Influence of antibiotics and food intake on liver glutathione and cytochrome P-450 in septic rats

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Experimental sepsis in rats induces a restriction in spontaneous food intake and a drop in liver glutathione, cytochrome P-450 (P-450) and aminopyrine demethylase (AD) activity. The present study was designed to assess the effects of antibiotics alone or when combined with food deprivation on these variables. Eighty-nine male Sprague–Dawley rats were assigned to six groups: control (C), acute infection (experimental pyelonephritis, I), acute infection with antibiotics and food given *ad lib.* (IA), control with antibiotics (CA), acute infection with antibiotics pair-fed to I (IAR), and sham-operated pair-fed to I (SR). Liver glutathione, P-450 and AD activities were reduced by 45·2, 79·8 and 41·2% respectively in group I. Glutathione and AD significantly increased only in those infected rats given antibiotics and allowed free access to food. P-450 did not normalize within the study period in infected rats receiving antibiotics and food repletion. The risk of drug hepatotoxicity in acute septic states is therefore closely related to the nutritional status. From this point of view, nutritional support is almost as important as treatment of infection.

Experimental sepsis: Glutathione: Cytochrome P-450: Rat

Metabolism and detoxification of lipid-soluble xenobiotics and endogenous steroids take place, for the most part, in the microsomal fraction of the liver cell. They involve mainly cytochrome P-450 (P-450)-dependent mono-oxygenases (Omura *et al.* 1965; Remmer, 1972). In some cases xenobiotic metabolism generates toxic reactive metabolites, which can bind covalently to vital liver macromolecules and lead to liver cell necrosis (Pessayre *et al.* 1979; Sherlock, 1979; Boyd *et al.* 1983). Liver cells are protected from these toxic metabolites by antioxidant factors, amongst which the tripeptide glutathione is the most important (Meister & Tate, 1976; Kaplowitz, 1981; Moldeus & Quanguan, 1987; Fouin-Fortunet *et al.* 1990). The hepatotoxicity of reactive metabolites thus depends on a balance between liver activity of P-450 and liver content of glutathione. Furthermore, glutathione deficiency impairs cell immune function and wound healing in critical illness and increases the incidence of multisystem organ failure (Robinson *et al.* 1992).

Both P-450 and glutathione are very sensitive to food intake. Glutathione is very susceptible to protein malnutrition (Edwards & Westerfeld, 1952; Pessayre *et al.* 1979; Warnet *et al.* 1987; Beutler, 1989; Fouin-Fortunet *et al.* 1989*a, b*; Hum *et al.* 1992). A short

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fasting period decreases liver glutathione to one third of the control value in the rat (Maruyama *et al.* 1968; Tateishi *et al.* 1974; Cho *et al.* 1981). Liver P-450-dependent mono-oxygenase (*EC* 1.14.14.1) activities, for example ethoxycoumarine *O*-de-ethylase (Lorenz *et al.* 1984), aniline *p*-hydroxylase (Campbell, 1977), ethylmorphine *N*-demethylase (Campbell, 1977; Wade *et al.* 1985) or aminopyrine *N*-demethylase (AD; Fouin-Fortunet *et al.* 1989*b*) are altered in protein malnutrition. As a consequence, pharmacokinetics of drugs metabolized by liver P-450-dependent mono-oxygenases are also altered (Kato *et al.* 1968; Mehta *et al.* 1975; Feldman *et al.* 1980; Yang *et al.* 1992).

