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Reduced Response to Intravenous Endotoxin Injections Following Repeated Oral Administration of Endotoxin in the Pig

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Holst, H., L.-E. Edqvist and H. Kindahl: Reduced respoonse to intravenaus endotoxin injections following repeated oral administration of endotoxin in the pip. Acta vet. scand. 1993, 34, 405-419. – Three prepubertal gilts were each given 100 mg of endotoxin (ET) in their ordinary feed rations, twice daily for 6 days; 3 other gilts received standard feed. Following ET feeding, all animals were injected intravenously (i.v.) with ET (1.0 µg/kg b.w.) once daily for 5 days. Blood samples were collected and analysed for hematology and total serum bile acids (S-BA), glutamate dehydrogenase (S-GLDH), calcium (S-Ca), iron (S-Fe), zinc (S-Zn) and a blood plasma metabolite (15-ketodihydro-PGF2a; P-PG) of prostaglandin F2a. The animals showed no apparent clinical symptoms following ET-feeding, neither did the blood analyses reveal effects of oral ET. However, when iv ET injections were given, the ET-fed animals showed fewer clinical signs of endotoxemia following the 2nd to 5th injection. S-BA and S-GLDH increased markedly in the standard-fed group following the first injection, while the ET-fed animals showed a much smaller increase in S-BA and no change in S-GLDH on that day. The difference in response may be explained by a direct uptake of ET from the gastrointestinal tract in the ET-fed pigs, making them less sensitive to the injected ET

prepubertal gilts; lipopolysaccharide; endotoxin tolerance; bile acids; glutamate dehydrogenase; diurnal variation; calcium; iron; zinc.

Introduction

Lipopolysaccharide (LPS), or endotoxin, is a constituent of the cell wall of Gram-negative bacteria. The term endotoxin (ET) is often used instead of LPS when discussing its biological effects. Endotoxin plays an important role in the pathogenesis of many infections with Gram-negative bacteria.

The biological effect of ET as a pyrogen is well known.

Repeated iv injections with ET are known to induce pyrogenic tolerance of a dual nature. The early phase tolerance is antibody-independent, develops within hours, is transient and requires continuing endotoxin infusion for maximum maintenance. The late phase tolerance is antibody dependent, its onset requires several days to appear and it persists for weeks *(Greisman 1983,* review). Tolerance to both the lethal and the metabolic effects of ET can also be induced *(Sanchez-Cantu et al.* 1989, *Lang & Spitzer* 1987).

The occurrence of ET is abundant in the natural environment, since Gram-negative ba-

cteria grow on vegetation and other organic matter. Large amounts of ET (about 60 mg/kg of feed) can be present in feed that has been harvested and stored under unsuitable conditions *(Rylander* 1989). Much interest has been focused on the question of whether or not ET can penetrate the intact gastrointestinal mucosa, and there are results to support both contentions *(Ravin et al.* 1960, *Truszcynski & Pilaszek* 1969, *Wray & Thomlinson* 1972, *Gans & Matsumoto* 1974, *Huber et al.* 1979, *Cort et al.* 1990). Recent investigations claim that, under normal circumstances, small amounts of ET are constantly being absorbed from the gut into the portal vein, passed to the liver where it is removed, mainly by the Kupffer cells, and detoxified (reviews *Nolan* 1988, *Fox et al.* 1990). Intravenously injected LPS is also detoxified to a large extent in the liver *(Freudenberg & Galanos* 1988). On the other hand, *Berczi et al.* (1968) found no evidence of ET absorption from the rat intestine and oral administration of ET did not induce tolerance to the lethal effect of iv injected LPS. However, these authors studied mortality rate, which is a rather crude estimate of ET influence. In various gastrointestinal disorders, uptake of ET from the small intestine is of importance. *Wessels et al.* (1987) showed that dogs with hemorrhagic enteritis had increased ET concentrations in peripheral blood.

In an earlier study *(Holst et al.* 1993), we found that oral administration of ET to prepubertal gilts did not cause any clinical signs of endotoxemia except for a slight increase in rectal temperature. Furthermore, an increased concentration of S-BA was detected, which might indicate that ET was absorbed but then detoxified in the liver. The aim of the present study was to investigate the effect of repeated oral administration of ET on the subsequent response to iv injected LPS in the pig.

