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POLYMYXIN B SULPHATE PROTECTS CATS AGAINST THE HAEMODYNAMIC AND METABOLIC EFFECTS OF *E. coli* ENDOTOXIN

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1 The intravenous administration of *E. coli* endotoxin (2 mg/kg) in cats anaesthetized with pentobarbitone resulted in an initial acute increase in right atrial pressure and a transient systemic hypotension. Later (from 1 h onwards) there was a progressive decrease in cardiac output, a reduced right atrial filling pressure, systemic hypotension and a profound metabolic acidosis (lactate of 30 ± 1 mg/100 ml at 5 h compared with 5.1 ± 0.5 mg/100 ml pre-endotoxin). Only one of eight animals so treated survived 8 h.

2 Polymyxin B sulphate, given intravenously (1 min before endotoxin) as a bolus injection (5 mg/kg) followed by a continuous intravenous infusion (additional 5 mg/kg given over a 30 min period) prevented the endotoxin-induced pulmonary (right atrial) hypertension but not the acute systemic hypotension.

3 Polymyxin B sulphate reduced the delayed haemodynamic effects of endotoxin (systemic hypotension, decrease in cardiac output); all the eight animals so treated survived 8 h compared with only 1/8 of the controls.

4 Polymyxin B did not prevent the initial (1–3 h) and marked metabolic acidosis following endotoxin; however, after 3 h, arterial lactate levels returned towards control whereas in the endotoxin-alone group they continued to increase until death.

5 The mechanism of this marked protective effect of the antibiotic and the possible clinical repercussions are discussed; the most likely explanation for the protection is in chemical combination with the lipid A moiety of the endotoxin.

Introduction

Recent studies have demonstrated that the cyclic polypeptide antibiotic, polymyxin B sulphate, prevents a number of the biological effects that result from the administration of gram-negative bacterial endotoxins. These effects include the febrile response to endotoxin in goats (Van Miert & Van Duin, 1977; 1978), the generalized Shwartzmann reaction in rabbits (Rifkind & Hill, 1967; Corrigan, 1969; Corrigan & Bell, 1971) and the lethal effects of purified endotoxin and of *Serratia marcesens* and *Neisseria meningitidis* organisms in mice (Rifkind, 1967; Bannatyne, Harnett & Cheung, 1977; Bannatyne & Cheung, 1979).

In the cat, the response to the administration of a lethal dose of *E. coli* endotoxin consists of an acute phase (occurring within 1–3 min of intravenous administration) characterized by pronounced pulmonary changes (hypertension, reduced pulmonary compliance and an increase in airways resistance) and by a transient systemic hypotension. This is followed by a delayed shock phase characterized by prolonged systemic hypotension, a reduced cardiac output and severe metabolic acidosis (Parratt, 1973; Parratt, Coker, Hughes, McDonald, Ledingham, Rodger & Zeitlin, 1981).

Previous unpublished work in this department (B.R. Madan & J.R. Parratt) demonstrated that polymyxin B, in an optimal dose of 5 mg/kg administered 1 min before endotoxin, abolished the acute post-endotoxin responses. The main purpose of the present studies was to investigate the effects of polymyxin on the development of the delayed (fatal) shock phase in this model. A preliminary account of these studies was presented at a meeting of the British Pharmacological Society (Hughes, Madan & Parratt, 1980).

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Methods

Twenty seven cats (mean weight 2.1 ± 0.1 kg) of either sex were fasted overnight and anaesthetized with sodium pentobarbitone (30 mg/kg administered intraperitoneally). Polythene cannulae were placed in the right atrium and the aortic arch for pressure measurements.

The trachea was cannulated and the animals allowed to breathe spontaneously. When necessary (e.g. immediately after endotoxin administration) the animals were ventilated with a positive pressure respiration pump (Palmer) using room air at a rate of 20 strokes/min and a stroke volume of 25 ml/kg. A femoral vein was cannulated for drug and endotoxin administration.

All pressures were recorded with appropriate Elema-Schönander or Statham transducers and recorded on a Mingograph 81 ink-jet writing recorder, together with the electrocardiogram (usually lead II). Cardiac output was measured by a thermodilution technique using room temperature saline (0.9% w/v NaCl solution) (Parratt 1973). Body temperature was monitored from direct recording thermocouples in the oesophagus and rectum. Arterial blood samples were analysed for blood gases and pH using appropriate electrode systems (Instrumentation Laboratories) and arterial lactate was measured enzymatically (oxidation by NAD) using a Boehringer test combination. All cats received heparin (100 iu/kg) at the start of the operative procedures.

