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Research Article

SUSCEPTIBILITY OF RATS WITH HORMONAL HYPERTENSION TO EXPERIMENTAL PYELONEPHRITIS [1, 2]

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Congenital malformations and urinary tract obstructions are accepted predisposing factors in *human* pyelonephritis. Ureteral ligation (1, 2), mechanical trauma (3), scar formation in the kidney after recovery from severe staphylococcal infection (4), and localized thermal injury to the renal medulla (5) have been shown to predispose to the development of acute, hematogenous, *Escherichia coli* pyelonephritis in the *experimental animal.* It is conceivable that other renal insults such as arteriolar nephrosclerosis and necrotizing arteriolitis might similarly increase susceptibility to infection. There are no experimental data to support this supposition. The postmortem examination of "end stage" kidneys and analysis of clinical charts do not yield evidence which clearly establishes the *sequential* relationship of hypertension and pyelonephritis. The present investigation was therefore designed to test by experiment the hypothesis that animals which are made hypertensive and which have renal arteriolar disease may have an increased susceptibility to pyelonephritis.

MATERIALS AND METHODS

Animals. White female Sprague-Dawley strain rats, weighing 50 to 60 Gm., were used.

Induction of hypertension. Experimental animals were subjected to left nephrectomy and three to four fortnightly injections of a long-acting derivative of desoxycorticosterone (DCA)[3] in 25 mg. doses. They drank 1 per cent NaCl. This method of inducing hypertension was chosen because of its relatively predictable course, reproducible occurrence, and the development of diffuse arteriolarsclerosis, most evident in the kidney. Arterial pressure is usually increased by about 30 mm. Hg in 2 weeks while manifestations of cardiovascular disease occur in 6 to 12 weeks. Knowlton and Loeb (6) have shown that rats made hypertensive by this method exhibit a striking lowering of carcass potassium. In order to prevent this, 0.49 per cent KCl was added to the drinking water.

Control animals had a left nephrectomy but received no DCA and drank ordinary tap water. Both experimental and control animals ate Purina Laboratory Chow. The animals of Study A were not pair-fed and there was a 30 Gm. difference between the average weights of the experimentals (156 Gm.) and the controls (195 Gm.). In Study B, the animals were pair-fed and resulting weight curves were practically identical.

Blood pressure measurement. Blood pressures were measured according to the method of Friedman and Freed (7) but with the modification that the animals were neither anesthetized nor immobilized with curare. Normal values obtained by this modified technique ranged from 20 to 40 mm. Hg higher than those obtained on anesthetized animals. Readings were obtained at 7 to 10 day intervals.

Bacteriological techniques. Study of the susceptibility of the kidneys of these animals to infection by *E. coli* injected intravenously was based on the work of Guze and Beeson (2). Strains of *E. coli* to be used for intravenous inoculation were isolated from the urine of patients with active pyelonephritis. After identification, the organism was injected intravenously into a rat with a ligated ureter, subsequently recovered from the infected kidney, and inoculated into tubes containing 10 ml. of sterile broth. The tubes were incubated for three hours, sealed with parafilm, frozen, and stored at $-20°$ C. Colony counts were plotted against Klett-Summerson colorimeter readings on a broth culture at frequent intervals in order to simplify the determination and standardization of inoculum size for future experiments. When a group of animals were to be injected with *E. coli,* a frozen culture was warmed to 37° C., incubated for 12 hours, and a small inoculum placed in 150 ml. of sterile broth. When growth produced marked turbidity, the culture was diluted with sterile broth to the desired colorimeter reading, *i.e.,* equivalent to the number of million bacteria to be injected. Then 20 to 25 ml. was removed, centrifuged and the sediment resuspended in an equivalent volume of sterile saline. One ml. of this saline suspension was then injected into the tail veins of

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[2] Presented before the Annual Meeting of the American Federation for Clinical Research, Atlantic City, May 4, 1958.

