Experimental Pyelonephritis in Rats with Aminonucleoside-Induced Nephrotic Syndrome.* (26578)

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(Introduced by Henry D. Moon)
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The normal kidney is usually quite resistant to infection by organisms known to cause pyelonephritis. The susceptibility of the kidney to experimental infection, however, is increased by injuries such as induced ureteral obstruction(1,2), renal massage(3), scar formation(4), lesions of potassium depletion(5) and treatment with desoxycorticosterone acetate-saline(6). Clinically, urinary tract obstruction has long been recognized as predisposing to pyelonephritis and various other predisposing factors have been suspected.

The clinical observation of a high incidence of urinary tract infections in patients with the nephrotic syndrome(7) prompted us to investigate the susceptibility to pyelonephritis in rats with experimentally induced nephrosis. Many of the methods for production of pyelonephritis employ extrarenal or intrarenal urinary obstruction as the predisposing factor(8). Recent studies, however, have shown that a nephrotic syndrome can be induced in experimental animals by administration of the aminonucleoside, 6-dimethyl-aminopurine 3-amino-d-ribose (AMN) (9), and that the renal changes, as seen by light and electron microscopy(10), closely resemble those found in many cases of nephrosis in humans. In an attempt to clarify factors other than obstruction that might predispose the kidney to infection, rats pretreated with AMN were used in the present study. After induction of bacteremia, incidence of pyelonephritis and rate of disappearance of the injected organisms from the kidneys was determined by histologic examination and quantitative bacteriologic culture of renal tissue from experimental and control animals.

* This work was supported by U. S. Public Health Service Grants and by funds from the McKee Fund, allocated by Univ. of California Committee on Research, and from Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

Materials and methods. Long-Evans rats weighing 150 to 200 g were used. Nephrosis was induced by subcutaneous injection on 2 consecutive days of a 0.5% aqueous solution of AMN in doses of 0.3 ml per 100 g of body weight. An 18-hour broth culture of *E. coli*,† containing approximately 10^8 organisms per ml, was administered in a dose of 0.5 ml into the jugular vein.

The rats were divided into 4 groups. Group I consisted of 12 normal, untreated animals. The remaining 3 groups were comprised of 60 animals each. The rats in Group II were injected with *E. coli* and sacrificed in sets of twelve 2, 7, 14, 21 and 30 days after injection. The animals in Groups III and IV were treated with AMN. Urinary protein excretion was measured daily by the sulfosalicylic acid method. The rats developed 4+ proteinuria by the 7th to 10th day after injection of AMN. The rats in Group III were sacrificed in sets of twelve 2, 7, 14, 21 and 30 days after appearance of 4+ proteinuria. The rats in Group IV were injected with *E. coli* as soon as they developed 4+ proteinuria, and were sacrificed in sets of twelve 2, 7, 14, 21 and 30 days after injection.

The rats were killed with ether and autopsied immediately. The kidneys were removed under sterile conditions, and each was cut into 6 slices. Alternating slices were used for quantitative bacterial cultures and for microscopic sections stained with hematoxylin and eosin.

Bacterial cultures were prepared by grinding 0.5 to 1.2 g of kidney in a sterile mortar and adding broth (2 ml/g). Serial dilutions were prepared, plated on blood agar and incubated for 24 hours. Bacterial colonies were

† Gray strain, obtained from Dr. A. I. Braude, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

TABLE I. Incidence of Pyelonephritic Lesions and Presence of Coliform Organisms per Rat Kidney.

	Histologic examination			Bacteriologic examination					
					E. coli/g kidney				Significant non-coliform organisms
Group & interval	Neg.	"Non-specific"	Pyelo-nephritis	Neg.	$<10^3$	10^3-10^4	10^4-10^5	$>10^5$	
I (normal control)	21	3	0	24					
2 days									
II (*E. coli*)	9	15	0	0	0	5	10	9	0
III (AMN)	15	9	0	24	0	0	0	0	0
IV (AMN & *E. coli*)	4	20	0	0	0	8	4	12	0
7 days									
II	16	8	0	4	15	1	1	3	0
III	13	11	0	24	0	0	0	0	0
IV	0	21	3	6	3	4	3	7	1
14 days									
II	10	13	1	14	9	0	0	0	1
III	17	6	1	24	0	0	0	0	0
IV	1	12	11	13	8	1	0	2	0
21 days									
II	23	1	0	19	4	1	0	0	0
III	16	8	0	24	0	0	0	0	0
IV	3	14	7	19	3	0	0	2	0
30 days									
II	18	5	1	24	0	0	0	0	0
III	20	4	0	24	0	0	0	0	0
IV	5	8	11	17	3	1	0	3	0

then counted, and number of organisms per g of renal tissue was calculated. All coliform organisms were subjected to antibiotic sensitivity tests to determine whether they were identical with the injected strain. Occasional cultures contained small numbers (usually less than 20 per g of tissue) of organisms such as nonhemolytic *Staphylococcus albus* or *Alpha streptococcus,* which were considered to be contaminants and were disregarded.