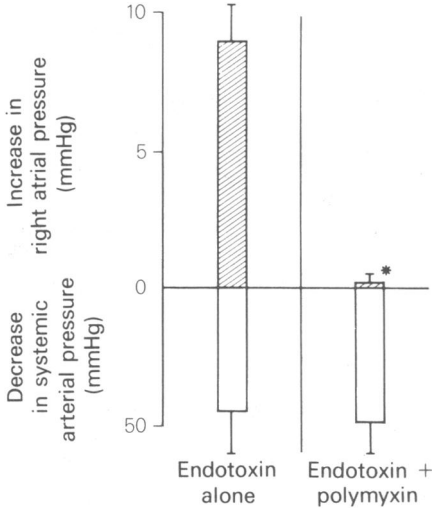

Figure 1 Abolition, by pretreatment with polymyxin B sulphate, of the acute (pulmonary) hypertensive response that occurs 1–3 min after *E. coli* endotoxin administration. The systemic hypotensive response was unaffected by polymyxin.
*$P < 0.05$.

The cats were divided into three groups:
Group (1) These cats ($n = 8$) were given *E. coli* endotoxin (0.55 B5 lipopolysaccharide B Difco, prepared by the Boivin method) in a dose of 2 mg/kg, suspended in saline, by intravenous injection. This is an LD_{90} in this species (Parratt, 1973).
Group (II) These cats ($n = 11$) were given polymyxin B sulphate (Aerosporin, Wellcome Laboratories) 5 mg/kg (total dose) dissolved in saline and infused intravenously for 30 min (i.e. at a rate of $0.17\ mg\ kg^{-1}\ min^{-1}$ beginning 5 min before endotoxin administration). A further 5 mg/kg was injected as a bolus 1 min before endotoxin.
Group (III) The third group of cats ($n = 8$) received polymyxin B alone, administered as for the Group II animals.

For the determination of significant differences between control (endotoxin alone) and treated (endotoxin plus polymyxin B) groups, Student's *t* test for paired data was used.

Results

Effect of polymyxin B sulphate pretreatment on the initial (acute) response to E. coli *endotoxin*

Within 1–3 min of endotoxin administration the animals not pretreated with polymyxin B (Group I) exhibited responses typical of this model (Parratt, 1973). There was a marked increase in right atrial pressure of 9 ± 1.3 mmHg (from 1.3 ± 0.6 to 10.4 ± 1.2 mmHg). In some cats there was a slight reduction in heart rate and ventricular arrhythmias frequently occurred.

In the group of cats pretreated with polymyxin B, endotoxin failed to increase right atrial pressure (1.7 ± 0.3 pre-endotoxin to 2.0 ± 0.6 mmHg) and there were no arrhythmias. However, the initial (immediate) reduction in arterial blood pressure (of 49 ± 9 mmHg) was similar to that in the endotoxin alone group (Figure 1) and also to that observed in (Group III) cats given only polymyxin (137 ± 9 to 110 ± 9 mmHg during the infusion period).

Effects of polymyxin B sulphate pretreatment on the delayed response to E. coli *endotoxin*

Haemodynamic responses The delayed cardiovascular effects of *E. coli* endotoxin (Group I) are summarized in Table 1. After an initial hypotension during the first hour (e.g. diastolic arterial blood pressure 114 ± 10 (control) to 50 ± 7 mmHg at 1 h; $P < 0.05$), the blood pressure returned towards pre-endotoxin levels at 3 h, declining thereafter. There were significant reductions in cardiac output and in stroke volume (Table 1 and Figure 2).

Table 1 The haemodynamic effects of *E. coli* endotoxin (2 mg/kg, i.v.) in anaesthetized cats (Group I).

