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In vivo effects of *Escherichia coli* endotoxin on sulfobromophthalein clearance in the guinea-pig

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Summary. In vivo studies indicated that the primary effects of *E. coli* endotoxin on hepatic clearance of sulfobromophthalein were at the excretory level. Newborns were more sensitive to the LPS than older animals.

Cholestatic jaundice associated with gram-negative bacterial infections has been reported in humans, especially neonates[3-6], but the pathogenesis of the clinical entity is uncertain. Many of the effects of these bacteria have been attributed to their lipopolysaccharides (LPS)[7]. Recently, we reported that a purified LPS from *E. coli* reduced bile flow and sulfobromophthalein (BSP) excretion and increased BSP storage in the isolated perfused rat liver (IPRL)[8,9]. In addition, endotoxins from various species of bacteria have been described to inhibit the clearance of BSP from the blood of rats, rabbits, baboons and humans[10-13]. Since the incidence of cholestatic jaundice appears to be more prevalent in newborns than adult humans[3-6], it seemed of interest to compare the effects of an endotoxin on the excretory processes of animals of different ages. Guinea-pigs were chosen for the study as they are relatively large at birth and are sensitive to LPS[14]. Furthermore, the hepatic mechanisms for the clearance of BSP in these animals have been extensively studied[15,16] and are mature at 7 days[15].

Materials and methods. Hartley guinea-pigs (both sexes) at 1, 3 and 7 days were utilized in these studies. LPS from *E. coli* (055:B5) was purchased from Difco Laboratories. It was dissolved in sterile pyrogen-free saline just prior to use and administered (35 µg/100 g b.wt, i.p.; saline only in controls) between 9.30 and 10.30 h. 2 h later, BSP (1.2 mg/100 g[15]) was given by intracardiac injection. To

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Effect of *E. coli* endotoxin (LPS) on sulfobromophthalein (BSP) retention, storage in the liver and release of aspartate aminotransferase (GOT)*

Age in days	Treatment	Percent BSP retention at 30 min	BSP concentration in liver µg/g liver	BSP in liver as percent of applied dose	Serum GOT (IU/l)
1	None (6)	1.94 ± 0.29	67 ± 20	21 ± 6	48 ± 9
1	LPS (8)	6.39 ± 0.83***	136 ± 20**	52 ± 5***	82 ± 10**
3	None (6)	2.85 ± 0.58	13 ± 3	4 ± 1 (3)	60 ± 9
3	LPS (7)	6.58 ± 1.00***	95 ± 15*** (6)	31 ± 5*** (6)	82 ± 11 (4)
7	None (7)	1.61 ± 0.36	14 ± 5	4 ± 5	45 ± 3
7	LPS (8)	2.17 ± 0.39	35 ± 6**	11 ± 6**	66 ± 11 (4)

* All data are expressed as means ± SE. The number in parentheses are the number of experiments in each group. ** $p < 0.05$. *** $p < 0.01$.

insure complete administration, blood was withdrawn into the syringe before and after injection of BSP. Any animal that appeared to be traumatized by the procedure was not used. Pentobarbital sodium (50 mg/kg, i.p.) was given 25 min after the BSP. 5 min later, the abdominal cavity was opened and blood collected from the heart. Then, the liver was removed, blotted with a gauze pad and weighed. Serum levels of BSP were determined by the method of Seligson[17]. To calculate the 100% BSP retention, the plasma volume was assumed to be 5.7% of the body weight[18]. BSP storage was estimated by the method of Whelan et al.[19] and the level of serum aspartate aminotransferase (GOT) was assayed as described previously[20].

Results and discussion. At the dose used in this study, the endotoxin (LPS) killed 4 of 8 treated 1-day-old guinea-pigs within 48 h, but did not cause any mortality in the 3-day-group (5 animals). There were no differences in % BSP retention, at 30 min, among the control groups at 1, 3 and 7 days. The storage of BSP in the liver decreased from 1 to 7 days, indicating maturation of hepatic conjugative processes[15]. At 1 day, BSP in the liver accounted for about 21% of the applied dose, but at 3 and 7 days, only about 4% (table). These data are in agreement with those reported by other authors[15,16].

Administration of the LPS increased the percent BSP retention in the 1- and 3-day-groups, but not the 7-day-group. At all ages studied, the LPS caused an increased storage of BSP in the liver, whether expressed in μg/g liver or as percent of the applied dose (table). The data suggested that the LPS interfered with hepatic function as the excretory level as there were increased levels of BSP in the serum and liver in the 1- and 3-day-old guinea-pigs. Although it is possible the LPS reduced dye excretion by inhibiting BSP conjugation, the observation that LPS inhibited the excretion of indocyanine green[8], indicates this is unlikely. The results of the present study are in agreement with those reported for the IPRL[8,9], that the LPS exerts adverse effects on hepatic excretory mechanisms. There was a moderate rise in serum GOT in the 1-day-group, but not in the 3- and 7-day-groups (table). The above results indicated that newborn guinea-pigs are more sensitive to LPS than are older animals.

These results may possibly have relevance to the clinical syndrome of intrahepatic cholestasis associated with non-hepatic gram-negative bacterial infections. Since this entity is more prevalent in neonates[3–5] than adults[6], the newborn human, as the guinea-pig, may be more sensitive to LPS than adults. The data of this study are consistent with the hypothesis[8] that the impairment of hepatic excretory processes is a factor in the development of cholestasis often seen during gram-negative bacterial infections.

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Interaction between aldosterone and renomedullary prostaglandins. Competitive action between aspirin and spironolactone

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Summary. Aldosterone injected i.m. decreased the release of renomedullary PGEs and the index (urinary Na/K ratio) in conscious normotensive intact and adrenalectomized rats. Coadministration of spironolactone increased the release of PGEs as well as the index (urinary Na/K ratio). The effect of spironolactone was partly inhibited by aspirin injected in a ratio 5:1 (aspirin:spironolactone), an effect which could be reversed by the infusion of a synthetic prostaglandin (PGA_2) in a subhypotensive dose.

In a previous study in the man[2], we found a positive, statistically significant correlation between urinary PGEs and the index (urinary Na/K ratio) ($Y = 0.015\,X + 0.53$; $r = 0.672$, $p < 0.001$) and it had been suggested that the phenomenon was an antagonistic result between these substances (PGEs) and the aldosterone system. These results enable us to investigate whether the administration of aldosterone in experimental animals could decrease the renomedullary PGEs synthesis and/or release and the coadministration of an antagonist of aldosterone, the spironolactone[3], could increase the release of the natriuretic PGEs. The simultaneous administration of aspirin, a well-known inhibitor of PG synthesis and/or release[4], could further clarify whether the natriuretic effect of spironolactone was only the antagonistic result between these substances and aldosterone or whether it could be mediated (at least in part) by the potent natriuretic renomedullary PGEs[5].

Our results are suggestive of an inhibitory effect of aldosterone on renomedullary PGEs synthesis and/or release and of a stimulating effect of spironolactone on PGEs synthesis.

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