[3] Desoxycorticosterone trimethylacetate kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

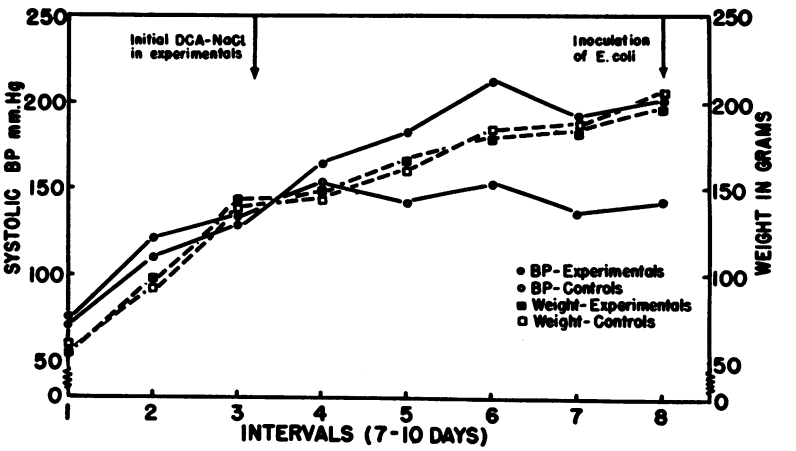


FIG. 1. AVERAGE WEIGHTS AND SYSTOLIC BLOOD PRESSURES OF A GROUP OF FOUR EXPERIMENTAL RATS AND THEIR PAIR-FED CONTROLS

a group of rats with hypertension of six to eight weeks duration and their controls. In Study A, 120 to 325 million organisms were injected into each animal but in Study B only 75 million of a more virulent strain was given to most animals. Estimations of the number of bacteria injected, based on the colorimeter, were always checked by incubation of serial dilutions in desoxycholate pour plates followed by colony counting. There was close agreement by the two methods.

The mean survival time for animals in which DCA-saline hypertension is induced by this method is about 117 days (8). Rats in this study were inoculated with *E. coli* 50 to 60 days after the regimen was started; most appeared to be vigorous at the time of inoculation, and reference to Figure 1 shows that they were steadily gaining weight. Three of 45 hypertensive rats died prior to injection of *E. coli*. Others died during the night following injection, presumably of bacteremic shock, and were usually lost to the study. The remaining 33 animals were sacrificed at intervals varying from 2 to 15 days. They were not sacrificed less than 2 days after injection unless it became apparent that they were dying. Since all animals had been uninephrectomized, pyelonephritis was poorly tolerated when it occurred. A randomly selected control was sacrificed with each experimental rat, or if a control animal appeared to be dying she was sacrificed with a randomly selected experimental. The purpose of these experiments was to determine presence or absence of pyelonephritis and no attempt was made to study survival time. The right kidney was removed, using sterile technique, and its macroscopic appearance noted. The kidney was then sliced in half in its long axis. One half was fixed in either 10 per cent formalin or Helly's fluid for histologic sections.

TABLE I

Study A—Colony counts and descriptions of kidneys of hypertensive and control animals at intervals after injection of E. coli

Time interval	Kidney of hypertensive	Description*	Kidney of control	Description*
Hours				
5	1,290	0	820	0
10	> 1,000,000	0	870	0
17	> 10,000,000	0	1,500	0
23	420	0	2,540	0
Days				
1	> 30,000,000	0	6,140	0
3	40	0	380	0
3	> 30,000,000	++	20	0
4	>100,000,000	+	40	0
6	> 15,000,000	+	660	0
6	10	0	0	0
6	0	0	150	0
15	> 20,000,000	++	0	0

* 0, no cellular infiltrate; +, cellular infiltrate; ++, microscopic abscesses; +++, macroscopic abscesses.

