress are in direct contrast to the 36 per cent incidence of glomerulonephritis and the 100 per cent incidence of endocarditis (group 2) that was observed in the dogs with large increases in cardiovascular stress (bilateral arteriovenous fistulas) receiving bacterial doses of comparable magnitude. It should also be noted that, as anticipated, in the two dogs with femoral fistulas of only four and six days duration (dogs 120, 1918) it

Two additional animals (dogs 745, 98) in this group (3b) with bilateral arteriovenous shunts received intravenous injections of a coagulase negative staphylococcus, and although one developed endocarditis, neither showed glomerulonephritis.

In the four dogs with a single short femoral arteriovenous fistula (group 4), the daily intravenous injection of a 0.5 ml. of a 24 hour broth culture* of beta hemolytic streptococcus

* Mean colony count = 1,500,000,000 organisms per ml.

---

**Table 5.—Bacterial Injection into Normal Dogs***

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Type of Bacteria</th>
<th>Dose (24 Hr. Broth Culture) ml./day IV</th>
<th>No. of days injected</th>
<th>Results</th>
<th>Glomerulonephritis or Endocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1162</td>
<td>Beta Hem. Strep.†</td>
<td>0.5</td>
<td>7</td>
<td>Sacrificed</td>
<td>None</td>
</tr>
<tr>
<td>198</td>
<td>Beta Hem. Strep.†</td>
<td>2.0</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>1887</td>
<td>Beta Hem. Strep.†</td>
<td>0.5</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>386</td>
<td>Beta Hem. Strep.†</td>
<td>50.0</td>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>277</td>
<td>Beta Hem. Strep.†</td>
<td>50.0</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>294</td>
<td>Beta Hem. Strep.†</td>
<td>50.0</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>963</td>
<td>Coag. Neg. Staph.‡</td>
<td>0.5</td>
<td>16</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Coag. Neg. Staph.‡</td>
<td>1.0</td>
<td>34</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>989</td>
<td>Coag. Neg. Staph.‡</td>
<td>0.5</td>
<td>48</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Alpha Hem. Strep.§</td>
<td>0.5</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>1327</td>
<td>Alpha Hem. Strep.§</td>
<td>0.5</td>
<td>15</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>1362</td>
<td>Alpha Hem. Strep.§</td>
<td>0.5</td>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>40</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* Animals of group 5 in text
† Lancefield group D, "Strain I.F."; mean colony count = 1,500,000,000 organisms per ml. of a 24 hour broth culture
‡ Mean colony count = 1,500,000,000 organisms per ml. of a 24 hour broth culture
§ Mean colony count = 83,000,000 organisms per ml. of a 24 hour broth culture

* No. of normal dogs injected = 10; No. of normal dogs developing glomerulonephritis (or endocarditis) = 0
### Table 6.—Blood Cultures in Dogs with Glomerulonephritis Produced by Large Arteriovenous Fistula Loads and Bacterial Injection

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Amount of Cardiovascular Stress</th>
<th>Type of Bacteria</th>
<th>Total IV. Bacterial Dose* (24 hr. Broth Culture)</th>
<th>Dates of Bacteria Injections</th>
<th>Results of Blood Cultures</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1194</td>
<td>Bilateral Iliac A.V.F.</td>
<td>Beta Hem. Strep.†</td>
<td>0.035</td>
<td>7/19-7/25 inc.</td>
<td>7/14 Negative</td>
<td>596 Beta Strep. 1800 Beta Strep. 1600 Beta Strep. 8/15-8/20 sacrificed 8/14, Endocarditis</td>
</tr>
<tr>
<td>60</td>
<td>Bilateral Iliac A.V.F.</td>
<td>Beta Hem. Strep.†</td>
<td>0.10</td>
<td>7/15-7/16 inc.</td>
<td>7/14 33 Gram + Cocci</td>
<td>15 Beta Strep. 272 Beta Strep. 124 Beta Strep. 340 Beta Strep. 4266 Beta Strep.</td>
</tr>
<tr>
<td>70</td>
<td>Iliac and Femoral A.V.F.</td>
<td>Beta Hem. Strep.†</td>
<td>3.5</td>
<td>1/3-1/9 inc.</td>
<td>1/2 Negative</td>
<td>27 Beta Strep. 639 Beta Strep. 1457 Beta Strep.</td>
</tr>
<tr>
<td>100-x</td>
<td>Iliac and Femoral A.V.F.</td>
<td>Beta Hem. Strep.†</td>
<td>3.5</td>
<td>12/13-12/19 inc.</td>
<td>12/22 2102 Beta Strep.</td>
<td>5040 Beta Strep. 3148 Beta Strep. 2556 Beta Strep.</td>
</tr>
<tr>
<td>1042</td>
<td>Bilateral Iliac A.V.F.</td>
<td>Alpha Hem. Strep.‡</td>
<td>0.35</td>
<td>7/17-7/23 inc.</td>
<td>7/17 No growth</td>
<td>120 Alpha Strep. 62 Alpha Strep. 413 Alpha Strep.</td>
</tr>
<tr>
<td>1318</td>
<td>Iliac and Femoral A.V.F.</td>
<td>Alpha Hem. Strep.‡</td>
<td>0.035</td>
<td>7/17 and 7/20-7/25 inc.</td>
<td>7/17 Negative</td>
<td>2 Alpha Strep. 54 Alpha Strep. 74 Alpha Strep. 2 Alpha Strep. 1 Alpha Strep.</td>
</tr>
</tbody>
</table>