Experimental nutritional studies usually reproduce protein malnutrition or protein–energy malnutrition. Metabolic and nutritional status during sepsis-related malnutrition in human pathology can obviously not be inferred from those observed in cases of deliberate and isolated starvation (Petit *et al.* 1991). Previous work in our laboratory showed that various models of experimental acute infection in rats induce both decreases in spontaneous food intake and increases in muscle protein breakdown, thus leading to severe malnutrition. In these conditions, P-450 drops, but the levels of glutathione are less decreased than might have been expected from food restriction alone (Lescut *et al.* 1991). We therefore suggested that glutathione liver synthesis might be stimulated as a part of the acute-phase response, or that the release of S amino acids, which are the limiting factors for glutathione synthesis (Prescott *et al.* 1976), from the muscle in septic animals accounted for increased plasma levels (Freund *et al.* 1978) and for the preservation of liver glutathione. The significant decrease in liver glutathione observed in septic rats treated by α-ketoisocaproate, a compound that reduces muscle proteolysis, is an indirect argument for this hypothesis (Petit *et al.* 1991).

The present study was therefore designed to assess the respective effects of nutritional repletion and antibiotics on hepatic P-450 and glutathione levels during the treatment of acute pyelonephritis in rats.

MATERIALS AND METHODS

Animals

Handling and care of laboratory animals conformed to the standards established by the Animal Studies Committee of the University of Rouen. Male Sprague–Dawley rats, weighing 200–225 g, were obtained from Charles River (Saint-Aubin-les-Elbeuf, France). They were allowed 4 d to acclimatize to our laboratory conditions (constant humidity and temperature: 21°, with a 12 h light–dark cycle) in community cages, with free access to water and food, before the experiments. Diet composition (Autoclave A113 pellets; UAR, Villemoisson sur Orge, France) was as follows (g/kg): casein 230, glucose 580, fat 50, cellulose 60, minerals 70, vitamins 10.

On day 1, rats were assigned to six groups: C (controls, rats fed *ad lib.* and not operated upon); I (infected rats, sepsis achieved by acute retrograde pyelonephritis); IA (infected rats treated with antibiotics and fed *ad lib.*); CA (same as C but treated with antibiotics), IAR (infected rats given antibiotics, pair-fed to I); SR (rats which were sham-operated and food-restricted, pair-fed to I).

Operative procedure

Rats fasted for 18 h were anaesthetized by an intramuscular injection of atropine (0·04 mg/kg body-weight (BW)), ketamine (28·5 mg/kg BW) and acepromazine (0·3 mg/kg BW). Administration of these drugs results in an increase in the activity of drug-metabolizing enzymes, but only when they are given for long periods of time (Cooper *et al.*

1976; White *et al.* 1982). We had verified that one injection given 3 d before slaughter did not modify liver factors to be studied (V. Colomb and J. Petit, unpublished results).

Acute retrograde pyelonephritis was achieved using the method described by Brooks *et al.* (1974) and later modified by Glauser *et al.* (1978). Briefly, a median laparotomy was performed and 1 ml of a 5×10^3 colony-forming units (cfu)/ml culture of *Escherichia coli* was injected into the bladder. The injection was followed by partial ligation of the left ureter for 18 h. Sham-operated rats were treated identically, except that the bladder injection consisted of 1 ml sterile normal saline (9 g NaCl/l), and that the suture was passed into the retroperitoneal space but away from the ureter.

Experimental procedure

Following surgery, rats were maintained without food and water for 3 h. Thus the total period of fasting before re-feeding was approximately 21 h. All the rats were then housed individually in metabolism cages and allowed free access to food and water.

CA, IA and IAR rats were given separate intramuscular injections of amoxicillin (300 mg/kg BW twice daily) and tobramycin (2·5 mg/kg BW twice daily), a combination of antimicrobial agents which was active *in vitro* against the strain of *E. coli* used in this study. Antibiotic treatment was initiated 5 h postoperatively.

Rats were killed by cervical dislocation on the 4th day.