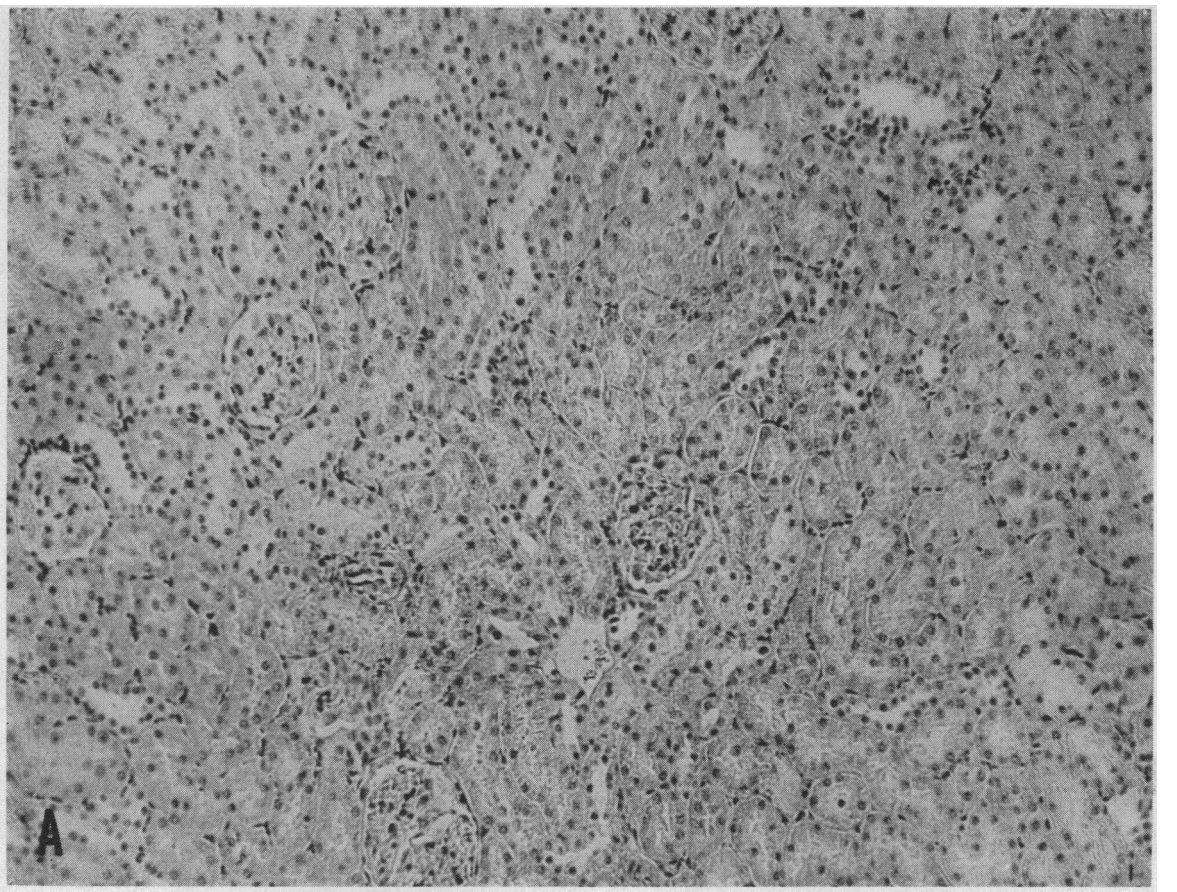


FIG. 2. ILLUSTRATIONS OF NORMAL AND

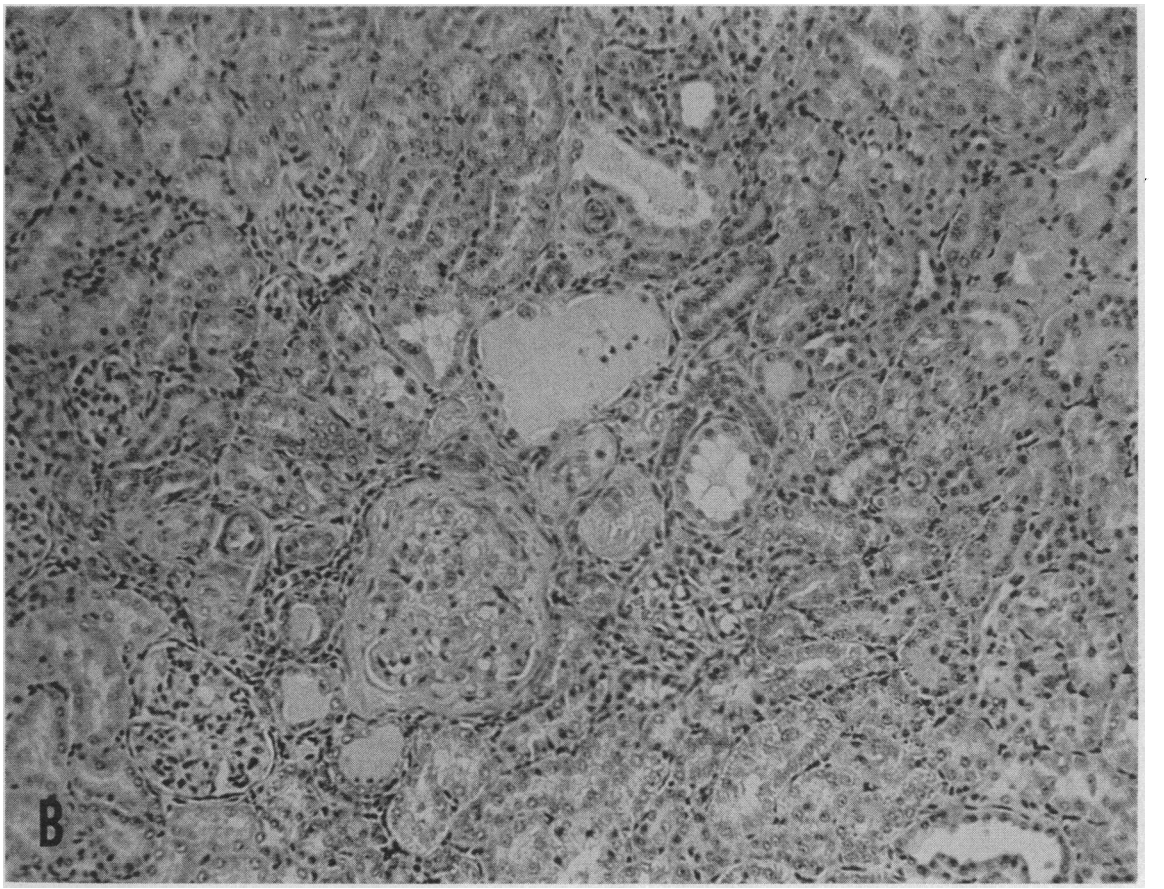
A, kidney of control rat (previously uninephrectomized). B, kidney from a hypertensive rat inoculated with *E. coli* but revealing no cultural or histological evidence of infection. Glomerular and tubular changes character-

The remaining half was placed in peptone water in a sterile graduate cylinder, its volume measured, and a dilution of 1 : 10 made. It was next ground with sterile sand in a mortar and pestle, serial dilutions made, and 1 ml. of the several dilutions added to desoxycholate agar for preparation of pour plates. These were incubated at 37° C. for 48 hours and colony counts made.

RESULTS

Study A

On the DCA-saline regimen, the experimental rats developed hypertension in about two weeks and, soon thereafter, vascular disease, most marked in the kidney. The inoculum of *E. coli* varied in size from 120 to 325 million organisms which were considerably less virulent than those of the strain used in Study B. Of those animals available for study, 7 of 12 of the hypertensive group and none of the 12 controls showed evidence of renal infection by colony counts (Table I). Normal kidneys were expected to arrest up to 20 to 30 thousand bacteria per Gm. of kidney tissue for about a week (2). Significantly higher colony counts were considered indicative of bacterial multiplication and infection. The seven hypertensive rats with renal infection had colony counts in excess of one million, whereas no control rat had a count of more than 6,200 and most had only a few hundred. None of those with infected kidneys by bacterial counts had macroscopic abscesses, but the four sacrificed more than 24 hours after inoculation had microscopic evidence of infection. None of the kidneys of control animals showed histologic abnormality.



PATHOLOGICAL HISTOLOGY ENCOUNTERED

istic of the DCA-saline regimen are demonstrated. C, extensive infiltration of mononuclear cells. D, renal cortical abscess. Hematoxylin and eosin (× 115).