Material and methods

Animals

Six clinically healthy prepubertal Yorkshire X Landrace X Hampshire crossbred gilts were used. The animals came from three different litters (2+2+2; nos.1+4,2+5,3+6) and their body weights (bw) ranged between 24 and 30 kg on arrival one week before surgery. The animals were fed twice daily (9 am, 3 pm) with 0.5 kg of commercially prepared pelleted feed and were kept in individual pens on a strawbed. The feed was mixed with water (200 ml/0.5 kg) to a consistency of stodgy porridge. Water was provided *ad libitum.*

One week before the experiments, a jugular venous catheter was surgically inserted *(Rodriguez & Kunavongkrit* 1983) under general anesthesia (halothane). Trimethoprim-sulfadoxin was given, 15 mg/kg bw, iv for 3 days after surgery. The catheters were flushed twice daily with heparinized physiological saline (25 IE/ml NaCl, Kabi, Stockholm, Sweden) with an additive of 1 mg benzyl-penicillin-potassium/ml of NaCl (Novo Nordisk, Bagsvaerd, Denmark) until the experiments were completed.

Experimental design

Both the control and the experimental group consisted of 3 animals, one from each of the 3 different litters that were used. For 6 days (days 1-6), at 9 am and 3 pm, the experimental animals were each given 0.5 kg of standard feed containing 100 mg LPS from *Escherichia coli* (055:B5, phenol-extracted, batch 17F-40191, Sigma, St. Louis, USA). Meanwhile, the control animals received 0.5 kg of standard feed. This was checked for background content of unspecified ET with the Limulus amoebocyte lysate test (LAL) originally developed for plasma measurement *(Goto & Rylander* 1987) and was found to contain 12.0

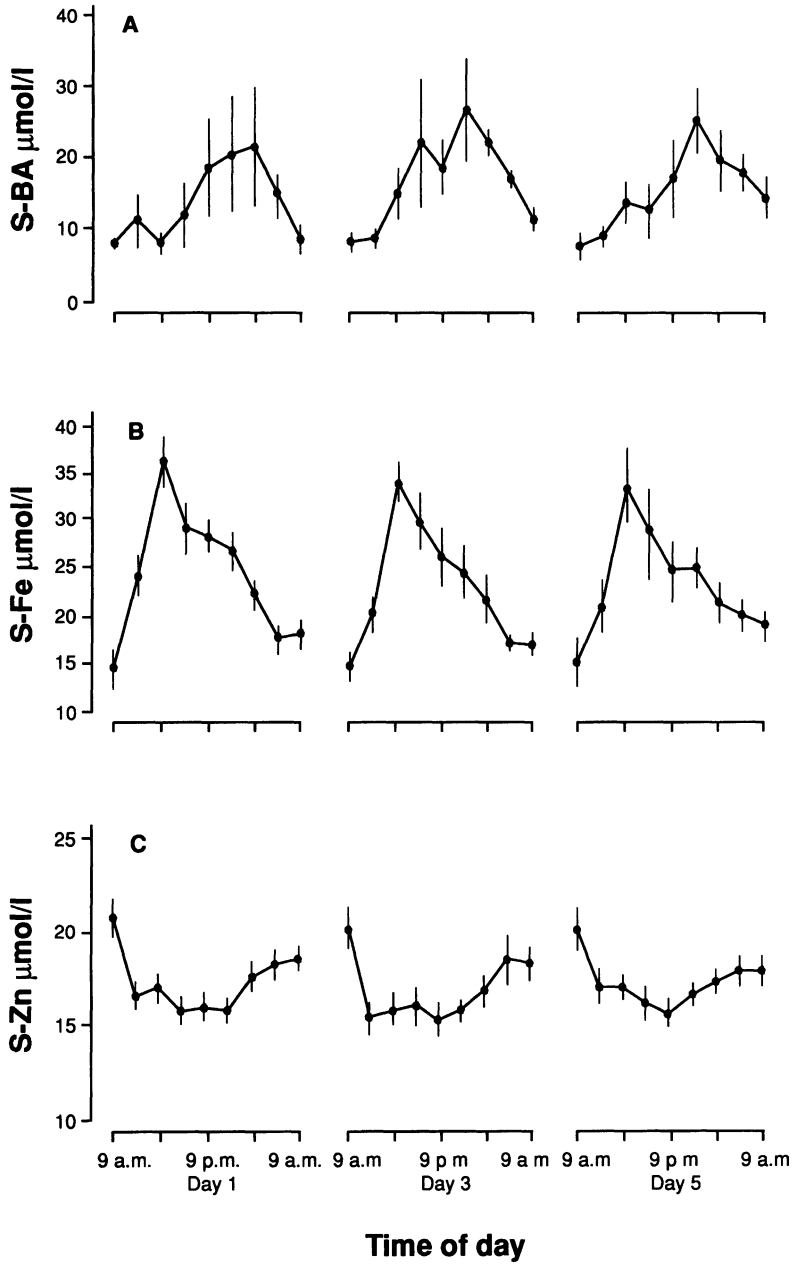

Figure. 1A-C. Profiles of daily variation in S-BA (*A*), S-Fe (*B*) and S-Zn (*C*). Data for ET- and standard-fed gilts (n=6) are pooled together and shown as mean values during three 24 h periods. Vertical bars represent ±SEM.

mg/kg. The ET feed given each day was prepared the day before by dissolving 600 mg of ET in 1200 ml water which was mixed with 3.0 kg feed and kept cool overnight. The final mixture of ET feed was shown to contain 82 mg of ET/kg as measured with the LAL test. Except for the addition of ET, the standard feed was pre-treated in the same way. In every case, all the feed was consumed.