Tissue preparation

Blood was withdrawn from the mid-aorta, centrifuged immediately, and the serum was frozen at $-20°$ until analysis. The right and left kidneys were rapidly excised and stored in sterile flasks at $4°$ until culture. The liver was rinsed with ice-cold normal saline through the portal vein, rapidly excised, blotted, and weighed. A sample of liver (1 g) was minced and homogenized in a Braun® potter homogenizer with normal saline for 60 s at $4°$. This homogenate was used to determine the liver content of soluble proteins. Another 1 g liver sample was similarly homogenized in three volumes of trichloracetic acid (50 g/l). The homogenate was centrifuged at $10\,000\,g$ for 20 min at $4°$. The supernatant was used for immediate glutathione assay. A further portion of liver (2 g) was homogenized in three volumes 0·1 M-Tris-HCl buffer containing 0·154 M-KCl, pH 7·4, then centrifuged at $10\,000\,g$ for 20 min. The supernatant was centrifuged at $100\,000\,g$ for 60 min. The microsomal fraction was frozen immediately at $-30°$ until assays were carried out.

Nutritional assessment

Body weight and food consumption were measured daily.

Urinary N was determined by the Antek chemiluminescence technique (Antek Instruments, Houston, TX, USA; Ward *et al.* 1980) on 24 h urine samples collected on day 3. Except for some diarrhoea, few operated animals had any stools during the postoperative period. The N balance was therefore calculated as being the difference between input and urinary excretion without taking N losses in stools into account.

Serum protein concentration was measured by the biuret method (Gornall *et al.* 1949). Serum transferrin was determined using specific anti-rat transferrin antibodies; control values were converted to 100 % and all other values expressed as relative concentrations to take into account both experimental precision of measurements and inter-individual variability.

Carcass fat was estimated from carcass water (the difference in carcass weight before and after carcass drying for 6 weeks at $60°$), using the Cox formula:

$$\text{carcass fat} = 95·96 - (1·272 \times \text{carcass water}),$$

Table 1. *Effects of intramuscular administration of amoxicillin (300 mg/kg body-weight (BW) twice daily) and tobramycin (2·5 mg/kg BW twice daily) for 3 d on nutritional variables and liver glutathione and cytochrome P-450 in rats†*

(Mean values and standard deviations)

	Group C, controls (n 17)		Group CA, treatment (n 9)	
	Mean	SD	Mean	SD
Food intake (g/d)	22·4	2·2	18·7*	2·7
Weight change (g/3 d)	43·5	10·0	38·0	4·7
Liver:body weight ratio	0·48	0·05	0·45	0·04
Liver glutathione (μmol/g)	6·2	1·5	5·7	1·4
Liver cytochrome P-450 (nmol/g)	20·1	5·9	18·4	3·5

* Mean value was significantly different from that of the control group: $P < 0.05$.
† For details of procedures, see pp. 100–102.

where carcass fat and water are expressed as a percentage of the initial body weight (Cox *et al.* 1985).

Analytical procedure for liver variables

Soluble liver proteins and the protein content of the liver microsomal suspension were determined using the method of Lowry *et al.* (1951). Liver glutathione was measured as described by Ellman (1959), the cytochrome P-450 content as described by Omura & Sato (1964), and aminopyrine demethylase activity using the method of Mazel (1971).

Infection criteria

Successive dilutions of the homogenate of the left kidney were cultured. The number of cfu after incubation for 24 h at 37° was expressed using a logarithmic transformation.

The bacteriological inclusion criteria were a positive culture (log cfu > 2) of the left kidney for group I, and a negative culture for groups IA, IAR, and SR. Of the rats which were injected with *E. coli* and did not receive antibiotics, 20 % were excluded for sepsis failure; 14 % of the infected rats given antibiotics had a slightly positive kidney culture and were excluded.

Finally, eighty-nine rats were included: group C, *n* 17; group I, *n* 14; group IA, *n* 17; group CA, *n* 9; group IAR, *n* 17; group SR, *n* 15.

Statistical analysis

Results are presented as the mean and standard deviation. Differences between groups were tested using the Mann–Whitney test, with $P < 0.05$ as the critical limit.

RESULTS
Effects of antibiotics alone

As shown in Table 1, control rats given antibiotics alone had a 16·5% lower food intake than control rats that did not receive antibiotics or any other intramuscular injection. They also had slightly lower, but not significantly different, weight gain, liver:body weight ratio, liver glutathione and P-450.

