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Potassium, Lethal and Tumor-Necrotizing Properties of Klebsiella Polysaccharide.* (27265)

JAMES R. CROOK, WERNER K. OTTO AND RUSSELL S. JONES
Department of Pathology, University of Utah College of Medicine, Salt Lake City

Endotoxins of gram-negative bacteria produce a variety of biologic responses including fever, hyperglycemia, shock, tumor-necrosis, death(1,2) and increased corticosteroidogenesis(3). The interrelationships and mechanisms of the varied biologic effects are uncertain. Some responses appear to oppose one another. For example, augmented corticosteroidogenesis, like exogenous glucocorticoids, may inhibit tumor-necrotizing(4,5), delayed lethal(5,6), and pyrogenic(7) properties of the endotoxins. Alterations in glycolysis and oxidative phosphorylation may provide a partial explanation for some of the biologic effects. Changes in liver glycogen, blood glucose and lactic acid levels of animals following injection of endotoxins(2,8) are accompanied by alterations in inorganic and organic phosphates, pyruvate and α ketoglutarate(9). Mitochondria from animals treated with endotoxin showed decreased esterification of inorganic phosphate(10) and the addition *in vitro* of non-lethal, haptenic bacterial polysaccharide to mitochondria from rat and mouse liver and guinea pig kidney decreased oxygen uptake and oxidative phosphorylation(11). In the present study, potassium (K), which plays an important but obscure role in cellular energy production and transfer mechanisms(12), has been found to markedly augment the tumor-necrotizing and delayed lethal effects of relatively non-toxic, haptenic bacterial polysaccharide.

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Materials and methods. The haptenic, capsular polysaccharide of Klebsiella pneumoniae, type B, was extracted with 0.1 N NaOH(13) and dissolved in either 5% glucose of 0.85% NaCl in 1, 10, 100, and 1,000 mg% concentration and injected intravenously, .01 to 10 mg/100 g body weight, into young male Holtzman rats, each 120-140 g, and both with and without Murphy-Sturm lymphosarcomas. The lymphosarcoma was transplanted by subcutaneous injection over lumbospinal muscles of approximately 300,000 tumor cells suspended in Earle's solution. Size of the tumor and weights of the rats were recorded daily. Control and other groups of tumor-bearing rats were observed for spontaneous regression—*i.e.*, reduction in size and disappearance of the transplantable tumors. Surviving rats in all tumor-bearing groups were observed for 6 or more weeks. The time of appearance of palpable tumors, rate of tumor growth and accompanying loss of body mass of rats in the present study were similar to those previously reported(14). Because the delayed lethal toxicity of the polysaccharide increased with tumor size, animals with tumors of 25 ± 2 mm mean diameter were used.

Before, during and after polysaccharide administration, KCl in either 5% glucose or in 0.85% NaCl, was injected intravenously, 0.001 to 0.5 meq K/l/100 g body weight. KCl solutions were injected within 3 to 5 seconds. Appropriate dilutions were made

TABLE I. Enhancement by Potassium of Delayed Lethal Toxicity (LD_{50}) of Klebsiella Polysaccharide in Normal Rats.

Polysaccharide mg/100g body wt	.85 in glucose 2.20 in NaCl	.4†	.3	.2	.1	.05	.025	.01	None
KCl, meq/ 100 g in 5% glucose	—	.00013	.00025	.00080	.0014	.055*	.065*	.070*	.070*
.85% NaCl	—	.00020	.00040	.00088	.0035	.070*	.075*	.070*	.075*

* Immediate death due to K intoxication produced by these values; delayed death characteristic of lipopolysaccharide endotoxins did not occur in rats surviving the immediate hyperkalemia or in rats receiving lower doses of KCl in combination with .05 mg or less of polysaccharide.

† Polysaccharide in either 5% glucose or .85% NaCl corresponding to KCl solutions, with uniform .1 ml/100 g injection volume by adjustment of polysaccharide concentration.

to permit injection of comparable fluid volumes. Control animals received a single injection of the varying concentrations of either KCl, NaCl, glucose or polysaccharide. Cortisol acetate in aqueous vehicle (Merck Sharp and Dohme) in several dosages was injected subcutaneously 1 hour before intravenous injections of a standard (LD_{60}) combination of polysaccharide and KCl. Ten or more animals were used for each concentration of injected materials. K and Na were determined by flame photometry of voided urine collected over a 12-hour period and in non-hemolyzed plasma of a separate group of tumor-free rats, killed 3 hours after injection of various solutions. All rats received water *ad libitum* and purina laboratory chow.

Results. The mild lethal toxicity of Klebsiella polysaccharide in normal rats was increased by solutions containing K. The delayed lethal toxicity of the polysaccharide in K-free solutions was greater when dissolved in 5% glucose than in 0.85% NaCl, LD_{50} being 0.85 and 2.2 mg/100 g body weight, respectively. There was an inverse relation between amount of polysaccharide injected, whether in glucose or NaCl solution, and quantity of K required to produce delayed death. With minimal quantities of polysaccharide, K was without effect, normal rats tolerating this electrolyte up to amounts which, when given alone, lead to paresis and death within a few minutes following intravenous injection (Table I). The enhancement by K of delayed lethal toxicity persisted when either K or polysaccharide was given within 24 but not 48 hours before the other. With near lethal doses of K in combination with polysaccharide, the larger doses of cortisol, subcutaneously injected, only slightly decreased while the smaller doses may have increased the delayed lethal effect (Table II).