After 6 days of ET feeding, all animals were injected iv with 1.0 μg/kg bw of LPS from *Escherichia coli* (055:B5, phenol-extracted and chromatographically purified, batch 38F-4043, Sigma) at 9 am (day 7). This LPS was dissolved in physiological saline immediately before injection. The injections were repeated on the next 4 days at 9 am (days 8-11). During this part of the experiment, all animals received standard feed mixed with water (9 am, 3 pm). The animals were slaughtered the day after the last LPS injection, whereupon autopsy and a fecal parasitological examination were performed.

Blood sampling and analytical methods

On days 1, 3 and 5, the first blood sample was taken in connection with the 9 am feed, then every 3 h for 24 h. A small amount of standard feed was given at each sampling. The animals were familiar with the procedure and showed no signs of stress. On days 7 to 11, blood samples were taken immediately before iv LPS injection, then 1, 2, 3, 4, 6, 9 and 12 h afterwards.

At each blood sampling, jugular vein blood was aspirated through the venous catheter into a plastic syringe (Becton & Dickinson, Franklin Lakes, USA) and transferred to plain, heparinized and EDTA vacutainer tubes (Becton & Dickinson). The hematological analyses were completed within 1 h of blood sampling. The methods and the precisions for the analyses: total white blood cells (B-WBC), hemoglobin, packed cell volume, total red blood cells, S-BA, S-GLDH, S-Ca, S-Fe, S-Zn and P-PG are described in full elsewhere *(Holst et al.* 1993). In addition, blood platelets (B-PLT) were analysed (Sysmex F-800, TOA Medical Electronics, Japan). The interassay coefficient of variation was 4.9%. Classification of white blood cells into polymorphonuclear (neutrophils, basophils and eosinophils) (PMN) and mononuclear (monocytes, macrophages and lymphocytes) (MN) was done by microscopic counting of 200 cells from May-Grünwald/Giemsa stained blood smears.

Clinical examination

Rectal temperature was measured and clinical status recorded in connection with the blood samplings. When iv LPS injections were given, rectal temperature was also measured 30 min after injection and the animals were kept under continuous observation the first 2 h after injection and in connection with blood sampling.

Data analysis

The data were analysed by calculating the differences between data generated from each of the experimental gilts and the corresponding data from the littermate gilt in the control group i.e. "experimental animal minus littermate control". This was done for each time point on all experimental days, thereby generating a difference-curve for each littermate-pair. For parameters where consistent and systematic differences occurred, the changes are described in Fig. 2 with a summary measure: + or $-\bar{X}_{diff}$ = mean value of the difference curve (i.e. the difference between the 2 gilts in each pair at all time points during a certain experimental day). A positive value indicates that the experimental (ET-fed) animal during that period had a higher mean value than its littermate in the control group and