Table 2. Effects of infection, antibiotics, and food restriction on basic nutritional measurement in rats‖

(Mean values and standard deviations)

	Group C (n 17)		Group SR (n 15)		Group I (n 14)		Group IA (n 17)		Group IAR (n 17)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Food intake (g/d)	22.4	2.2	5.2*	0.7	4.0*	1.9	11.0*†‡	3.3	5.5*†§	0.6
Weight change (g/3 d)	43.5	10.0	−10.5*	4.1	−19.1*	9.7	14.9*†‡	10.4	−11.7*§	5.3
Nitrogen balance (mg/d)	543	125	82*	69	11*†	11	393*†	238	57*†§	80
Serum proteins (g/l)	54.6	4.1	49.1*	3.6	53.3†	4.2	52.9†	4.1	52.5†	3.3
Transferrin (% control)	100	2.0	81.4*	4.1	92.0*†	13.6	86.8*†	5.3	83.9*‡	6.9
Body fat (g/kg)	100	18	87*	11	66*†	32	72*	34	84*‡	12

* Mean values were significantly different from those of group C, $P < 0.05$.
† Mean values were significantly different from those of group SR, $P < 0.05$.
‡ Mean values were significantly different from those of group I, $P < 0.05$.
§ Mean values were significantly different from those of group IA, $P < 0.05$.
‖ Group C, controls; group I rats infected with *Escherichia coli*; group IA, infected rats given antibiotics and fed *ad lib.*; group IAR, infected rats given antibiotics and pair-fed to group I; group SR, sham-operated rats pair-fed to group I. For details of procedures, see pp. 100–102.
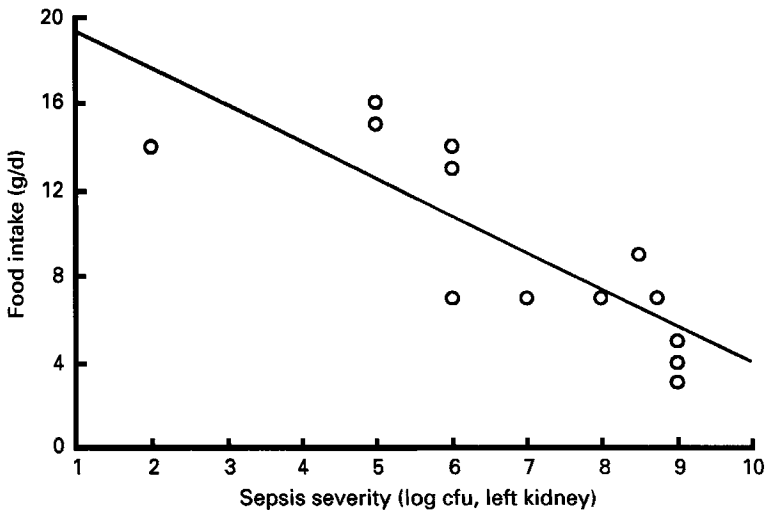
Fig. 1. Relationship between sepsis severity (expressed using a logarithmic transformation of the number of colony forming units (cfu) from the left kidney) and food intake in infected rats which were not given antibiotics (Group I). For details of procedures, see pp. 100–102. The regression line is described by the equation $y = -1.8 x + 21$ ($r = 0.83$).

Basic nutritional measurements (Table 2)

Spontaneous food intake was strongly reduced by infection. A negative correlation was found between spontaneous food intake and sepsis severity in group I (Fig. 1). In group IA, food intakes were higher than in group I, but still remained lower than in C. Rats from groups C and IA gained weight, while those from other groups lost weight. N balance dropped in group I and increased when infected rats were given antibiotics and allowed free access to food (group IA). Serum proteins were higher in all infected groups than in group SR, and serum transferrin was higher in group I than in groups IAR and SR.