Study B

Rats in this study were pair-fed. Weight and systolic blood pressure curves of a representative experimental group with their controls are shown in Figure 1. The first two groups (of eight each) were inoculated with approximately 100 million bacteria of a new strain of *E. coli*. Three of the experimentals died within 12 hours. In addition, two of the controls developed abscesses of the kidney. It was concluded that this strain was more virulent than that used in Study A. Therefore, a group of normal rats were divided into subgroups of three each and injected with decreasing numbers of organisms (100, 75, 50 and 25 million). On the basis of results, 75 million bacteria was chosen as the highest dose which would not infect a significant number of controls, and was used in all succeeding experiments. Of the remaining 16 controls, only 1 developed renal infection.

As is shown in Table II, 16 of 21 hypertensive and only 3 of 21 control animals developed pyelonephritis. Of the 16 infected hypertensives, 9 had macroscopic abscesses, 2 microscopic abscesses, and 3 infiltrates of inflammatory cells. The 2 rats sacrificed less than 24 hours after injection had no histologic evidence of infection. Two of the 3 infected controls had macroscopic abscesses, and the third, sacrificed 13 hours after injection, had no microscopic evidence of infection. Organs other than the kidneys were never macroscopically infected at the time of sacrifice. Colony counts of cultures from liver and spleen in a small but representative group of hypertensive and control rats confirmed the absence of infection in these or-