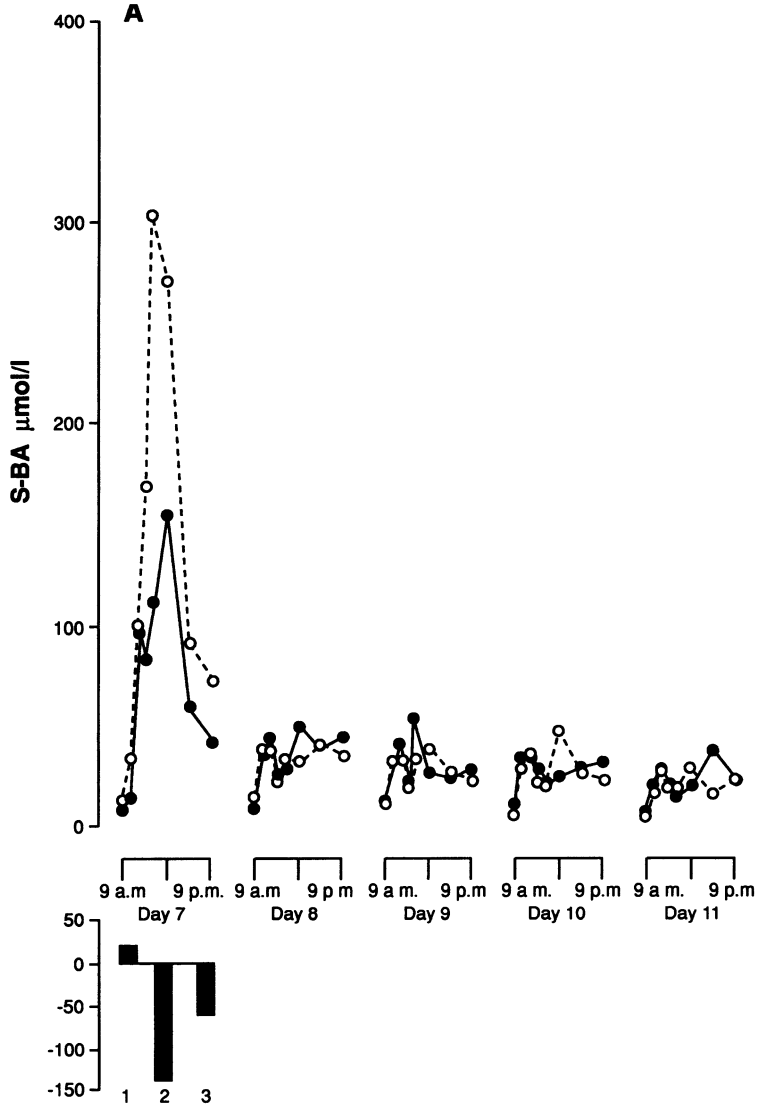

Figure 2A-D. Effect of repeated iv administration of *E.Coli* LPS (1.0 µg/kg; 9 am on days 7-11) on some blood parameters in ET-fed (•——•)(*n*=3) and standard-fed gilts (○------○)(*n*=3) shown as mean values within group during five 12 h periods. *Panel A:* S-BA; *B:* S-GLDH; *C:* B-WBC; *D:* PMN. For S-GLDH, only days 7-8 are shown and ET-fed gilts are omitted as the values were kept constantly below the detection limit (20 nkat/l). The summary measures shown as bars in each panel ($\bar{X}_{diff}$) represent the mean value of the difference, for the concentration/activity of the measured parameter, between the 2 gilts in each pair (pairs 1,2,3) for all time points during an experimental period (9 am to 9 pm) where consistent differences occurred. A positive value indicates that the ET-fed animal during that period had a higher mean concentration/activity than its standard-fed littermate and vice versa. Experimental periods without bars indicate absence of consistent differences.

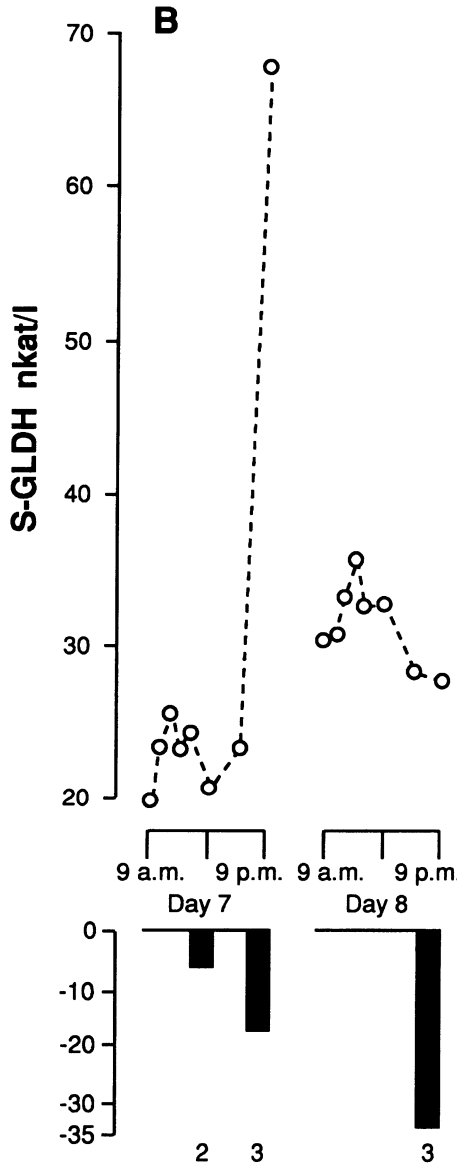

Figure 2 B. For text please see Fig. 2 A.

vice versa. For ease of interpretation, the mean value curves for the control (n=3) and the experimental group (n=3) are shown, together with the summary measures in Fig. 2.

Results

Endotoxin in feed

The animals showed no clinical signs of endotoxemia following repeated oral administration of ET. There were no differences between the experimental and the control group concerning the serum biochemical or hematological parameters. However, several parameters investigated, S-BA, S-Fe and S-Zn, showed pronounced diurnal variations and in describing these variations the data from both groups were pooled (n=6).