Liver variables (Table 3)

Liver:body weight ratio was lower in groups SR and IAR than in C, I, and IA. Soluble liver proteins were higher in group I than in group SR.

Total liver glutathione was decreased by 66.4% in group SR and by 45.2% only in group I. Antibiotics induced a rise in liver glutathione when rats were allowed free access to food (IA) and a drop when they remained malnourished (IAR).

Similar results were found for liver microsomal proteins. Cytochrome P-450 decreased to a greater extent in infected rats than in sham-operated pair-fed rats. A highly significant negative correlation was found between P-450 and sepsis severity in group I (Fig. 2). P-450 was not increased in antibiotic-treated rats, whatever their food intake. AD activity was reduced by 41.2% in infected and by 57.0% in sham-operated pair-fed rats, but no statistical difference was found between group I and group IA nor between group IAR and group SR.

DISCUSSION

Protein and protein–energy malnutrition states induce a drop in liver variables involved in drug metabolism such as glutathione and cytochrome P-450 (Edwards & Westerfeld, 1952; Kato et al. 1968; Mehta et al. 1975; Feldman et al. 1980; Warnet et al. 1987; Beutler, 1989; Fouin-Fortunet et al. 1989b). Infection states induce a malnutrition pattern which is not

Table 3. *Effects of infection, antibiotics, and food restriction on liver factors involved in xenobiotic metabolism in rats*‖

(Mean values and standard deviations)

	Group C (n 17)		Group SR (n 15)		Group I (n 14)		Group IA (n 17)		Group IAR (n 17)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver weight (g)	13·2	1·1	7·7*	0·9	9·3*†	1·3	9·8*†	1·0	7·5*†§	0·8
Liver:body weight ratio	0·48	0·05	0·36*	0·03	0·45†	0·08	0·42*†	0·04	0·35*†§	0·03
Soluble liver proteins (mg/g)	81·6	27·8	73·6	18·1	91·7†	20·2	73·2	28·5	81·8	28·9
Glutathione (μmol/liver)	80·7	17·3	27·1*	9·8	44·2*†	18·3	58·6*†‡	13·2	29·2*†§	9·4
Microsomal proteins (mg/liver)	518	144	233*	55	315*†	57	374*†	123	253*†§	83
Cytochrome P-450 (nmol/liver)	265·6	83·2	167·5*	44·3	122·9*†	48·6	142·2*	43·8	153·7*	55·6
Ad (nmol/min per liver)	1057·2	208·6	454·6*	141·1	621·4*†	120·0	694·2*†	243·8	445·7*†§	133·5

AD, aminopyrine *N*-demethylase.

* Mean values were significantly different from those of group C, $P < 0.05$.
† Mean values were significantly different from those of group SR, $P < 0.05$.
‡ Mean values were significantly different from those of group I, $P < 0.05$.
§ Mean values were significantly different from those of group IA, $P < 0.05$.
‖ Group C, controls; group I rats infected with *Escherichia coli*; group IA, infected rats given antibiotics and fed *ad lib.*; group IAR, infected rats given antibiotics and pair-fed to group I; group SR, sham-operated rats pair-fed to group I. For details of procedures, see pp. 100–102.
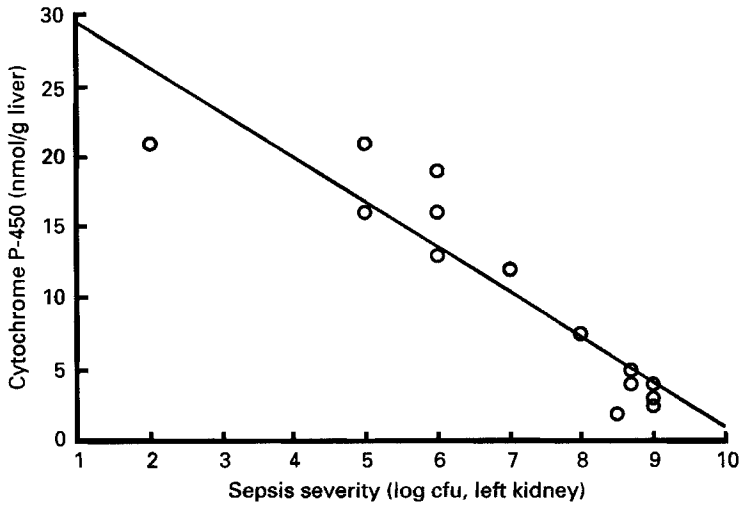
Fig. 2. Relationship between sepsis severity (expressed using a logarithmic transformation of the number of colony forming units (cfu) from the left kidney) and liver cytochrome P-450 concentration in infected rats which were not given antibiotics (Group I). For details of procedures, see pp. 100–102. The regression line is described by the equation $y = -3 \cdot 2\ x + 32 \cdot 6$ $(r = 0 \cdot 92)$.

