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EFFECT OF ACUTE POTASSIUM DEFICIENCY ON SUSCEPTIBILITY TO INFECTION WITH PARTICULAR REFERENCE TO THE KIDNEY‡

Recent clinical reports describe the association of pyelonephritis and potassium deficiency, and the suggestion has been made that potassium depletion enhances the susceptibility of the kidney to infection.[11, 12]

There are both structural and metabolic alterations in potassium deficiency favoring renal infection. It has been well demonstrated in animals that potassium deficiency produces obstruction of collecting tubules in the medulla secondary to epithelial proliferation and interstitial fibrosis.[12] This results, in effect, in an internal hydronephrosis with stasis of tubular fluid. In addition, potassium is essential in many cellular processes. Lack of this ion could adversely affect the inflammatory process through impaired function of phagocytic cells or cellular mechanisms responsible for the elaboration of humoral factors. Furthermore, it has been shown that increased production of ketone bodies stimulates bacterial growth in tissues.[4, 5] The development of ketosis might be enhanced in potassium deficiency owing to inhibition of carbohydrate metabolism[7] and extracellular alkalosis.[10]

Evidence presented in this report suggests that in the presence of enterococcus, acute potassium deficiency appears to increase the susceptibility of rats to infection in general, with no primary effect upon the kidney.

MATERIALS AND METHODS

White male Sprague-Dawley strain rats weighing 100 to 250 gm. and white male Swiss strain mice weighing about 30 gm. were used .

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The potassium-deficient diet contained approximately 79 per cent carbohydrate, 15 per cent protein, and 5 per cent fat with vitamin supplementation and a basic salt mixture deficient in sodium, potassium, and chloride. The control diet for pair-fed animals had balanced quantities of potassium salts added. Animals on these diets received normal saline *ad libitum*. All other animals were fed Purina Lab Chow.

The strain of *Escherichia coli* employed was originally isolated from the urine of a patient with pyelonephritis. The method of preparing and maintaining this organism has been described.[9] Bacteria for intravenous injection were incubated in beef heart infusion broth at 37° C. for four hours. The volume injected was 0.5 cc. in rats and 0.25 cc. in mice.

The strain of enterococcus (*Streptococcus faecalis,* Lancefield Group D) was obtained from a patient with a urinary tract infection. Bacteria for injection were incubated in beef heart infusion broth at 37° C. for 24 hours. The volume injected was 0.5 cc. of a 1 : 10 dilution.

To enumerate each inoculum, tenfold dilutions in saline were incubated in agar pour plates. The tail vein was used for all intravenous injections. The organisms were identified by colony characteristics, Gram-stain, pigment production, and the use of special media such as Simmon's citrate and Kligler's iron agar.

Organs for bacterial counts were removed under pentobarbital anaesthesia. The abdomen or thorax was opened and the organs removed and placed in a Ten Broeck grinder. The whole left kidney was employed, whereas with lung or spleen about 1 gm. of tissue was removed. The tissue was ground in 9 ml. of saline until a smooth suspension was obtained. Subsequent tenfold dilutions were made in saline, and agar pour plates were prepared from a few of these dilutions, depending on the expected number of bacteria. Colony counts were taken 48 hours after incubation.

The right kidney in all animals and portions of lung, liver, spleen, pancreas, and bowel from some animals were fixed in 10 per cent formalin. All histological sections were stained with hematoxylin and eosin, and the Brown-Brenn method for identification of bacteria in tissues was used when indicated. In animals with high bacterial counts, two or more sections of kidney were examined histologically. Evidence for infection was the association of high bacterial counts with histological findings of acute inflammation, i.e., the exudation of plasma and polymorphonuclear neutrophils and tissue destruction.

Blood was obtained by direct puncture of the abdominal aorta, and serum potassium was determined with a Baird internal standard flame photometer.