Histologic criteria. Lesions were considered to be pyelonephritic when inflammatory cells, consisting of lymphocytes and varying numbers of polymorphonuclear leukocytes and macrophages, extended in sheets between the renal tubules, and were also present to a variable degree within the tubules and the renal pelvis. Older lesions contained occasional plasma cells.

Infiltrates were considered "nonspecific" for any disease process when they consisted of small numbers of cells, mostly lymphocytes, located within the interstitial tissues but not within the tubules and pelvis.

Results and discussion. The results of serial histologic and bacteriologic examinations in the 4 groups of animals are summarized in Table I and graphically presented in Fig. 1 and 2.

The normal control rats (Group I) showed no evidence of pyelonephritis. A "nonspecific" infiltrate was observed microscopically in 3 kidneys, but no *E. coli* were demonstrable by quantitative cultures.

The rats injected with AMN alone (Group III) developed degenerative and regenerative changes of the renal tubules. Hyaline casts were frequent, and in some cases small numbers of chronic inflammatory cells were seen surrounding the affected tubules (Fig. 3). In this group, only one kidney showed a minute focus of pyelonephritis on microscopic examination. No evidence of pyelonephritis was obtained by bacteriologic cultures. Bacteremias probably occurred in these animals, but evidently not to a sufficient degree to cause pyelonephritis. If the experiment had been carried out for a longer period, the incidence of spontaneous pyelonephritis in this group might have been greater.

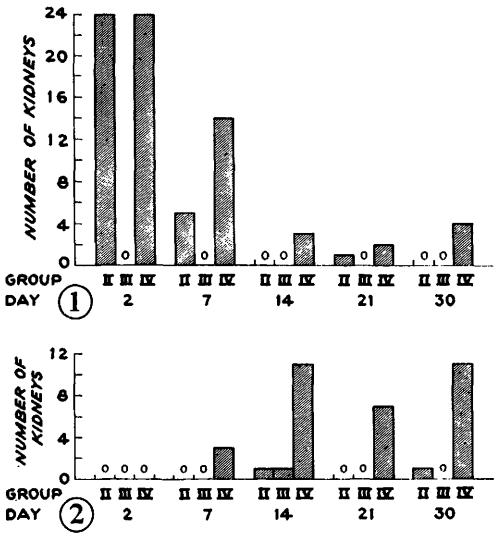

FIG. 1. No. of kidneys with bacterial counts $> 10^3$ *E. coli*/g of tissue in Groups II, III, IV.
FIG. 2. No. of kidneys with histologic lesions of pyelonephritis in Groups II, III, IV.

The rats with AMN-induced nephrosis (Group IV) showed a significantly increased susceptibility to hematogenous pyelonephritis when challenged with *E. coli*, compared to the rats injected with *E. coli* alone (Group II). The difference between Groups II and IV became evident on histologic examination 7 days after injection of *E. coli*, with occurrence of 3 cases of pyelonephritis in the nephrotic rats and none in the controls injected with *E. coli* only. The difference between Groups II and IV became even more marked with time. At 14, 21 and 30 days after injection of *E. coli*, 11, 7 and 11 cases of pyelonephritis, respectively, had occurred in Group IV, compared to 1, 0 and 1 cases in Group II. Analysis by the Chi-square test, using the Yates correction, showed these differences to be highly significant ($p<.01$).

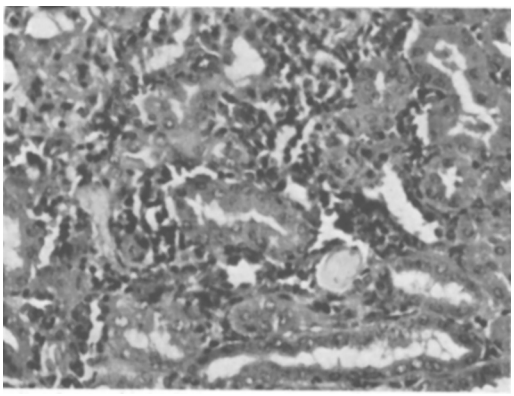

FIG. 3. Kidney of rat from Group III 7 days after onset of 4+ proteinuria, showing a "nonspecific" infiltrate, presence of protein within tubules and degenerative tubular changes. 204 ×.