**Note:**
- *Total Bacteria Dose = daily dose of a 24 hr. broth culture in ml. multiplied by the no. of days injected.
- † Lancefield group D, "Strain I.F. "; mean colony count = 1,500,000,000 organisms per ml. of a 24 hr. broth culture.
- ‡ Mean colony count = 83,000,000 organisms per ml. of a 24 hr. broth culture.
- § Animal receiving antibiotic therapy at time blood culture was drawn.
is quite likely that the fistula became the site of a localized infection. This conclusion is based on the fact that in both of these animals the blood cultures (table 7) indicated a significant elevation (up to 381 organisms per ml. in dog 1918, and up to 24 organisms per ml. in dog 120) above the usual degree of bacteremia. Nevertheless, even in the presence of this temporary septicemia without an appreciable increase in cardiovascular stress, there was no tendency for the bacterial infection to involve the endothelium of the kidneys or heart, and the blood cultures gradually became sterile again.

The results (table 5) of the injection of these same bacterial strains into normal dogs (group 5) are also in distinct contrast to the results observed in dogs with bilateral arteriovenous shunts. In the normal dogs, the intravenous injection of several different species of bacteria as indicated in table 5 caused no apparent ill effects except the transient occurrence of fever. All of the animals remained in excellent health, and it was necessary to sacrifice them for autopsy. There was no glomerulonephritis or endocarditis found. It will be noted (table 7) that the blood cultures in these animals nearly always revealed a low-grade bacteremia for the first few days immediately after the cessation of a course of bacterial injections. However, after this short interval, their blood streams became sterile again in all cases. It should be noted that the total numbers of beta hemolytic streptococci (group D, "Strain I.F.") organisms injected into these normal control animals were as much as 10,000 times greater than the minimum number of organisms necessary to produce glomerulonephritis in the dogs with bilateral arteriovenous shunts. Likewise, in the case of the alpha hemolytic streptococcus the numbers of bacteria that were injected into the normal dogs were as much as 620 times the minimum number of bacteria necessary to cause glomerulonephritis in the dogs with bilateral arteriovenous fistulas. These comparisons in numbers of organisms provide striking evidence of the importance of the physiologic alterations associated with large arteriovenous fistulas in promoting endothelial susceptibility to infection.

As indicated in table 1, the gross and microscopic study of the kidneys and hearts from 36 consecutive dogs (group 6) from our animal colony which had been used in a wide variety of other experiments did not disclose any instance of glomerulonephritis or endocarditis. A number of these dogs were subjected to surgical operations and some of them also had complicating wound infections with no apparent tendency for localization of the infection to the kidney glomeruli or heart valves. Our findings of the rarity of glomerulonephritis in normal dogs and the relative frequency of the easily distinguishable interstitial nephritis were similar to those of others.7, 8

**DISCUSSION**

Several comprehensive surveys of the literature on experimentally induced lesions of the kidney have been published9, 10 in recent years, which indicate the large number of attempts that have been made to reproduce the human disease, glomerulonephritis. Of the numerous methods that have been tried for the experimental production of glomerulonephritis, those involving bacteria or the products of their growth and those involving some form of hypersensitivity to a protein appear to have invoked the greatest interest and produced the closest approach to human lesions. However, Simonds and co-workers11 and McNider,9 among others, have expressed the general discouragement with the results obtained by the use of either living or dead bacteria or their toxins for the experimental production of diffuse glomerulonephritis. These unsatisfactory results are made even more difficult to understand because of the intimate association clinically of bacterial infection with the pathogenesis of glomerulonephritis. Loeb12 and Horn10 concluded from a review of the literature that diffuse glomerulonephritis satisfying all of the criteria required for the diagnosis of the human disease had not yet been produced experimentally by any method.