	Control	*Time after endotoxin* (h) 1	2	3	4	5 h
Systolic blood pressure (mmHg)	141 ± 12	79 ± 10*	126 ± 6	138 ± 7	115 ± 16	73 ± 25*
Diastolic blood pressure (mmHg)	114 ± 10	50 ± 7*	93 ± 6	102 ± 5	83 ± 16	49 ± 8*
Right atrial pressure (mmHg)	1.36 ± 0.02	0.02 ± 0.6*	0.8 ± 0.08	0.02 ± 0.05*	0.04 ± 0.05**	0.05 ± 0.01*
Heart rate (beats/min)	206 ± 6	153 ± 30	214 ± 7	220 ± 7	202 ± 14	164 ± 10
Cardiac output (ml/min)	386 ± 53	288 ± 53**	268 ± 53*	213 ± 32*	178 ± 36*	170 ± 43*
Stroke volume (ml/beat)	2.06 ± 0.09	1.50 ± 0.14*	1.50 ± 0.11*	1.03 ± 0.11*	1.09 ± 0.22*	0.885 ± 0.14*
No. of survivals	8	7	7	7	5	4

Values quoted are means ± s.e.mean., with the number of observations shown as number of survivors.
*Significantly different from control (pre-endotoxin) levels; $P < 0.05$; **$P < 0.01$.

In the polymyxin and endotoxin treated group (II), there was a similar initial hypotension (diastolic arterial pressure decreasing from 110 ± 8 to 60 ± 6 mmHg at 1 h; $P < 0.05$, see Figure 1), the diastolic blood pressure returning towards pre-polymyxin levels (105 ± 5 mmHg) at 3 h and being maintained thereafter (Table 2). Although there were reductions in cardiac output (e.g. 398 ± 56 to 252 ± 45 ml/min at 5 h) and in stroke volume, these were similar to those observed in the cats given only

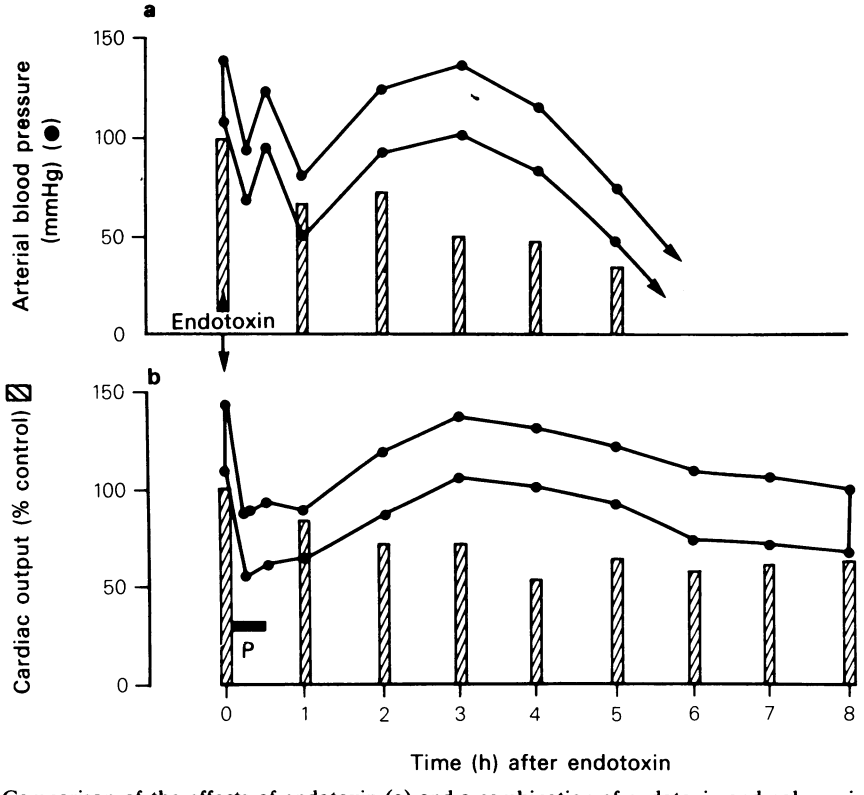

Figure 2 Comparison of the effects of endotoxin (a) and a combination of endotoxin and polymyxin B (b) on arterial blood pressure (●, mmHg, systolic and diastolic) and on cardiac output (hatched columns, as a % of values quoted in Tables 1 and 2) in anaesthetized cats. Only one of the endotoxin-alone cats survived 6 h; all of those pretreated with polymyxin B survived 8 h.