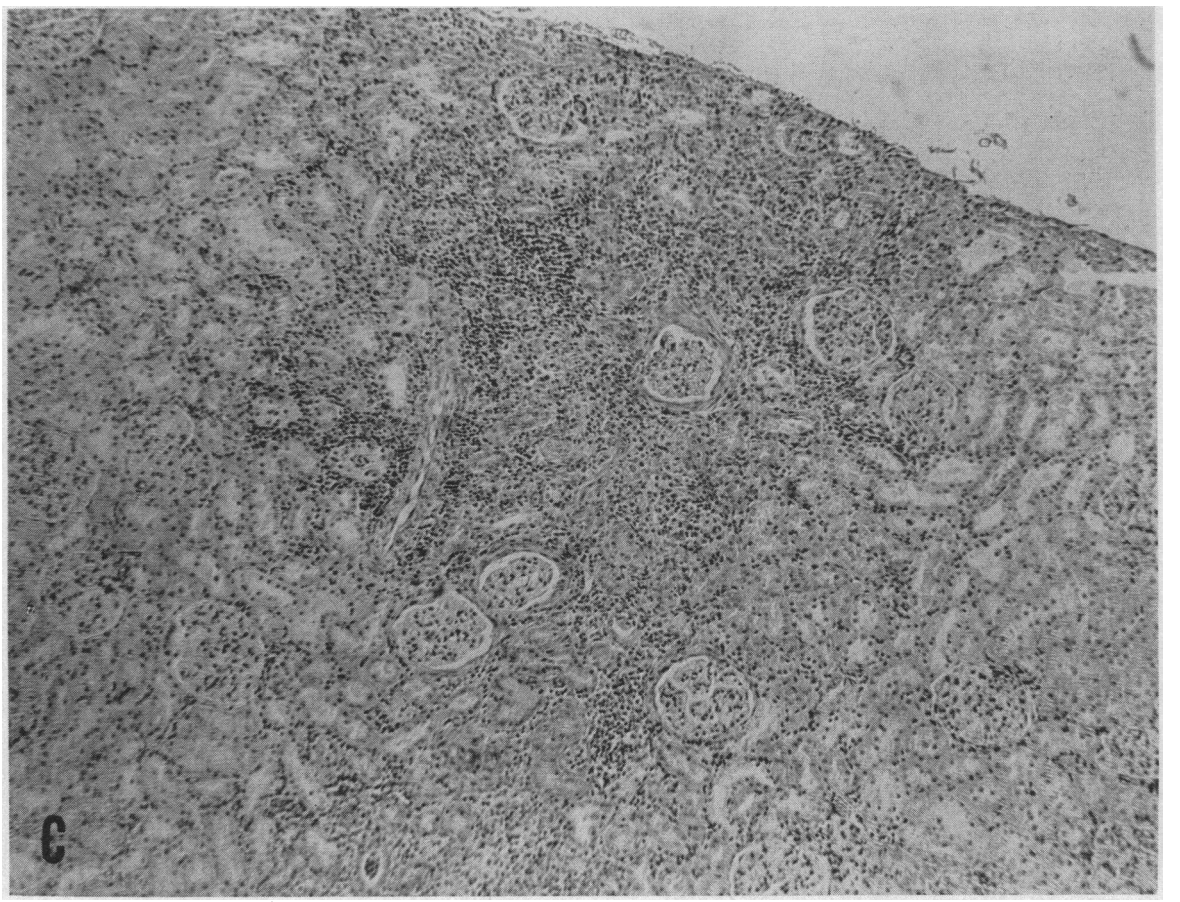


FIG. 2. (*For description*

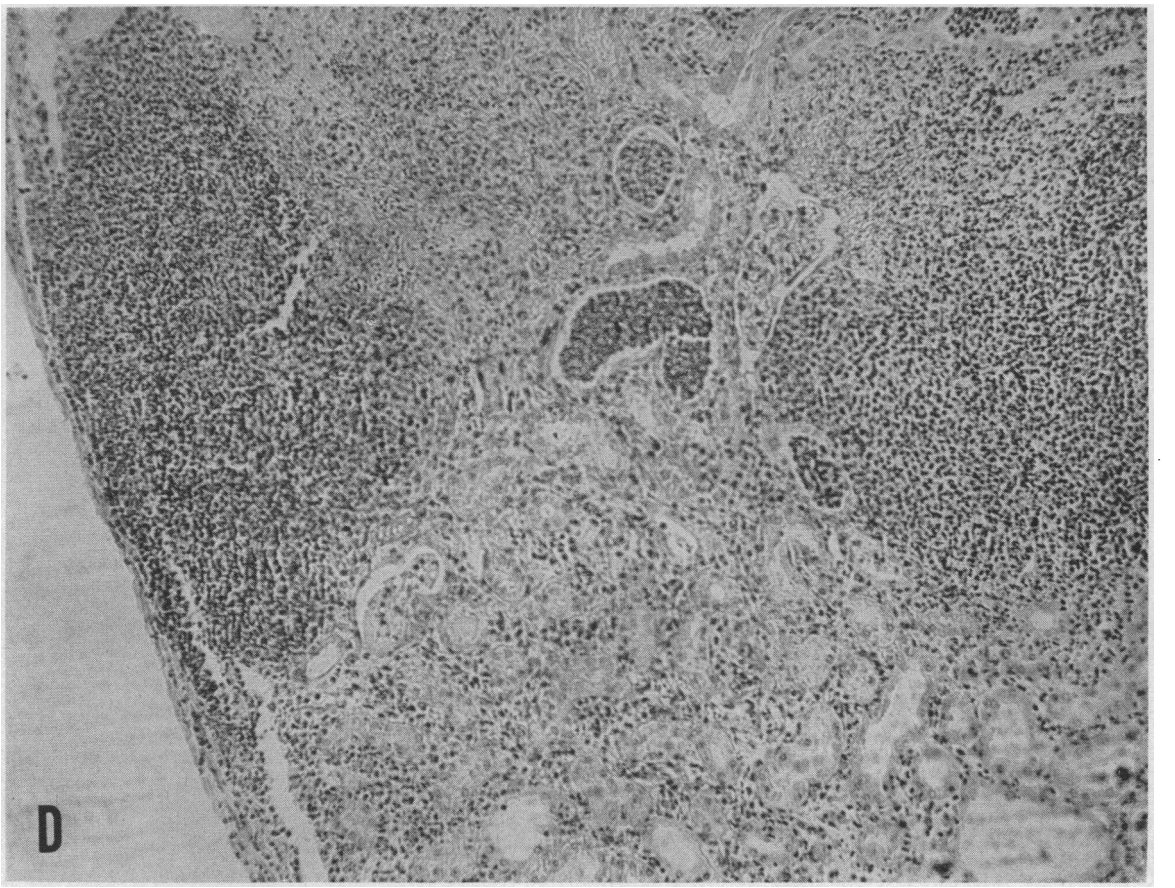
gans. Illustrations of kidneys of normal (uninephrectomized) rats, of those with DCA-saline induced hypertension, and of those with superimposed infection are shown in Figure 2.