Plasma levels of K and Na at the critical 3-hour interval were not modified significantly by K alone or in various combinations. Urinary excretion of K and Na, although limited in value by lack of dietary control of these electrolytes, appeared unrelated to polysaccharide injection (Table III).

Both the delayed lethal action of the polysaccharide and the enhancing effect of K were greatly increased by the presence of rapidly growing but relatively small lymphosarcomas (Table IV). As noted with the tumor-free rats, the larger the non-lethal dose of polysaccharide, the smaller the quantity of K required to produce death. For example, with non-lethal doses of polysaccharide, 50% of the tumor-bearing rats died when 0.0001 meq of K/100 g body weight was also injected. This minute quantity of K added to the 4 ml plasma volume (for 100 g body weight) would be expected to yield a maximal increase of only 0.025 meq in the normal plasma K of 5.0 meq.

Similar small amounts of K, injected intravenously, also augmented the action of the polysaccharide upon the lymphosarcomas

TABLE II. Effect of Cortisol Upon Potassium Enhancement of Lethal Toxicity of Klebsiella Polysaccharide in Normal Rats.

Cortisol* (mg/100g body wt) 1.0	.5	.1	.05	None
8/10 —(40%)	10/20 —(50%)	16/20 —(80%)	17/20 —(85%)	9/15 —(60%)

* Cortisol acetate subcutaneously 1 hr before intravenous injection of polysaccharide, .2 mg, and KCl, .002 mEq/100g body weight, in 5% glucose.

TABLE III. Plasma Levels and Urinary Excretion of Potassium After Injection of Polysaccharide With and Without Other Solutions.

Polysaccharide*	Concomitant† solution	Total electrolyte injected mEq/100 g		Plasma‡ mEq/L		Urine mEq/12 hr			
		K	Na	K	Na	K		Na	
						per L	total	per L	total
in .15 M NaCl	—	—	.015	5.03	146	240	.589	217	.561
"	150 mEq/L NaCl	—	.165	4.95	154	258	.712	188	.516
"	1 mEq/L KCl in NaCl	.001	.165	5.10	151	258	.798	192	.615
in 5% glucose	—	—	—	4.75	134	251	.251	114	.114
"	5% glucose	—	—	5.45	157	370	.612	186	.308
"	KCl in glucose	.001	—	4.48	142	240	.711	174	.525
None	glucose	—	—	5.33	144	357	.179	365	.183
"	NaCl	—	.150	5.90	150	422	.422	338	.338
"	KCl in NaCl	.001	.150	4.60	152	390	.975	227	.568
"	" " glucose	.001	—	5.22	148	225	1.013	127	.570

* .1 mg (.1 ml)/100 g body weight.
† 1 ml/100 g body weight.
‡ 3 hrs after injections.

(Table IV). Four daily injections of 0.02 mg/100 g body weight resulted in 35.9% regression in comparison to 88.8 and 70% when 0.0001 and 0.00005 meq K, respectively, were also injected. Glucose, KCl and NaCl solutions given without polysaccharide did not increase the low incidence of tumor regression in control rats.

Comments. The data of the present study indicate that K potentiated the delayed lethal toxicity of Klebsiella polysaccharide in normal and especially in tumor-bearing rats.

The enhancing effects of K do not appear attributable to hyperkalemia, *per se,* because: a) plasma K levels were not increased by polysaccharide alone, b) large quantities of K injected intravenously without polysaccharide produced immediate and not delayed death, and c) minute quantities of K added to otherwise non-lethal doses of polysaccharide produced delayed death. It is possible that K, injected intravenously, modified the interrelationship of plasma constituents and the bacterial polysaccharide. The toxicity of lipopolysaccharides, ascribed to the lipoid component, is reduced by incubation with plasma(15,16). Shear and coworkers(16) found that divalent cations, particularly calcium, inhibited the detoxifying action of plasma. The decrease in corticosteroidogenic capacity of the lipid-free polysaccharide used in the present study has been associated with binding to plasma proteins (17). However, studies with C^{14} labeled polysaccharide indicate that it is removed from the plasma by many tissues before appreciable "detoxification" would have occurred(13,17). Further studies are needed to determine whether the biologic effects of this particular polyelectrolytic, polyhydrogen-bonding macromolecule may be modified by small quantities of electrolytes, including K, either within the plasma, on the surfaces of cells or within the cytoplasm of certain cells.

Because death does not occur for several hours after injection of polysaccharide, either with or without K, a progressive metabolic derangement is suggested. Cellular alterations induced by the bacterial polysaccharide could be augmented by minute changes in K gradients within cells or between cells and their fluid environment. The latter possibility might be related to the greater toxicity of polysaccharide in 5% glucose as compared to .85% NaCl, Na favoring the usual gradient across the cellular membrane and the increased cellular utilization of glucose favor-

TABLE IV. Enhancement by Potassium of Lethal and Tumor-Regressing Properties of a Uniform Small Quantity* of Bacterial Polysaccharide in Rats With Lymphosarcoma.