S-BA increased from 9 am to around 12 pm and then declined (Fig. 1A). A daily variation in S-Fe was seen, where low morning concentrations (9 am) were followed by a peak at 3 pm The values then declined gradually until 9 am (Fig. 1B). The highest S-Zn concentrations were always detected at 9 am and were followed by a sharp decrease until around noon. From 12 pm, an increase toward the high morning levels was seen (Fig. 1C).

Intravenous injections of LPS

Following the first iv LPS injection (day 7), all animals were severely depressed, showing symptoms such as fever, vomiting, anorexia and increased respiratory rate (RR). After the next 4 LPS injections, however, the previously ET-fed animals showed consecutively fewer symptoms each day as compared with the control animals (Table 1). The fever response was most pronounced on the first day (day 7) but there was no difference between the groups (Fig. 3F).

The concentration of S-BA increased sharply following the first LPS injection on day 7, with peak values measured 3-6 h after LPS. The 4 following days, much smaller increases were measured. The response on day 7 was noticeably lower in the experimental group than in the control group (Fig. 2A).

The activity of S-GLDH increased above

Table 1. Clinical symptoms after repeated daily iv administration of LPS from *E. coli* (1.0 μg/kg) in 6 gilts as a subjective score.

Group	Standard-fed				ET-fed			
Gilt no	1	2	3	Sum	4	5	6	Sum
Day 7	-++	+++	+++	8	+++	+++	-++	8
8	-++	+++	+++	8	-++	-++	---	4
9	-++	--+	-++	5	---	-+-	---	1
10	-++	---	-+-	3	---	-+-	---	1
11	-++	---	-+-	3	-+-	---	---	1

+++ denoting respectively vomiting; anorexia; increased RR
--- denoting respectively no vomiting; no anorexia; no increased RR

the detection limit of 20 nkat/l in the control group following the first 2 LPS injections (in 2 and 1 animal respectively). This was not the case in the experimental group (Fig. 2B).

A distinct fall in B-WBC was seen in both groups, much more pronounced after the first than the subsequent LPS injections. Within 12 h, the B-WBC counts were approximately back to the pre-injection values. This leukopenic response to LPS diminished gradually for each LPS injection. Following the 4th LPS injection on day 10, the fall in B-WBC was of shorter duration in the experimental group than in the control group. This was due to the difference between only 1 of the 3 littermate pairs (Fig. 2C).

An initial decrease in PMN cells was seen immediately after the LPS injections in both groups. This change became smaller for each day and was gradually replaced by an increase (Fig. 2D). On the first day that LPS was injected, the control group had increased PMN counts 9-12 h afterwards, in contrast to the ET-fed animals. Following the 4th LPS injection, the experimental animals showed a more marked increase in PMN than the control group 2-4 h after the LPS injection. A drop in MN cells was seen each day following injection and this pattern was the same in the 2 groups and somewhat less pronounced with each injection (Fig. 3E).

Following iv injected LPS, S-Fe, S-Zn, S-Ca, P-PG and B-PLT did not show any differences when comparing experimental and control animals. The results below describe the effects of repeated LPS injections without regard to the pre-treatment with ET. Therefore, the results from all 6 animals have been pooled. This is also the case with the rectal temperature and MN data above.

Serum-Fe concentration started to decrease 4 h after the first LPS injection, a change not seen on the following 4 days (Fig. 3A). In contrast to the pattern seen earlier, where the concentration had started to decrease at 6 pm (Fig. 1B), S-Fe increased continuously until 9 pm.

The concentration of S-Zn showed the largest decrease following the first LPS injection. As a consistent change following all injections, the concentration of S-Zn showed a more pronounced decrease than during the first part of the study (Fig. 3B).

A sharp decrease in S-Ca concentration occurred on the first day, but not on the 4 subsequent days when LPS was injected (Fig. 3C).