Summarizing the combined results of Studies A and B, 23 of 33 hypertensives but only 3 of 33 controls developed varying degrees of pyelonephritis. This difference is highly significant ($p < 0.00001$).

DISCUSSION

The induction of severe hypertension and diffuse arteriolarsclerosis in uninephrectomized rats given DCA and saline was first described by Selye, Hall and Rowley in 1943 (9). Of all varieties of experimental hypertension, it is said to resemble most closely the human disease in both its functional and anatomical aspects (10). The model is well suited to the purposes of this investigation except that a striking lowering of carcass potassium may occur (6). The production of potassium nephropathy by a marked deficiency of this ion has been shown by Oliver and co-workers (11). In order to avoid this complicating renal lesion, supplementary potassium was furnished the experimental rats. Subsequent analyses of muscle in eight hypertensive animals revealed a mean potassium concentration of 48.3 (S.D., 1.7) mEq. per 100 Gm. of fat-free dry solids, whereas the mean concentration in four controls was 49.7 (S.D., 1.5) mEq. per 100 Gm. Muscle potassium was thus not significantly different in the two groups ($p > 0.5$).

The behavior of the organism used in Study B is somewhat puzzling when compared with the results obtained by Guze and Beeson (2). These



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investigators used a strain of *E. coli* which did not infect normal kidneys yet which was consistently found in low numbers in these kidneys for periods of up to seven days. Study B, however, reveals that of the nine control animals sacrificed at five days or less, six had sterile kidneys while two were infected. The explanation of this variance is not apparent but this model differs from that used by the above investigators in three respects: The organism was "passed" through a rat only once after recovery from a patient with pyelonephritis, whereas theirs was "passed" 13 times; the inoculum size used in most animals was one-half or less that used by them which resulted in the arrest of fewer bacteria by the kidney; and, finally, the rats used in this study had a single kidney.

These data support the interpretation that DCA-saline induced hypertension renders rats more susceptible to hematogenous renal infection. This type of experimental hypertension is characterized by diffuse arteriolarsclerosis, most evident in the kidney, but, in addition, results in a tubular abnormality characterized by dilatation of lumina which are filled with homogenous material. The factor or factors causing increased susceptibility are not apparent from this study.

SUMMARY AND CONCLUSIONS

Rats with DCA-saline induced hypertension have been shown to exhibit increased susceptibility to experimental pyelonephritis.

The data may support the hypothesis that various injuries, including nephrosclerosis, predispose to human pyelonephritis and that pyelonephritis is superimposed on hypertensive disease more frequently than is usually suspected.

TABLE II

Study B—Colony counts and descriptions of kidneys of hypertensive and control animals at intervals after injection of E. coli

Time interval	Kidney of hypertensive	Description*	Kidney of control	Description*
Hours				
13	> 30,000,000	0	>30,000,000	0
20	79,000	0	0	0
Days				
5	> 20,000,000	+++	0	0
2	> 30,000,000	+++	>30,000,000	+++
3	121,000	+	0	0
5	> 10,000,000	+	0	0
6†	0	0	0	0
6	>100,000,000	+++	0	0
6	0	0	0	0
6	> 20,000,000	++	> 5,000,000	+++
6	0	0	0	0
6	>100,000,000	+++	80	0
6	40	+	10	0
6	30	0	670	0
2	>100,000,000	+++	0	0
7	312,000	+	0	0
7	3,500,000	+++	0	0
2	>100,000,000	++	5	0
5	> 20,000,000	+++	0	0
6	> 10,000,000	+++	0	0
6	> 10,000,000	+++	0	0

* 0, no cellular infiltrate; +, cellular infiltrate; ++, microscopic abscesses; +++, macroscopic abscesses.
† Inoculum reduced from 100 to 75 million organisms.

ACKNOWLEDGMENT

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