RESULTS

In the first group of experiments (Table 1), potassium-depleted rats were injected with a strain of *E. coli* which does not produce pyelonephritis in the normal kidney but causes infection in the obstructed kidney.[9] These animals weighed approximately 250 grams and were maintained on a potassium-deficient diet for two to eight weeks. Serum potassium was significantly low in all groups and the kidneys showed prominent structural alterations attributed to potassium depletion.[13] The intravenous inoculum contained 150 to 200 million organisms, and colony counts were made after intervals of three days to two weeks. Most kidneys contained no more

TABLE 1. COLONY COUNTS IN POTASSIUM-DEPLETED RAT KIDNEYS FOLLOWING INTRAVENOUS INJECTION OF E. COLI

Group	*Length of deficiency*	*Serum potassium mEq/L.*	*Time interval*	*Colony counts x 10^3*
1	4 weeks	2.0-2.6	3 days	400
				151
				111
				11
				10
				1
2	2 weeks	2.3-2.6	1 week	.19
				.10
				.02
				.01
				.01
				0
3	3 weeks	2.4-2.6	1 week	.30
				.20
				.01
				0
				0
4	4 weeks	1.7-2.6	1 week	168
				53
				38
				16
				15
				7
5	8 weeks	2.3-2.5	1 week	15
				1
				1
				.16
				0
6	4 weeks	2.1-2.4	2 weeks	.68
				.03
				.02
				0
				0
				0

organisms than were found in normal animals.[9] A few counts, those over 100,000 in Groups 1 and 4, were higher than those encountered normally;[9] however, evidence of infection was not seen histologically. Furthermore,

TABLE 2. COLONY COUNTS IN NORMAL AND POTASSIUM-DEPLETED MOUSE KIDNEYS FOLLOWING INTRAVENOUS INJECTION OF *E. Coli*

Time interval	*Normal x 10³*	*Potassium depleted x 10³*	*Serum potassium (K-depleted) mEq/L.*
0	1000		
	800		
	9		
3 hours	43		
	34		
	2		
8 hours	4	11	1.8
	3	9	
		.60	
24 hours	60	74	
	.27	9	2.3
		2	
4 days	.26	24	
	.14	9	2.1
	.10	.24	
1 week	1	3.70	1.7-1.8
	0	1.76	
	0	.78	
		.15	
		.13	
		.05	
		0	
		0	
		0	
		0	

these counts were much lower than those of obstructed kidneys with frank infections.[9]

Because of the negative results obtained after the injection of *E. coli* in potassium-deficient rats, it was decided to study the effect in potassium-depleted mice. Control mice and mice maintained on a potassium-free diet for four weeks were injected with 75 to 100 million organisms. The results are represented in Table 2. Up to one million organisms were recovered

in normal animals immediately post-injection, which represent chiefly bacteria in the blood circulating through the kidneys at this period. Over the next few hours and days the number diminished, and at one week viable bacteria were recovered in only one of three animals. Bacterial counts were not significantly different in potassium-depleted mice, and evidence of infection was not seen histologically. The kidneys showed prominent alterations secondary to potassium lack.

Data presented thus far indicate that both potassium-depleted rats and mice had no increased susceptibility to renal infection after the intravenous administration of *E. coli*. Amren,[1] while studying experimentally induced

TABLE 3. OCCURRENCE OF RENAL AND OTHER INFECTIONS IN CONTROL AND POTASSIUM-DEPLETED RATS INJECTED WITH ENTEROCOCCUS

	Control			*Potassium depleted*			*Serum*
Group	*No. of animals*	*Renal infection*	*Other* infection*	*No. of animals*	*Renal infection*	*Other* infection*	*potassium mEq/L.*
1	10	2	..	10	4	..	2.0-2.4
2	10	2	3	10	4	6	1.8-2.3
3	10	2	5	10	5	8	2.1-2.3
4	10	1	3	10	7	9	1.9-2.4
Total‡		5	11		16†	23†	