Table 2 The haemodynamic effects of *E. coli* (2 mg/kg) endotoxin in (Group II) cats pretreated with polymyxin B sulphate (total dose, 10 mg/kg).

	Control	*Time after endotoxin* (h) 1	2	3	4	5 h
Systolic blood pressure (mmHg)	141 ± 9	89 ± 7*	120 ± 5	138 ± 6	134 ± 7	154 ± 8
Diastolic blood pressure (mmHg)	110 ± 8	60 ± 6**	69 ± 6*	105 ± 5	102 ± 7	102 ± 7
Right atrial pressure (mmHg)	1.74 ± 0.32	0.96 ± 0.7	0.38 ± 0.36*	0.22 ± 0.36*	0.04 ± 0.36*	0.41 ± 0.55*
Heart rate (beats/min)	185 ± 13	187 ± 13	195 ± 11	207 ± 5	221 ± 4*	218 ± 3*
Cardiac output (ml/min)	398 ± 56	320 ± 71	305 ± 52	296 ± 87	209 ± 29*	252 ± 45
Stroke volume (ml/beat)	2.11 ± 0.4	2.27 ± 0.73	1.68 ± 0.41	1.62 ± 0.57	1.1 ± 0.15*	1.35 ± 0.26*
No. of survivals	8	8	8	8	8	8

Values are means ± s.e.mean., with 8 observations throughout.
Values significantly different from control: *$P < 0.05$; **$P < 0.01$.

polymyxin B (e.g. cardiac output, 330 ± 25 to 202 ± 46 ml/min at 5 h; Table 3); these decreases were certainly not as marked as those in the endotoxin-alone (Group I) cats where cardiac output was reduced from 386 ± 53 to 170 ± 43 ml/min (i.e. to 44% of control) at 5 h (Figure 2; Table 1).

The delayed haemodynamic effect in cats given only polymyxin B (total dose 10 mg/kg) are summarized in Table 3. There was no change in arterial blood pressure up to the end of the experimental period (5 h) but a fairly marked decrease in cardiac output and in stroke volume occurred, especially over the first 2 h after administration. The most likely explanation for this is a reduced circulating blood volume (reference the decrease in right atrial pressure) perhaps resulting from endogenous histamine release.

Metabolic responses The metabolic consequences of administering *E. coli* endotoxin (e.g. the metabolic acidosis) were also profoundly modified by polymyxin B pretreatment (Figure 3). In the endotoxin-alone (Group I) animals, arterial lactate increased markedly from 4.65 ± 0.6 to a maximum of 34.3 ± 4 mg/100 ml ($P < 0.01$) at 4 h; over the first 3 h this increased lactate did not result in marked changes in arterial pH (Figure 3) because of hyperventilation and a decrease in P_{CO_2}, but pH then fell at 4 and 5 h. Such a metabolic acidosis did not occur in those animals given polymyxin B as well as en-

Table 3 The haemodynamic effects of polymyxin B sulphate (5 mg/kg, bolus plus 5 mg/kg infused over a thirty minute period) in anaesthetized (Group III) cats.

	Control	*Time after polymyxin B administration* (h) 1	2	3	4	5 h
Systolic blood pressure (mmHg)	137 ± 9	131 ± 9	131 ± 7	130 ± 8	134 ± 9	133 ± 9
Diastolic blood pressure (mmHg)	107 ± 7	98 ± 8	99 ± 6	95 ± 6	100 ± 6	96 ± 6
Right atrial pressure (mmHg)	0.07 ± 0.4	0.37 ± 0.3	−0.11 ± 0.4	−0.01 ± 0.3	−0.01 ± 0.36	0.01 ± 0.50
Heart rate (beats/min)	202 ± 8	193 ± 8	203 ± 12	206 ± 12	213 ± 13	223 ± 14
Cardiac output (ml/min)	330 ± 25	252 ± 37	230 ± 17*	232 ± 33*	236 ± 49*	202 ± 46*
Stroke volume (ml/beat)	1.48 ± 0.09	1.43 ± 0.18	1.17 ± 0.11*	1.16 ± 0.17*	1.17 ± 0.21*	1.00 ± 0.2*
No. of survivors	8	8	8	8	8	8

Values quoted are means ± s.e.mean. ($n = 8$).
*Significantly different from control, $P < 0.05$.