The present experiments provide a new approach to this problem. By utilizing the cardiovascular stress of large arteriovenous fistula loads together with a bacteremia occurring either by the fortuitous entrance of bacteria...
## Table 7—Blood Cultures in Normal* Dogs and Dogs with Small* Arteriovenous Fistula Loads Receiving Bacterial Injections

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Amount of Cardiovascular Stress</th>
<th>Type of Bacteria</th>
<th>Total I.V. Bacteria Dose (24 Hr. Broth Culture) ml.</th>
<th>Dates of Bacteria Injections</th>
<th>Results of Blood Cultures</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Date</td>
<td>No. organisms per ml. blood</td>
</tr>
<tr>
<td>120</td>
<td>Single Short Femoral A.V.F.</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>12/3–12/9 inc.</td>
<td>12/11</td>
<td>Negative</td>
</tr>
<tr>
<td>1918</td>
<td>Single Short Femoral A.V.F.</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>11/30–12/6 inc.</td>
<td>12/7</td>
<td>209 Beta Strep.</td>
</tr>
<tr>
<td>119</td>
<td>Single Short Femoral A.V.F.</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>11/30–12/6 inc.</td>
<td>12/7</td>
<td>Negative</td>
</tr>
<tr>
<td>814</td>
<td>Single Short Femoral A.V.F.</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>11/30–12/6 inc.</td>
<td>12/7</td>
<td>1 Beta Strep.</td>
</tr>
<tr>
<td>1162</td>
<td>None (had a femoral A.V.F. which closed)</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>11/30–12/6 inc.</td>
<td>12/7</td>
<td>1–2 Beta Strep.</td>
</tr>
<tr>
<td>198</td>
<td>None (Normal Dog)</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>14.0</td>
<td>1/2–1/8 inc.</td>
<td>1/2</td>
<td>5 Beta Strep.</td>
</tr>
<tr>
<td>1887</td>
<td>None (Normal Dog)</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>3.5</td>
<td>12/2–12/8 inc.</td>
<td>12/11</td>
<td>1 Beta Strep.</td>
</tr>
<tr>
<td>386</td>
<td>None (Normal Dog)</td>
<td>Beta Hemolytic Strep.‡†</td>
<td>300</td>
<td>1/31–2/6 inc.</td>
<td>None</td>
<td>—</td>
</tr>
</tbody>
</table>

* No glomerulonephritis (or endocarditis) was observed in any of these animals.
† Total bacteria dose = daily dose (in ml.) of a 24 hour broth culture multiplied by no. of consecutive days injected.
‡ Lancefield group D, "Strain I.F.,," average colony count on a 24 hour broth culture of this organism = 1,500,000,000 bacteria per ml.
§ Average colony count on a 24 hr. broth culture of this organism = 1,500,000,000 bacteria per ml.
‖ Average colony count on a 24 hr. broth culture of this organism = 83,000,000 bacteria per ml.
into the animal's blood stream, or produced by the intravenous injection of small numbers of bacteria, it has been possible to produce in one-third of the dogs studied an acute diffuse proliferative glomerulonephritis which satisfied many of the criteria required for diagnosis of the human disease.

The fact that the glomerulonephritis occurred in a rather constant percentage of the dogs of groups 1, 2, and 3, in contrast to the close to 100 per cent incidence of endocarditis suggests that some rather constantly occurring, but at present unknown, factor is necessary for the occurrence of glomerulonephritis in addition to bacterial infection and cardiovascular stress. No studies have as yet been made to determine whether individual differences among the several animals in their immune reactions to foreign proteins of bacterial origin may account for failure of all dogs treated similarly to respond alike. Furthermore, there may be such large individual differences in the endocrine and metabolic responses to the conditions imposed as to account for the nonuniformity of behavior.