* Includes pleuritis, mediastinitis, peritonitis, pancreatitis, and septic spleen.
† Significantly different from control group ($p < 0.05$) by chi-square test.
‡ Total includes only Groups 2 to 4.

bacterial endocarditis, found that the strain of enterococcus employed was capable of producing renal infection in normal rats. It was of interest to determine whether this organism would produce a higher incidence of pyelonephritis in potassium-deficient animals. An inoculum containing 30 to 50 million organisms was used since this caused renal infection in about 20 per cent of normal rats. Animals used in this study weighed about 100 gm. and since they were in a phase of rapid growth, two weeks' maintenance on a potassium-deficient diet produced significant lowering of serum potassium (Table 3) and marked structural change in the kidney. Bacterial counts were done four to six days' post-injection, although in several instances they were done earlier when death occurred or when death appeared imminent.

During the course of the first experiment (Group 1, Table 3) it became evident that infections were occurring in sites other than the kidney. Complete bacteriological and histological data were not obtained in this group, so that only the incidence of pyelonephritis was ascertained. Infection was

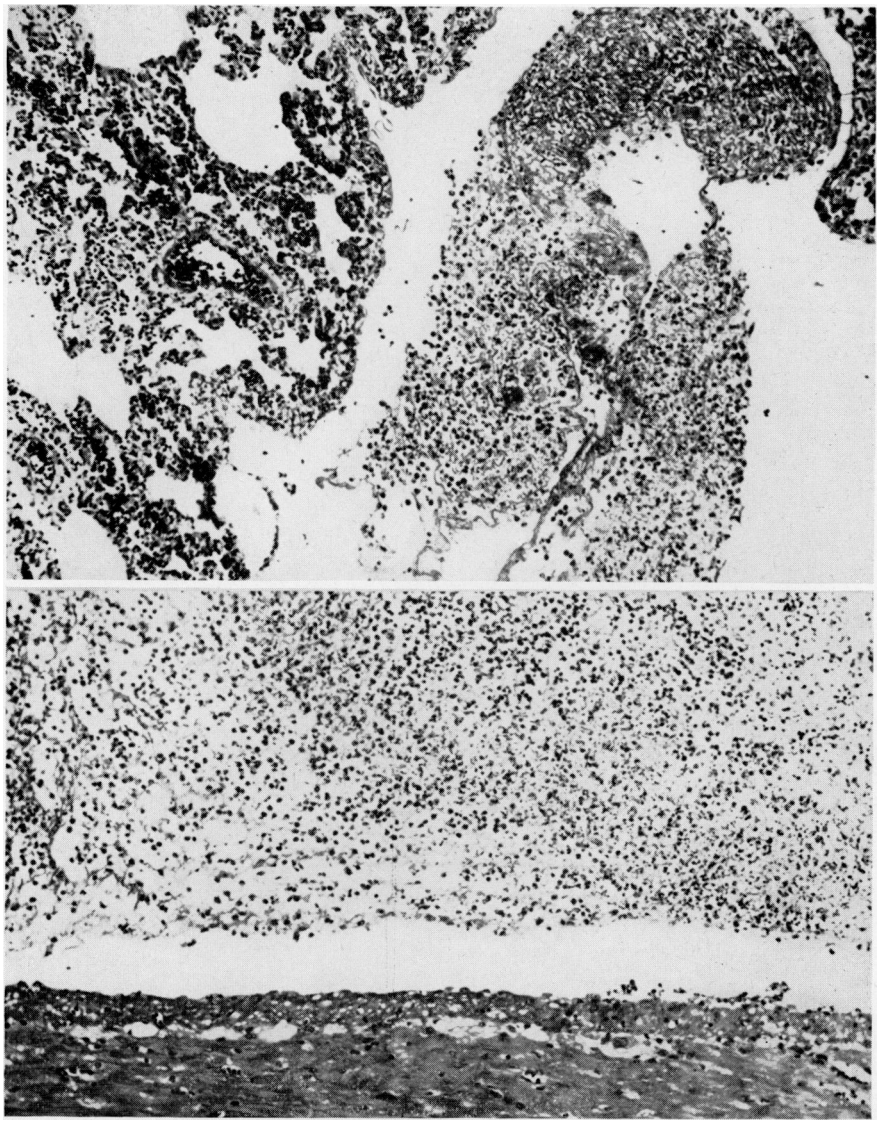

FIG. 1. Types of infections in potassium-depleted rats after the intravenous injection of enterococcus.