The lesions seen earlier in the experiment showed an admixture of polymorphonuclear leukocytes and lymphocytes to a variable degree, and occasional lesions consisted almost entirely of polymorphonuclear leukocytes (Fig. 4). Later, lymphocytes dominated the picture, with occasional plasma cells present (Fig. 5). Toward the end of the experiment, the lesions showed a tendency to heal.

The differences between Group II and IV on quantitative bacterial culture were less striking; however the kidneys of the Group IV animals tended to contain larger numbers of organisms than those of Group II. *E. coli* were gradually removed from the normal rat kidney (Group II). Small numbers of organisms were demonstrable by culture up to 21 days after the injection in a few animals, but were no longer detectable at 30 days. In contrast, large numbers of *E. coli* were still demonstrable in 3 kidneys of Group IV animals at 30 days, and small numbers of organisms were observed in 4 others. In all cases the coliform organisms recovered from the kidneys were identical with the injected strain. Significant numbers of organisms other than the injected strain of *E. coli* were found in only 2 kidneys, one from an animal in Group II and one from a animal in Group IV.

The focal nature of pyelonephritis probably accounts for the poor correlation between histologic and bacteriologic findings. The affected kidneys usually contained only one or a few small foci. The lesion, therefore, may have been present in the tissue examined histologically but not in the specimen used for bacterial culture, or vice versa.

"Nonspecific" renal infiltrates were found in all 4 groups (Table I). In the kidneys of rats injected with *E. coli* alone (Group II), the infiltrates consisted of a few polymorphonuclear leukocytes and macrophages within the medullary interstitial tissues near the

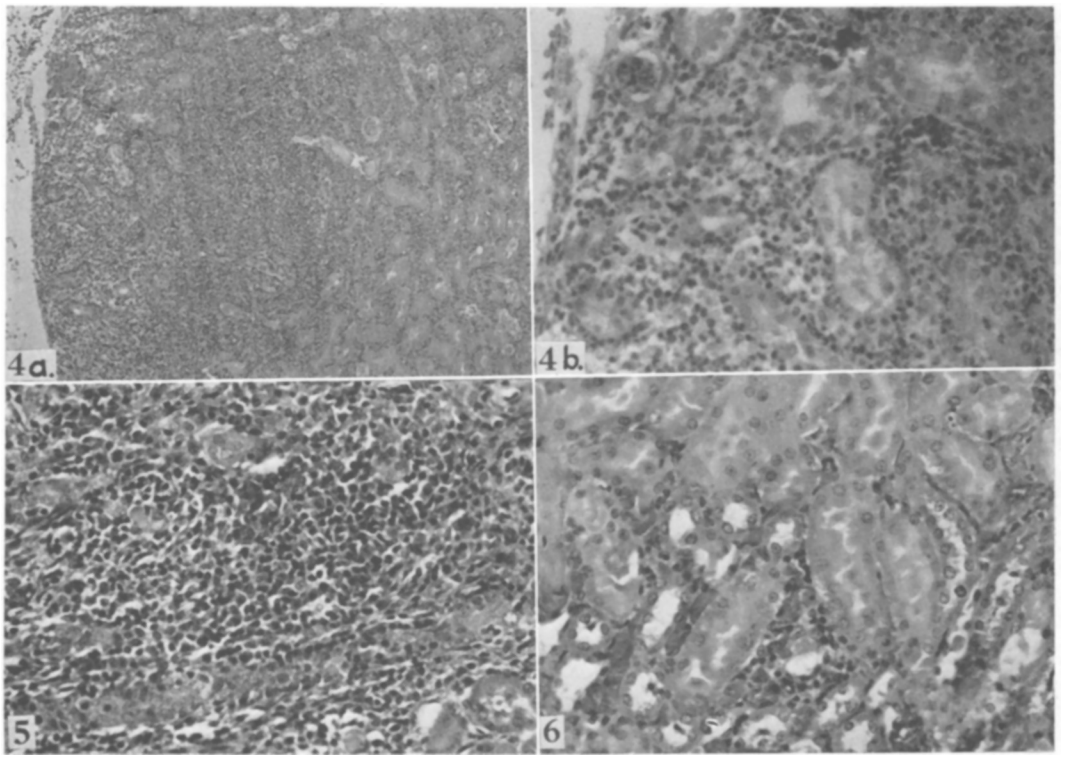

FIG. 4. a. Kidney of rat from Group IV 7 days after injection of *E. coli,* showing a typical cortical pyelonephritic lesion, consisting of a massive interstitial infiltrate of polymorphonuclear leukocytes, lymphocytes and macrophages. A tubule is also filled with inflammatory cells. 75 ×. b. Same lesion at higher magnification. 204 ×.

