position of the pyrimidine ring(13). Others (14,15) have obtained barbituric acid from uracil oxidation by strains of Nocardia, Corynebacteria, and Mycobacteria.

The biological oxidation of the hydroxymethyl group to an acid is not limited to pyrimidines. Pyridoxine is converted to 4-pyridoxic acid(16) and other data implies that pantothenyl alcohol gives rise to pantothenic acid (17).

It is of interest to note that the magnitude of antibacterial activity as measured in this study declines in sequence with the oxidation of methioprim *i.e.* $-CH_2OH > -CHO >$ COOH (inactive).

Summary. Rat liver slices convert methioprim to 2-methylthio-4-amino-5-formylpyrimidine and 2-methylthio-4-amino-5-pyrimidinecarboxylic acid. Another unidentified ultraviolet-absorbing material is also produced. The antibacterial activity of methioprim is greater than the 5-CHO derivative, whereas the 5-COOH derivative is inactive.

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Ascending Infection as a Mechanism in Pathogenesis of Experimental Non-Obstructive Pyelonephritis.* (25206)

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Experimental pyelonephritis has generally been produced by methods involving intra- or extra-renal obstruction to the flow of urine with subsequent injection of bacteria into the blood stream. Ureteral ligation(1,2), electrocauterization(3), renal massage(4), or the scars of antecedent staphylococcal infection (5) have resulted in the localization of bacteria in the traumatized kidney with subsequent development of acute pyelonephritis. In addition, *S. aureus* in rabbits and mice(6), *P. acruginosa* in mice(7), *S. fecalis* in rats (8) and *C. renale* in mice(9) have, after intravenous injection, produced infections of kidney in the absence of induced urinary obstruction. The experimental models involving obstruction and hematogenous dissemination have been widely studied. In the absence of a clear demonstration that infection of the kidney may ascend directly from the bladder, the concept has become increasingly accepted that the hematogenous route is the principal one for the spread of infection to the kidneys, although many investigators have indicated the lack of definitive experimental informa-

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tion(10). Recently, however, it has been shown that asymptomatic bacteriuria in man not only may be associated with unsuspected pyelonephritis, but also may precede development of pyelonephritis(11-14). These observations suggested that production of persistent bacteriuria might be followed by infection of the kidneys. The present studies demonstrate that when bacteriuria is produced in the urinary bladders of rats, ascending pyelonephritis occurs regularly in the absence of demonstrable obstruction.

Materials and methods. The test organism was a strain of *Proteus vulgaris* grown overnight in nutrient broth, chosen because it was the most pathogenic to rats of a large number of strains of gram negative rods studied. Bacteria were injected directly into the bladder after the abdomen had been opened. When glass beads were used, they were inserted through an incision in the dome of the bladder and the bacterial culture injected immediately afterward. The defect in the bladder was closed by double ligatures. The experimental animals were male white rats weighing 150-200 g; they were sacrificed using ether anesthesia and the kidneys removed aseptically through separate flank incisions. The kidneys were homogenized in sterile broth using glass homogenizers. Serial dilutions of the homogenate, in nutrient broth, were transferred to agar pour plates for colony counts. Heart's blood was obtained antemortem by percutaneous cardiac puncture and urine for culture was obtained by aspiration through the bladder wall after the abdomen had been opened aseptically.

Results. Preliminary experiments showed that when about 10^8 bacteria were introduced into urinary bladders of rats, 60% of the animals developed anatomic and bacteriologic evidence of pyelonephritis within 4 days. If, in addition, a glass bead was inserted into the bladder lumen, pyelonephritis occurred in 95% of the animals within 4 days. The infection was bilateral in 97% of animals. When 10^9 bacteria were injected intracardially, 15% of the animals had pyelonephritis within 4 days; when a glass bead was in the bladder, incidence of infection of the kidney after hematogeneous dissemination of the bacteria rose to 40%. In general, when there were morphologic lesions of active pyelonephritis, colony counts of the kidney were greater than 10^6.

When about 10^8 bacteria were inserted into the bladders without glass beads, the bacteria were found in 55-60% kidneys, 24 hours later (Table I). When one ureter was doubly ligated, sectioned between ligatures, and bacteria then inserted into the bladder, bacteria were found in 50% of kidneys with uninterrupted ureters, but were present in only 9% of kidneys with sectioned ureters. When the experiment was performed with a glass bead in the bladder, bacteria were found in 75% of kidneys on the intact side, and in 35% of kidneys on the interrupted side. In almost every instance in which bacteria were found in the kidneys with ligated ureters, the blood cul-

TABLE I. Effect of Ureteral Section on Bacterial Colony Counts in Kidneys of Rats after Administration of *Proteus vulgaris*.

Site of inj.	Bead in bladder	No. rats	Kidney	Bacterial colony count in kidney at 24 hr			
				$>10^6$	10^{4-6}	10^{0-3}	0
Bladder; ureters intact	No	29	Right	34%	0%	25%	41%
			Left	34	3	18	45
" left ureter sectioned	"	34	Right	26	12	12	50
			Left	0	0	9	91
" ureters intact	Yes	20	Right	70	0	0	30
			Left	70	0	10	20
" left ureter sectioned	"	28	Right	61	11	3	25
			Left	3	7	25	65
Heart; left ureter sectioned	No	21	Right	10	24	57	9
			Left	48	5	42	5
Idem	Yes	27	Right	4	4	33	59
			Left	30	7	26	37

tures were positive for Proteus.

The results are not due to greater susceptibility of the uninterrupted side to hematogenous infection; when 10^{6-9} bacteria were given intracardially, the kidneys with obstructed ureters were more susceptible to hematogenous infection than were the intact kidneys (Table I).

Discussion. The experiments cited here leave little doubt that, after induction of bacteriuria, ascending infection of the kidneys may occur. Whether this pathway is the most common one in man remains to be demonstrated, but there is strong circumstantial evidence to suggest that bacteriuria in man leads to pyelonephritis and that the most probable route is an ascending one(10,13,15).

Whether the ascension of infections in rats is by way of multiplication and diffusion of bacteria in the lumen containing urine, or is by way of tissue spaces and lymphatics remains to be seen.

The observations are consistent with the earlier observations of David(16). The reasons why earlier workers failed to establish bacteriuria experimentally are not clear. It is likely, on the basis of preliminary observations in this laboratory, that the ease with which persistent bacteriuria was produced was related to selection of a strain of bacteria that was highly virulent for the experimental animals. All too frequently, attempts to produce experimental pyelonephritis have involved the use of bacterial strains that were pathogenic to man and were but weakly pathogenic to the experimental animals used.

Summary. 1. When bacteriuria is induced in rats by instillation of *P. vulgaris* into the urinary bladder, pyelonephritis is produced consistently in the absence of demonstrable obstruction of the urinary tract. 2. Ligation and section of one ureter greatly impairs spread of bacteria to the kidney on affected side. Kidneys with ligated ureters are, however, more susceptible than unaffected kidneys to hematogenous spread of the organisms. 3. Thus, bacteriuria, as produced here, leads to ascending infection of the kidney in absence of demonstrable abnormalities of the urinary tract.

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