A. Mediastinitis. Heavy polymorphonuclear infiltrate in mediastinal tissues. H. and E. x100.

B. Peritonitis. Thick fibrinopurulent exudate adjacent to serosa. Bowel wall below. H. and E. x100.

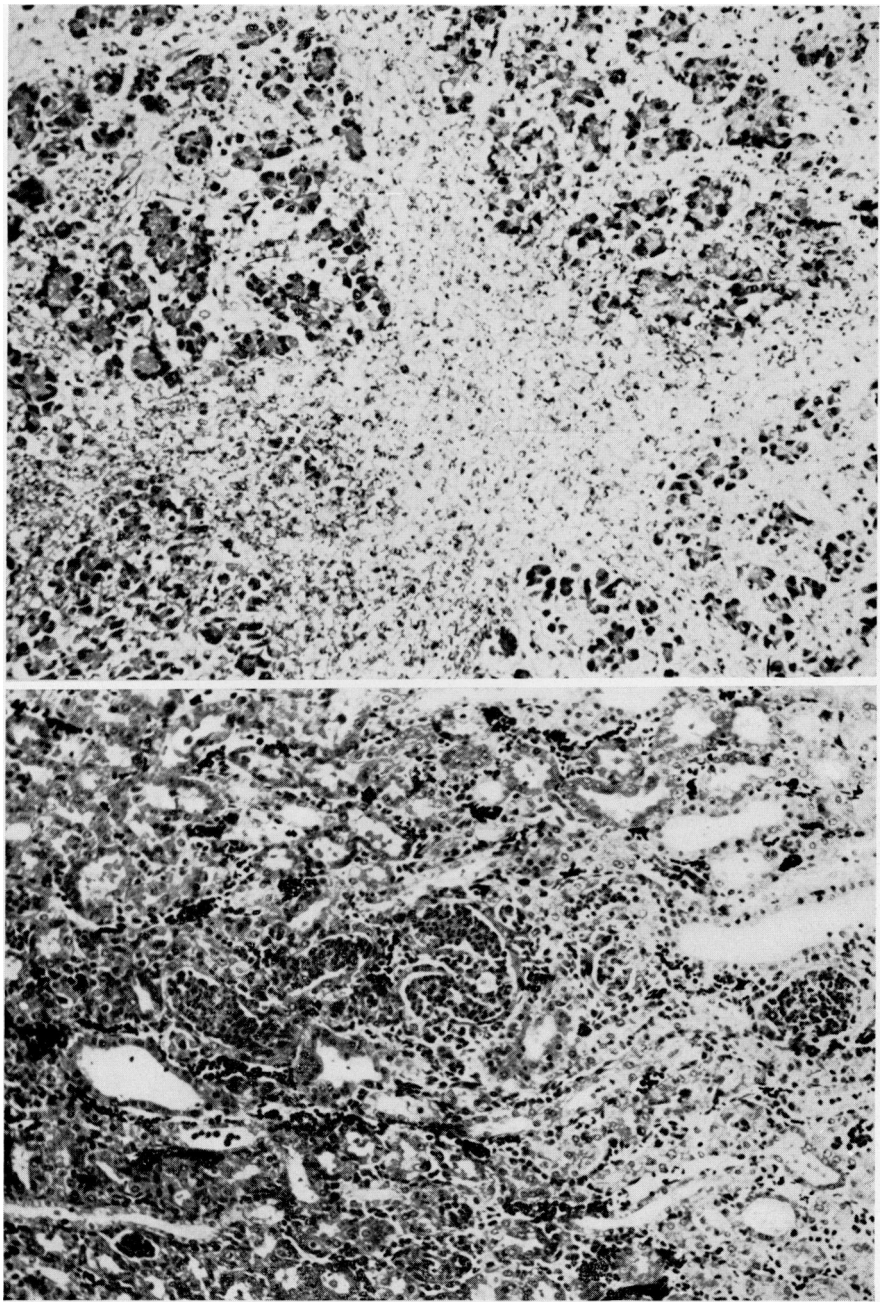

FIG. 1 (continued). Types of infections in potassium-depleted rats after the intravenous injection of enterococcus.

C. Pancreatitis. Extensive interstitial exudation and focal acinar necrosis. H. and E. x100.

D. Small focus in mid-medulla with slight acute inflammatory exudation in the interstitium and several necrotic tubules filled with masses of polymorphonuclear cells. H. and E. x100.

localized in the peritoneum, pancreas, retroperitoneum, spleen, pleura, and mediastinum. With such multiple involvement, evidence of infection was recorded as positive only when high bacterial counts were associated with histological evidence of an acute inflammatory process.

The incidence of pyelonephritis in potassium-depleted animals injected with enterococcus was three times that of simultaneously run controls (Table 3). Also, infections in tissues other than the kidney were much more numerous, and renal and extrarenal infections co-existed.

Growing animals on a potassium-free diet gain weight at a subnormal rate. Control animals in Group 4 were therefore pair-fed against potassium-depleted animals to determine if lack of an adequate caloric intake would alter susceptibility to infection.[17] The pair-fed controls gained an average of 46 gm., whereas other controls gained 72.9 gm. There was, however, no difference in susceptibility to enterococcus among the several control groups.