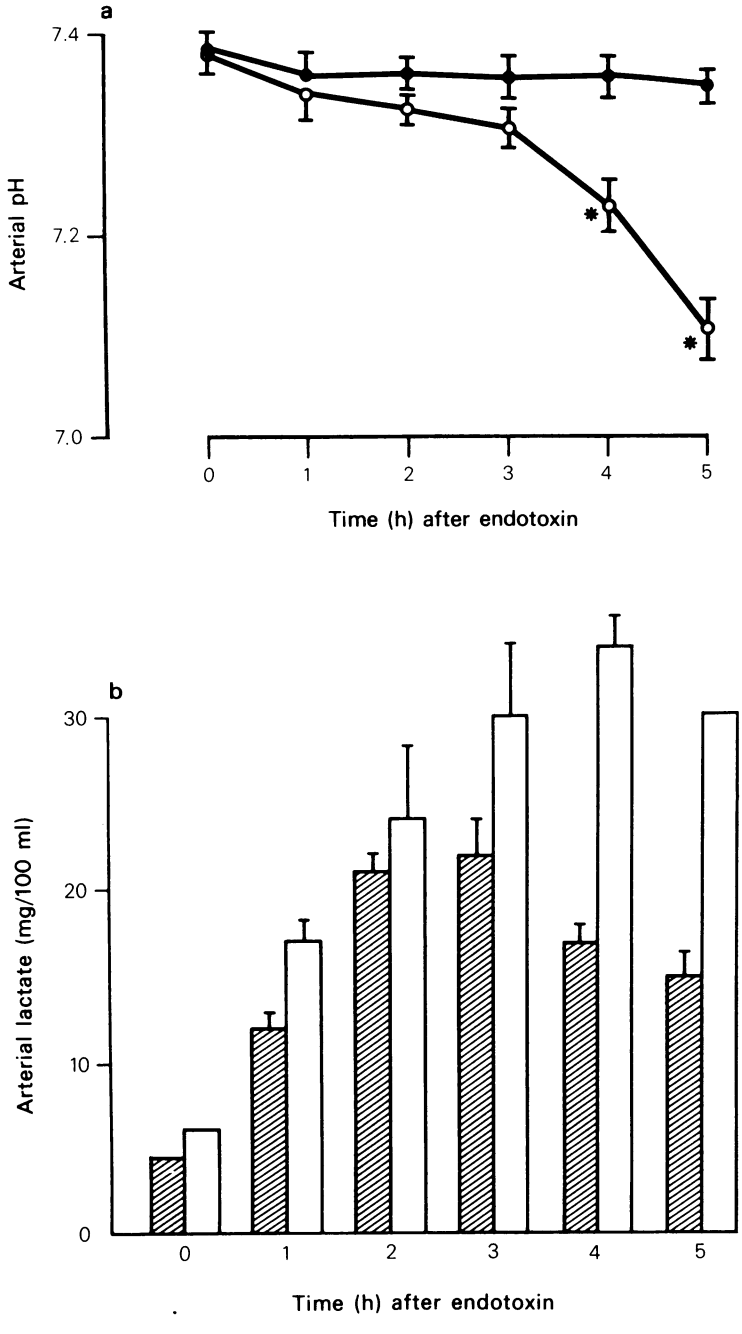

Figure 3 Comparison of the effects of endotoxin (○ and open columns) and a combination of endotoxin and polymyxin B (● and hatched columns) on arterial blood pH (a) and on arterial lactate levels (mg/100 ml) (b) in anaesthetized cats. Although metabolic acidosis was marked in both groups of animals up to 2 h, the lactate levels continued to rise until death ensued in the endotoxin-alone group whereas in those pretreated with polymyxin B there was considerable recovery towards normal by 5 h. $^{*}P<0.05$.

dotoxin (Group II), where there was no significant change in pH over the 5 h period and, although arterial lactate did increase initially (from 6.3 ± 0.6 to a maximum 21.9 ± 2.4 mg/100 ml at 3 h), the levels were returning towards control at 4 and 5 h (e.g. 13.3 mg/100 ml at 5 h; Figure 3). Polymyxin B itself exerted no metabolic effects. The improved metabolic and haemodynamic state of the endotoxin-treated cats given polymyxin B is reflected in the marked increase in survival. All these animals (8/8) survived the 8 h observation period. This is in marked contrast to the endotoxin-alone group in which only one animal out of the eight survived.