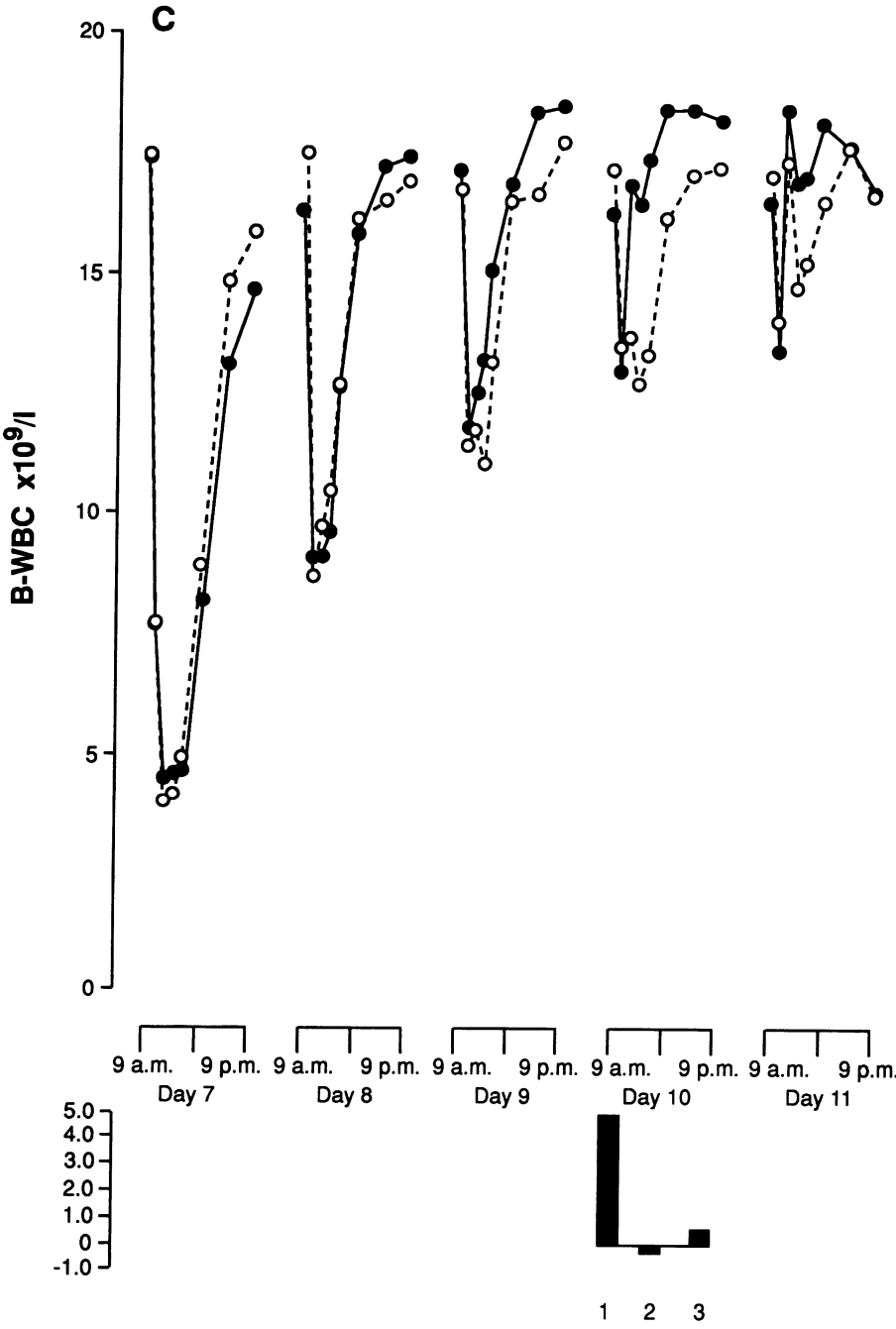

Figure 2 C. For text please see Fig. 2 A.