In both control and potassium-depleted animals, the nature and extent of infections in the kidney and other tissues requires special mention. Infection in the kidney was not visible grossly. Microscopically, there was no characteristic distribution, the cortex and medulla being involved in a random fashion, so that no specific relationship to the structural obstructive changes of potassium deficiency could be established. The infection consisted of small foci of interstitial exudation, predominantly polymorphonuclear, often with local tubular necrosis (Fig. 1-D). Brown-Brenn stains demonstrated the presence of Gram-positive cocci. In general, the pyelonephritis was relatively minor with little tendency toward extensive involvement of renal parenchyma.

Infection in other tissues was grossly visible. Mesothelial surfaces, such as the pleura and peritoneum, were thickened and dull and often coated with fibrinopurulent exudate. Fibrofatty tissues, such as mediastinum and retroperitoneum, were swollen and edematous. Histologically, the infection was suppurative (Fig. 1-B), frequently with marked necrosis. Although involvement of pleura and mediastinum was often widespread, infection was not seen in the lung parenchyma (Fig. 1-A). The pancreatitis was interstitial with regions of acinar necrosis (Fig. 1-C). The infective process tended to spread through the retroperitoneum, so that involvement of perinephric fat was not uncommon. Spleens from animals with well-established infectious disease showed proliferation of reticulo-endothelial elements and foci of polymorphonuclear cells occasionally with local necrosis. Examination of the endocardium with a dissecting microscope revealed no evidence of endocarditis. The infectious process in these sites tended to be extensive and progressive, which in about one quarter of the potassium-

depleted animals led to death. These findings are somewhat similar to those reported by Gray *et al.*[8] on staphylococcal-induced infections in mice. Their mortality was 15 per cent, and they found abscesses in kidney, heart, liver, intestinal mucosa, pancreas, and brain.