limited to severe reduction in food intake. Other nutritional and metabolic characteristics of sepsis include accelerated muscle proteolysis and selective stimulation of liver acute-phase protein synthesis (Schreiber *et al.* 1982; Baracos *et al.* 1983; Clowes *et al.* 1983; McCarthy *et al.* 1985; Roh *et al.* 1986; Long, 1988).

The aim of the present study was to assess the respective roles of antibiotic treatment and restricted feeding in normalizing the liver variables involved in xenobiotic metabolism and detoxification, in experimental acute retrograde pyelonephritis in rats.

The antibiotics amoxicillin and tobramycin were chosen because the *E. coli* strain used was susceptible. Both are primarily excreted by the kidney. They are not known to potentiate or to inhibit liver microsomal P-450 (Vasko & Brater, 1989). We observed that antibiotics given by the intramuscular route were associated with a significant 16·5% decrease in food intake. Weight gain, liver glutathione and cytochrome P-450 were also slightly, but not significantly, lower in rats given antibiotics (by 12·6, 8·0 and 8·4% respectively) than in control animals who did not have intramuscular injections. These decreases were of a far lower magnitude than those observed in sepsis, or than the changes seen in food-deprived and sham-operated rats. They might have been related to a loss of appetite or to the stress of iterative intramuscular injections. However, whether the difference between groups CA and SR was due to the dietary restriction or the effects of the sham operation cannot be ascertained from the current experiment.

Changes of body weight in infected animals given antibiotics depended on food intake. Treated rats which were allowed free access to food increased their spontaneous food intake and gained weight. Conversely, treated food-restricted rats lost weight, but to a less important extent than infected rats which were not administered antibiotics. Such a finding is not surprising, as cytokines, which have proved to mediate the nutritional consequences of sepsis (Baracos *et al.* 1983; Clowes *et al.* 1983; McCarthy *et al.* 1985), are secreted by monocytes and macrophages proportionally to inflammation intensity (Schreiber *et al.* 1982; Long, 1988).

Liver weight and liver:body weight ratio were 17·2 and 20% higher respectively in

infected than in pair-fed non-infected rats. We previously observed, using the same model, that the water content of the liver was 4·3 and 2·6% higher in septic rats than in controls and pair-fed non-septic animals respectively (Petit *et al.* 1991). Similar results were reported in experimental sepsis in rats (Roth *et al.* 1985) and in septic critically ill patients (Clemens *et al.* 1984). The increase in the water content does not therefore explain the increase in the liver weight. However, due to these changes in liver weight and liver water content, liver variables should not be expressed as concentrations per gram of fresh liver, but either as concentrations per gram of dry liver or as total liver content, the format we chose because it is the most significant from an overall metabolic point of view (Mitchell *et al.* 1973). We did not measure the fat content of the liver in the present study, because previous work using various experimental acute infection models in rats did not elicit any steatosis nor any increase in liver triacylglycerol content (Lescut *et al.* 1991). The main factor of liver weight increase is therefore the 19·7% increase of the liver protein content, a common finding in sepsis that has been related to increased acute-phase protein synthesis (Schreiber *et al.* 1982; Roh *et al.* 1986), as shown in the present study by higher serum transferrin levels in infected than in sham-operated pair-fed animals. However, the decrease in soluble liver proteins in group IAR did not reach the significance level, which could be explained by the short duration of the study when compared with half-lives of acute-phase proteins (Schreiber *et al.* 1982).