	K, mEq† (with uniform dose polysaccharide)*					K without poly mEq	NaCl (.15M) only mEq	Glucose (5%) only ml	Polysacch* only† mg	Control (no injections)
Quantity per 100 g body wt	.0001	.00005	.00001	.000005	.000001	.001	.015	.15	.02	—
Delayed Death‡	8 —(50%) 16	5 —(20%) 25	4 —(26.7%) 15	0 —(0%) 11	0 —(0%) 10	0 —(0%) 15	0 —(0%) 15	0 —(0%) 10	0 —(0%) 28	—
Tumor Regression	7 —(88.8%) 8	14 —(70%) 20	5 —(45.4%) 11	6 —(54.5%) 11	5 —(50%) 10	0 —(0%) 15	1 —(6.7%) 15	1 —(10%) 10	10 —(35.9%) 28	2 —(7.1%) 28

* .02 mg/100 g body weight for each of 4 consecutive days.
† Solutions in 5% glucose.
‡ Deaths with first but not subsequent injections in tumor-bearing rats.
LD_{50} of polysaccharide in 5% glucose, .27 mg and in .85% NaCl, .35 mg/100 g body weight tumor-bearing rat.

ing the uptake of K. However, in our unpublished studies large amounts of .85% NaCl injected either intravenously, intraperitoneally or subcutaneously, did not modify significantly the delayed lethal toxicity of the polysaccharide or the potentiating action of K; various commercial "balanced" electrolyte solutions given either intravenously or intraperitoneally did not alter the potentiating effect which remained proportional to the content of K and route of injection. It is also possible that glucose and K increased the cellular uptake of toxic polysaccharide in a manner comparable to the uptake of sucrose into protoplasts(18).

Endotoxins of gram-negative bacteria modify carbohydrate metabolism in experimental animals(8-11) but the primary site of this effect has not been established. The depletion of liver glycogen, attributed to activation of phosphorylases(19) and perhaps accompanied by blockage in glycogen synthesis (20) may follow and not precede other evidences of toxicity. Mager and Theodor(11) found no correlation between the *in vivo* toxicity, and certain *in vitro* effects of some bacterial products. A relatively non-toxic, haptenic polysaccharide, similar to that used in the present study, inhibited mitochondrial respiration and fixation of inorganic phosphate. The increased toxicity of Klebsiella polysaccharide in rats with rapidly growing tumor may be associated with impaired cellular production and transfer of energy. Dietary sources and conversion of host tissues are apparently inadequate to meet the nutritional demands of the tumor, which soon reduces the body mass of the rat(14). The unchanged oxygen consumption of the tumor-bearing rat (unpublished studies) may reflect the overall increase in anaerobic glycolysis due to the tumor. Metabolites produced by the high anaerobic glycolysis but not used by the tumor tissue(21), could be utilized by host tissue *via* respiratory pathways for its own energy requirement or could be converted with energy loss to glucose. Continued depletion of host tissue would suggest that the net result is the continued supply to the poor "economy" of the tumor. The introduction of bacterial polysaccharide

might further impair this metabolic imbalance and thereby lead to death of tumor or host.

The protective action of glucogenic corticosteroids against tumor necrosis and lethal toxicity(5) may be related to similar metabolic mechanisms. In the present study, cortisol, given in dosage and time relationship adequate to protect adrenalectomized rats from large doses of Klebsiella polysaccharide, did not protect normal rats against the lethal-enhancing action of K, suggesting that K and glucocorticoids may act through separate mechanisms in producing their opposing effects upon the toxicity of bacterial polysaccharide.

Summary. Potassium injected intravenously increased the mild delayed lethal toxicity of Klebsiella polysaccharide in normal rats. In rats bearing relatively small lymphosarcomas the delayed lethal and tumor-necrotizing actions of the polysaccharide were markedly increased by minute quantities of potassium.

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Effect of Protamine on Activity of Hog Pancreatic Amylase.* (27266)

ROBERT L. MCGEACHIN AND WILLIAM C. PAVORD
Department of Biochemistry, University of Louisville School of Medicine, Louisville, Ky.

Krebs(1) and Madsen and Cori(2) have reported that the protamine, salmine, strongly inhibited rabbit muscle phosphorylase at low concentrations of salmine. At higher concentrations of protamine, there was partial reactivation of the phosphorylase probably by solubilization of the protamine-phosphorylase complex. In studying the ability of various proteins to reverse the beryllium inhibition of certain mammalian amylases(3), we have found that not only does protamine fail to reverse or prevent beryllium inhibition but also that protamine alone may be inhibitory. Observations of the effect of protamine at various concentrations and of the modifying effect of heparin are reported here.

Materials and methods. Crystalline hog pancreatic amylase used in these experiments was obtained from the Worthington Biochemical Corp.(4), heparin (U.S.P. grade) from Hynson, Westcott and Dunning, Inc.

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