FIG. 5. Kidney of rat from Group IV 30 days after injection of *E. coli,* showing an older pyelonephritic lesion, consisting of a marked interstitial infiltrate of lymphocytes, macrophages and rare plasma cells. 204 ×.

FIG. 6. Kidney of rat from Group II 7 days after injection of *E. coli,* showing a small "non-specific" infiltrate. 204 ×.

corticomedullary junction (Fig. 6). Although these lesions were probably caused by bacteria, a diagnosis of pyelonephritis could not be made by pathological standards.

The results of this study show that AMN-induced nephrosis predisposes the rat kidney to pyelonephritis. Most of the factors previously shown to increase the susceptibility of the kidney to infection depend to a large extent on intrarenal or extrarenal obstruction of urine flow. In AMN-induced nephrosis, obstruction apparently is not a major predisposing factor to renal infection since the primary anatomic site of injury is in the glomerulus. Although tubular changes are present, the kidneys show no evidence of intrarenal obstruction. Thus, it is more likely that chemical or functional vascular alterations are the important factors in the pathogenesis of this type of pyelonephritis. A recent study of the enzymatic activity in homogenates of kidneys from rats with AMN-induced nephrosis(11) showed a decrease in alkaline phosphatase and an increase in hexokinase and glucose 6-phosphate-dehydrogenase. It remains to be seen whether the increased susceptibility to pyelonephritis in AMN-induced nephrosis can be related to changes in renal enzyme patterns, thus providing a common denominator for the various types of renal injury predisposing to infection.

Summary. Rats with nephrosis induced by treatment with aminonucleoside showed an increased susceptibility to pyelonephritis when challenged with *Escherichia coli.* The incidence of pyelonephritic lesions, determined histologically, was significantly greater

in these animals than in rats injected with *E. coli* only. The organisms were gradually removed from the normal kidney and were no longer demonstrable by culture 30 days after injection. In contrast, at 30 days, significant numbers of *E. coli* were still present in the kidneys of some rats with aminonucleoside-induced nephrosis. It is suggested that functional vascular alterations or, possibly, changes in renal enzyme patterns may be the important factors in the pathogenesis of this type of pyelonephritis.

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Received March 13, 1961. P.S.E.B.M., 1961, v107.

Oxidation of Palmitate-1-C^{14} by Red Blood Cells.* (26579)

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It is well established that many body tissues have the enzymatic equipment for oxidation of both glycerides and carbohydrates as a source of energy. Isotope tracer studies have shown that both these processes occur simultaneously in tissue cells, where either glucose or fatty acid oxidation may prevail to a different extent according to the experimental conditions. The non-esterified fatty acids (NEFA) of the blood plasma, present in water-soluble albumin complexes, have been shown to be highly active metabolites(1,2). They appear to be the prerequisite form for penetration of long-chain fatty acids into sites of oxidation(3,4) and the flux of calories carried by NEFA may reach more than twice the caloric value of glucose transport(5), although NEFA comprise only about 5% of the total fatty acids of the plasma.

It has long been known that red blood cells oxidize glucose as a source of energy(6). Although biosynthesis of fatty acids by red blood cells has been investigated by James, *et al.*(7), oxidation of fatty acids by red blood cells, to our knowledge, has not been examined.‡ However, oxidation of palmitate-1-C^{14} by skeletal muscle *in vitro* has been examined by Fritz, *et al.*(8), and its oxidation by hepatic and adipose tissues has been examined by Milstein and Briscol(9) and others. The aim of our investigation was to ascertain the existence of an enzymatic mechanism for fatty acid oxidation in red blood cells.

Methods. Normal, fed, adult, male rats, weighing between 200 and 400 g were killed by decapitation and rapid exsanguination. The blood was collected into about 15 to 20 times its own volume of modified Tyrode phosphate medium, (NaCl—0.137 M; KCl—0.004 M; Na_2HPO_4 buffered to pH 7.38—0.01 M). No anti-coagulants were used because this dilution of blood is sufficient to prevent formation of clots without their use.

* This research was supported in part by grants from U. S. Public Health Service.

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‡ A paper on "Incorporation of palmitate-1-C^{14} incorporation into blood cells" by Mines, C. J., Fillerup, D. L., Mead, J. F., *Nature* 1961, v190, 92, has just appeared as the present paper goes to press.