The rapid glutathione decrease in sham-operated pair-fed and in infected rats confirms the high sensitivity of this factor to the nutritional status. However, liver glutathione did not decrease to the same extent in sepsis. As proved by experimental studies in rats (Tateishi *et al.* 1974; Kaplowitz, 1981), and by the efficacy of the administration of sulphydryl compounds in replenishing hepatic stores of glutathione in acetaminophen overdosage (Prescott *et al.* 1976), the intracellular availability of cysteine is a limiting step in glutathione synthesis. While in food deprivation alone, low cysteine levels probably act, in part, by reducing liver glutathione synthesis, increased plasma levels of S amino acids such as methionine and cysteine (up to 200%) have been reported in severe infection (Freund *et al.* 1978). The increased muscle proteolysis accompanying sepsis (Lescut *et al.* 1991; Petit *et al.* 1991) could be responsible for such an increase, thus explaining why antibiotics, which reduced muscle catabolism as proved by reduced urinary N losses, also reduced liver glutathione when nutritional repletion was not simultaneously achieved. In the present study liver glutathione varied grossly as microsomal liver proteins did, therefore suggesting that glutathione is a positive part of the acute-phase response. Further studies are required to confirm this hypothesis and to assess the putative role of cytokines on glutathione synthesis. Mitchell *et al.* (1973) showed that acetaminophen-induced hepatic necrosis does not occur unless the liver glutathione content has been reduced to 30% of normal. In the present study the liver glutathione content was 72·6% of the control value when antibiotics were given to *ad lib.*-fed rats, and only 36·2% when food intake was restricted to that of group I. The conclusion to be drawn from these results is that the nutritional status is of major significance when considering glutathione availability and the treatment of severe sepsis.

Liver microsomal proteins were significantly lower in infected and sham-operated food-restricted rats than in controls. As was the case with glutathione, they were also higher in infected than in sham-operated rats. P-450 dropped in all septic groups. This decrease was found to be correlated to sepsis severity. Such results are in line with those of Renton & Mannering (1976), who showed in mice injected with *E. coli* lipopolysaccharide that microsomal enzyme activity was depressed in a dose–response fashion. Increasing evidence now exists to suggest that the sepsis effect on P-450 is mediated by cytokines (Renton & Mannering, 1976; Ghezzi *et al.* 1986*a, b*). On the other hand, endotoxins are known to

inhibit haem synthesis and to stimulate haem oxidase (*EC* 1.14.99.3; Bock *et al.* 1973; Bissel & Hammaker, 1976). Antibiotics did not reverse sepsis-induced P-450 decrease, a fact which might be explained partly by the short duration of the study period, and partly by the opposing effects of fasting on the various P-450 isoenzymes (Hong *et al.* 1987; Yang *et al.* 1992). The activity of liver AD, a mono-oxygenase requiring both P-450 and glutathione availability, was reduced in all infected and in food-restricted groups. Antibiotics significantly increased liver AD activity from 42·1 to 67·7% of control values when treated rats were allowed unrestricted intake.

The present study using experimental acute pyelonephritis in rats as a sepsis model proves that a short-term treatment with antibiotics alone does not reverse the drop in the liver content of P-450 and induce a decrease in the liver content of glutathione if food repletion is not provided. Antibiotics given without simultaneous nutritional support may therefore enhance the liver toxicity of xenobiotics. If the same holds true in infected patients given antibiotics, conclusions to be drawn from these findings include systematic need for food repletion, even over short periods of time, and careful use and monitoring of drugs cleared by the liver.

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