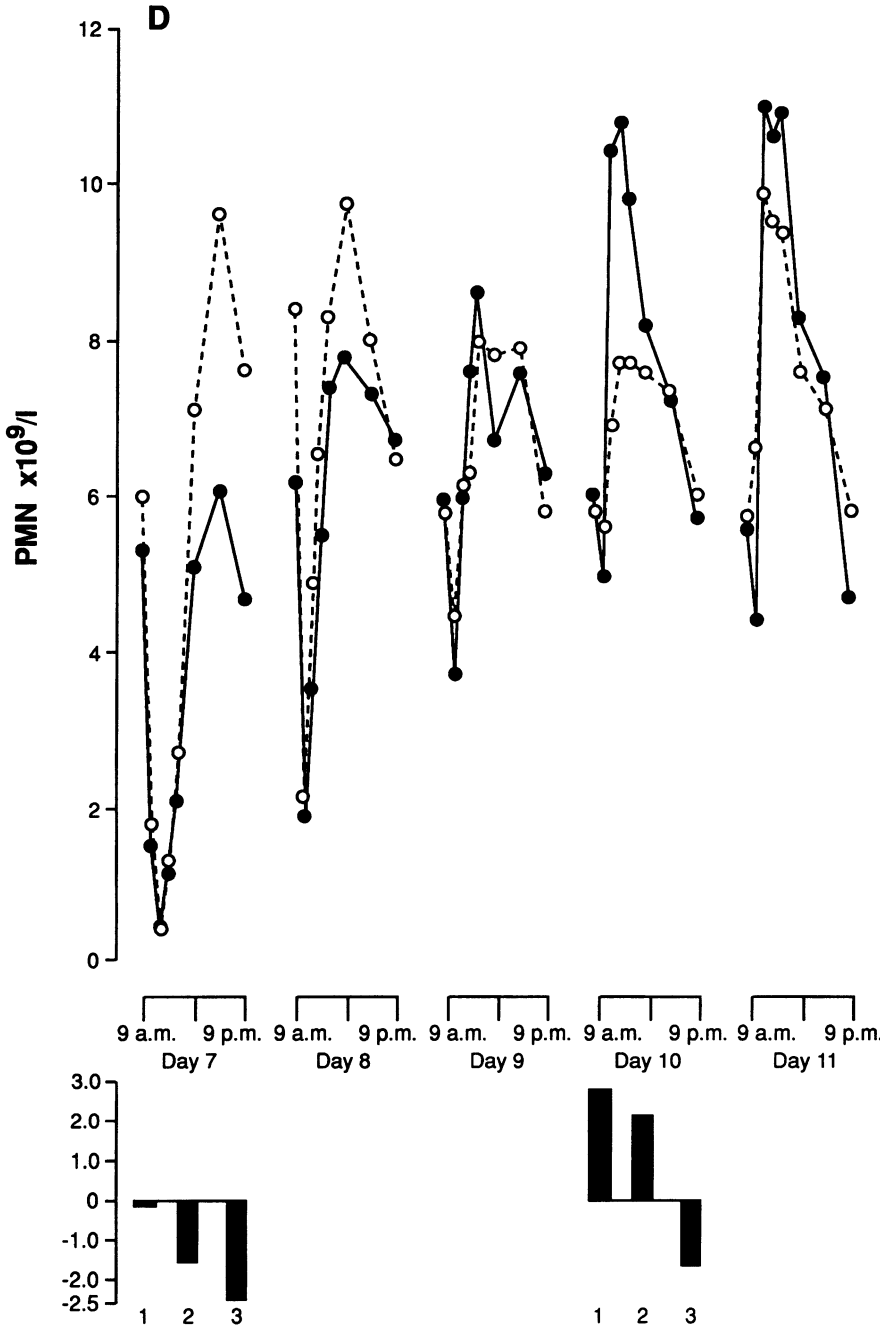

Figure 2 D. For text please see Fig. 2 A.

When the first LPS injection was given, P-PG increased more than tenfold within 1 h. The concentration had returned to the pre-injection level after about 10-12 h. The subsequent injections did induce P-PG increases, but not at all of the same magnitude (Fig. 3D).

An immediate decrease in B-PLT (data not shown) was seen in response to the first LPS injection. On the following days, no consistent changes were seen. The other hematological parameters were not affected by the LPS injections.

The autopsy did not reveal any macroscopic signs of disease except for mild chronic broncho-interstitial pneumonic changes in the apical lobes of animal nos. 2 and 4 *(Mycoplasma hyopneumoniae* shown with fluorescent antibody technique). The histopathological examination of the lungs showed, in all animals, hyperemia, moderate erythrophagia and a moderate amount of alveolar macrophages containing hemosiderin (no. 4 not examined). Furthermore, follicular hyperplasia of the spleen and paracortical hyperplasia of the mesenteric lymph nodes were seen in all animals. The parasitological fecal examination did not reveal presence of intestinal parasites.

Discussion

Effects of ET in feed and diurnal variations

In an earlier study *(Holst et al.* 1993), prepubertal gilts showed (although not fully consistent) slight temperature increases and increases in S-BA and S-GLDH following a single oral administration of ET-contaminated feed. This was not seen in the present experiment as a sequel either to the first or the subsequent ET-feedings. The lack of response after the first ET-feeding in this study is difficult to explain since, in both studies, comparable ET preparations, experimental animals and procedures were used. Biological variation, sub-clinical differences in state of health and earlier exposure to ET may well contribute. It is however conceivable that the repeated ET-feedings did not induce any response, as one could expect that the animals would gradually become refractory to the actions of ET.

Tracey et al. (1986) found that S-BA concentrations in the pig showed a peak value approximately 2-3 h after feed intake. The variation in S-BA concentration in the present study is consistent with earlier findings *(Holst et al.* 1993). Although we could not detect any distinct peak values during the hours after feeding, it is probable that the increase observed was of a postprandial nature. Diurnal variations in S-Fe in the horse have been described by *Stewart & Clarkson* (1969). In contrast to the pattern seen in the pig in the present study, these authors found lower S-Fe concentrations in the evening than in the morning sample. The U-shaped pattern of the S-Zn curve, which is consistent for all the three 24 h periods, is strikingly similar to that in man *(Markowitz et al.* 1985).