DISCUSSION

Milne *et al.*[11, 12] have suggested that there is a high incidence of pyelonephritis in patients with potassium depletion as a result of increased susceptibility of the kidney due to damage by potassium deficiency. Striking changes have been described in the nephropathy of potassium-depleted animals consisting of epithelial degeneration, necrosis, and proliferation of medullary collecting tubules, often leading to obstruction with dilatation proximally. Furthermore, Fourman *et al.*[6] observed prominent interstitial scarring which was progressive, even in the face of potassium repletion. Such lesions have not been seen in man. The usual finding is vacuolization of epithelial cells confined to the proximal convoluted tubules, the collecting system in most cases appearing normal.[14] In the absence of obstructive or other structural alterations, there is no obvious reason why degenerative changes in the proximal convolutions should enhance susceptibility to infection.

Relman and Schwartz[14] have recently reviewed the occurrence of pyelonephritis in reported cases of potassium depletion. In 47 cases of nephropathy caused by diarrhea, pyelonephritis was reported in 6 of 13 cases of primary aldosteronism. From this data they concluded that a predisposition to pyelonephritis was suggestive in the nephropathy associated with aldosteronism but unconvincing in diarrhea. In renal biopsies from patients with primary aldosteronism, interstitial scarring, glomerular hyalinisation, and tubular changes secondary to hypertension could be misinterpreted as evidence of pyelonephritis. More clinical observations must be compiled before definite conclusions can be drawn regarding the susceptibility of the potassium-depleted human kidney to infection.

Because of the marked differences in renal structural changes associated with potassium depletion in man and animals, interpretation of experimental data dealing with susceptibility to infection may not be directly applicable to humans. Moreover, it has been shown that renal scars, even when minute, enhance susceptibility of the kidney to infection in normal animals.[3, 16] Renal scars develop in the nephropathy of experimental potassium depletion and clinical aldosteronism and these lesions rather than potassium lack could be the chief factor causing infection. In order to minimize this in our animals, the degree of potassium depletion was of

sufficient severity to cause prominent structural alterations in the tubules with renal enlargement but without the development of significant interstitial fibrosis. In this manner, the effects of acute, severe potassium depletion upon susceptibility to infection could be studied without the complications of renal scarring and its secondary morphological changes.

It is not surprising that potassium-depleted rats and mice did not develop pyelonephritis after the intravenous injection of *E. coli*. Rocha[15] found that this organism produced no significant incidence of pyelonephritis in rats with severe injury following uric acid or mercuric chloride administration. In addition, *E. coli* failed to cause pyelonephritis in rats with vitamin D-induced nephrocalcinosis.[2]

Woods, et al.[18] have shown that rats with both acute and chronic nephropathy of potassium depletion have increased susceptibility to pyelonephritis produced by the intravenous administration of *E. coli*. Their strain of *E. coli* was highly virulent in rats, since it produced death or pyelonephritis in a large percentage of normal animals. When injected in small numbers, it is possible that this organism might reveal lesser degrees of susceptibility to infection than the strain of *E. coli* used in the present report.

In the present experiments, enterococcus produced a higher incidence of renal infection in potassium-depleted animals than in controls. Pyelonephritis was associated with infection elsewhere, the former being mild in comparison with the latter. The increased incidence of infection in potassium-depleted rats injected intravenously with enterococcus suggests that potassium deficiency may enhance susceptibility to some generalized infections. This is not unexpected since potassium is the principal intracellular cation and influences many cellular metabolic processes, possibly including those necessary for natural defense against infectious agents. Certain organic acids, including ketone bodies, have been shown to enhance the growth of bacteria in tissues. It is conceivable that the accumulation of such acids may be favored by a state of potassium depletion.

SUMMARY

E. coli failed to induce pyelonephritis in acutely potassium-depleted rats and mice. Enterococcus produced a higher incidence of generalized infection and pyelonephritis in potassium-deficient rats than in controls. Infections in extra-renal sites were extensive and often overwhelming, while those in the kidney were relatively mild. These data suggest that potassium depletion enhances generalized susceptibility to some infections with no primary effect on the kidney.

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