Discussion

These results demonstrate clear protection by polymyxin B against the lethal effects of purified lipopolysaccharide (*E. coli* endotoxin) in a severe endotoxin shock model. The initial pulmonary hypertension was completely prevented (Figure 1); systemic arterial pressure was well maintained into the severe and delayed shock phase (Table 2; compare Palmer & Rifkind's (1974) studies in a canine model of endotoxaemia) and the marked decreases in cardiac output and stroke volume that occur in this feline endotoxin shock model, were attenuated. More importantly, survival was dramatically increased, at least up to 8 h. However, despite this increased survival, not all of the effects of endotoxin were markedly reduced. For example, the immediate, transient systemic hypotension still occurred within the first 3 min (Figure 1); the metabolic acidosis between 1 and 3 h was just as marked in the antibiotic treated group as in the control (endotoxin-alone) group; and the reduction in right atrial filling pressure (probably a reflection of a decreased circulating blood volume as a result of increased capillary permeability) was also still present.

These results agree with those obtained in other experimental septic and endotoxin shock models. For example, in septicaemia induced in rabbits with *Pasteurella multocida* organisms, polymyxin improved survival but did not modify the leukopenia or the thrombocytopenia (Corrigan & Kiernat, 1979), whilst in a canine endotoxin shock model, pretreatment with polymyxin B largely prevented the delayed hypotensive phase and decreased lethality but did not modify either the early hypotensive phase (cf. Figure 1 of the present results) or the decreases in blood platelets and serum complement (From, Fong & Good, 1979). This presumably means that these particular endotoxin interactions with the components of the humoral defence system do not determine *ultimate* lethality or survival.

In the present experiments polymyxin itself caused marked initial reductions in systemic arterial blood pressure and more delayed decreases in cardiac output (Table 3). These effects may be the result of histamine release from mast cells (Bushby & Green, 1955; Parratt & West, 1957), ganglion blockade (Lee, Ricker & Katz, 1979) or a direct depressant effect on myocardial contractility (Sohn & Katz, 1979). It is possible that histamine release could further exacerbate shock induced by endotoxin or by sepsis; certainly histamine is released in the early phase of endotoxaemia (reviewed by Parratt, 1981). It could be that a less toxic polymyxin-like molecule could be produced which, whilst retaining an ability to prevent death resulting from endotoxaemia, does not cause either cardiovascular depression or histamine release.

The exact mechanism of the protective effect of polymyxin B against endotoxin is unknown. Our own initial studies (Madan and Parratt, unpublished) and those of others (Craig, Turner & Kunin, 1974; Corrigan & Kiernat, 1979) have shown that the timing of the polymyxin administration is crucial in modifying the biological effects of endotoxin. This could suggest that there is a chemical interaction between the two molecules. If they are mixed together *in vitro* before administration then the *in vivo* effects of endotoxin are still inhibited (Corrigan & Bell, 1971; Palmer & Rifkind, 1974 and our own earlier unpublished studies). This interaction appears to be between the cationic antibiotic and the lipid 2-keto-3-deoxy-octulosonate (KDO) region of the lipopolysaccharide (LPS) molecule (Morrison and Jacobs, 1976). This results, in the intact bacterial cell, in complete loss of structural integrity of the outer membrane (Brown & Tsang, 1978) as a consequence of a breakdown of the structure of the constituent LPS, an effect already demonstrated using electron-microscopic techniques by Lopes & Innis (1969) and by Wiegel & Mayer (1978). In intact organisms this interaction results in decreased endotoxin release (Goto & Nakamura, 1980; Ingoldby, 1980) and to reduced circulating blood endotoxin levels.

Results such as these have prompted studies in man. For example, endotoxin causes the release from human leucocytes of lysosomal enzymes, an effect also prevented by polymyxin B (Bannatyne, Harnett, Lee & Biggar, 1977). It is possible that polymyxin B (or a less toxic substitute) could inactivate endotoxin released from septic foci and promote neutrophil migration into such areas (Issekutz & Biggar, 1978). There is certainly some evidence that it improves renal function in cirrhotic patients (Liehr, Grün, Brunsing & Sautter, 1975), an effect possibly mediated by endotoxin neutralization.

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