It is important to emphasize that the dogs used in these experiments had a high protein diet of excellent quality. Smadel and Farr showed a significant correlation between protein intake and susceptibility to a nephrotoxic

### Table 7—Continued

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Amount of Cardiovascular Stress</th>
<th>Type of Bacteria</th>
<th>Total LV. Bacteria Dose† (24 hr. Broth Culture) ml.</th>
<th>Date of Bacteria Injections</th>
<th>Results of Blood Cultures</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Date</td>
<td>No. organisms per ml. blood</td>
</tr>
<tr>
<td>277</td>
<td>None (Normal Dog)</td>
<td>Beta Hemolytic Strep.†</td>
<td>350.0</td>
<td>1/10–1/16 inc.</td>
<td>1/19</td>
<td>1–2 Beta Strep. Negative</td>
</tr>
<tr>
<td>294</td>
<td>None (Normal Dog)</td>
<td>Beta Hemolytic Strep.†</td>
<td>350.0</td>
<td>1/21–1/30 inc.</td>
<td>2/2</td>
<td>9 Beta Strep. Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coag. Neg. Staph.‡</td>
<td>34.0</td>
<td>6/12–7/13 inc.</td>
<td>8/28</td>
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<tr>
<td></td>
<td></td>
<td>Beta Hemolytic Strep.†</td>
<td>7.0</td>
<td>8/8–8/21 inc.</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strep. Viridans</td>
<td></td>
<td></td>
<td>3.5</td>
<td>7/21–7/27 inc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta Hemolytic Strep.†</td>
<td>7.5</td>
<td>8/7–8/21 inc.</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>1327</td>
<td>None (Normal Dog)</td>
<td>Strep. Viridans</td>
<td></td>
<td></td>
<td>21.0</td>
<td>7/13–8/23 inc.</td>
</tr>
<tr>
<td></td>
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<td>Negative</td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>Strep. Viridans</td>
<td></td>
<td></td>
<td>2.0</td>
<td>7/13–7/16 inc.</td>
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</table>
lesion in rats. The other details of experimental technique in the study reported have been described fully because of the possibility that precisely similar conditions may be necessary to the reproduction of the results. Since the importance of many of the variables has not been tested by controlled experiments, it is impossible to state which of the conditions employed, other than those which have been analyzed, are or are not necessary to the result obtained.

In persons dead of bacterial endocarditis, nearly one-half show a moderate increase of glomerular endothelium with partial capillary obstruction (grade 1) and in a small percentage there is a severe capillary obstruction by endothelium (grade 3) which is clinical acute glomerulonephritis. Therefore, the acute glomerulonephritis in dogs must in some way be related to the bacteremia which was associated in most but not all of the animals with endocarditis. The intimate mechanism by which bacteremia produces glomerulonephritis is not understood; but, nevertheless, by combining the effects of a cardiovascular stress with bacteremia, a method has been found by which glomerulonephritis may be produced experimentally. This experimental glomerulonephritis corresponds entirely in its histologic features with the human lesion.

Conclusions

1. Acute diffuse proliferative glomerulonephritis has been found in about one-third of dogs with large arteriovenous anastomoses in which endocarditis occurred either with intentional or adventitious introduction of pathogenic organisms.

2. The majority of the dogs showed the lesions in "early" phases of the disease—two were moderately severe and two very severe—indistinguishable from those seen in patients dying from acute diffuse proliferative glomerulonephritis.

3. In a series of 36 control dogs without arteriovenous fistulas and without bacterial injection, no case of glomerulonephritis was found upon microscopic examination.

4. Ten unoperated normal dogs were given injections of 620 to 10,000 times the numbers of bacteria of the same species and strains as were followed by kidney lesions in dogs with bilateral large arteriovenous fistulas. There was in no case either endocarditis or glomerulonephritis in these normal dogs.

5. In dogs with single small femoral anastomoses receiving intravenous injections of bacteria in comparable numbers neither endocarditis nor glomerulonephritis occurred.

6. Glomerulonephritis was observed in association with infection by alpha and beta hemolytic streptococcus and coagulase positive staphylococcus. The lesion is therefore not limited in the dog to a single strain. No case was observed in association with Aerobacter aerogenes or coagulase negative staphylococcus endocarditis in six dogs.

7. Because of the absence of lesions in the unoperated normal dogs and dogs with small fistulas given the same bacteria in the same or much larger numbers than given to the animals with large fistulas subsequently showing glomerulonephritis, it is concluded that the cardiovascular stress plays a major role in determining the susceptibility of dogs to glomerulonephritis.

Acknowledgments

The authors wish to express their gratitude to Dr. Owen H. Wangensteen, Dr. W. W. Spink, and Dr. B. J. Clawson for their advice, aid and the use of their facilities which have made possible the work herein reported.

References


Experimental Production of Diffuse Proliferative Glomerulonephritis Utilizing Arteriovenous Fistula Stress with Bacteremia

C. WALTON LILLEHEI, J. R. R. BOBB, D. WARGO, M. B. VISSCHER, J. M. SHAFFER
and E. T. BELL

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