Reduced response to iv LPS following oral ET

Bile acids are normally efficiently extracted from the portal vein in the liver *(Erlinger* 1990, rev.) and increased peripheral concentrations have been found to be an indicator of hepatic dysfunction in several species, among them the mini-pig *(Kroker & Römer* 1984). The activity of S-GLDH is regarded as a marker for hepatocyte necrosis in the pig *(Hyldgaard-Jensen et al.* 1969). The increased S-BA concentration and S-GLDH activity following the first LPS injection in the present study might be consistent with mechanisms proposed by other investigators. *Spitzer & Deaciuc* (1990) showed an increased production of superoxide anions in vivo from the livers of ET pretreated rats and suggested that

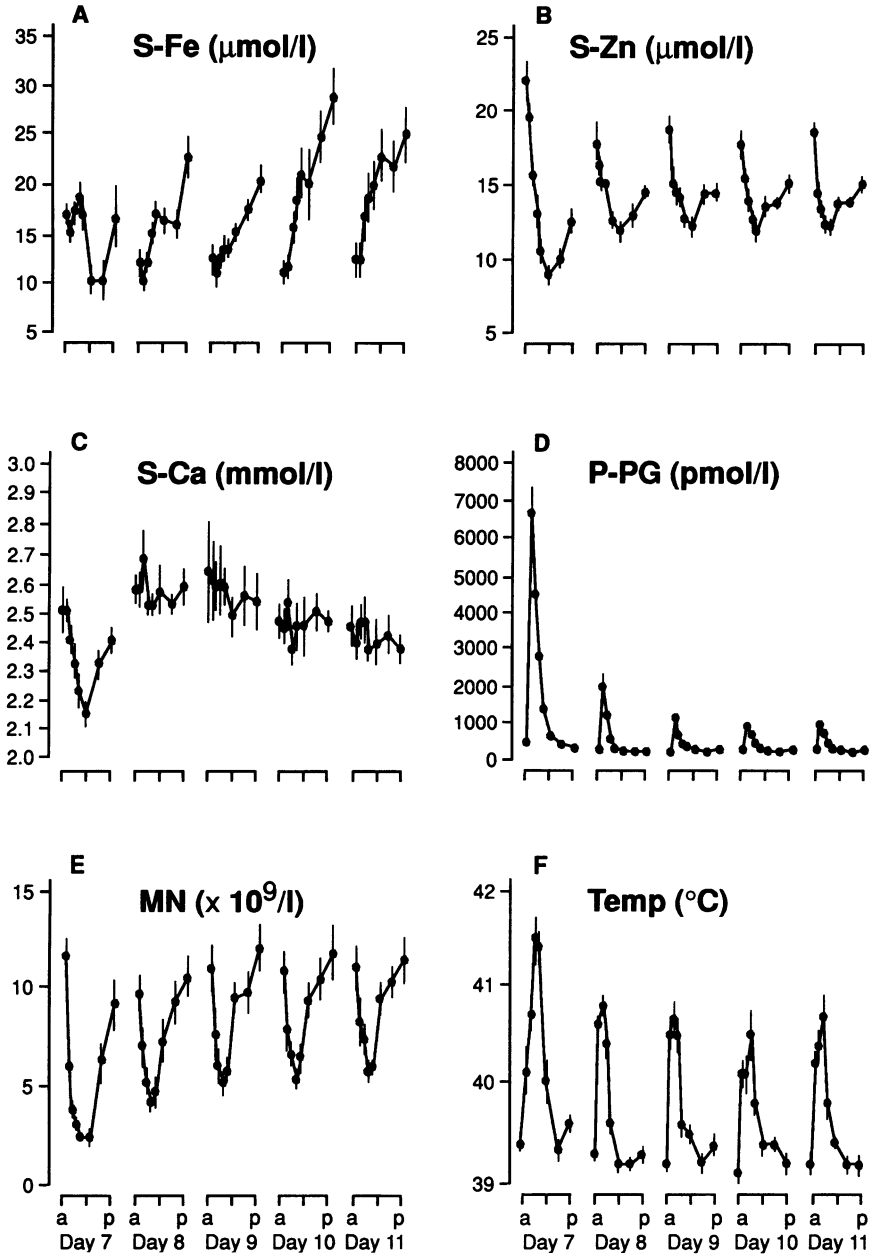

Figure 3A-F. Effect of repeated iv administration of *E.Coli* LPS (1.0 µg/kg; 9 am on days 7-11) in ET- and standard-fed gilts. Data for all animals (n=6) are pooled together. *Panel A:* S-Fe; *B:* S-Zn; *C:* S-Ca; *D:* P-PG; *E:* MN and *F:* rectal temperature. Data are shown as mean values during five 12 h periods from 9 am (a) to 9 pm (p). Vertical bars represent ±SEM.

these products might be toxic to intact adjacent cells and cause local damage. In addition, intracellular Ca^{2+} concentration increases in a dose-dependent fashion, together with an intracellular pH decrease in rat hepatocytes exposed to ET *(Portolés et al.* 1991) and these events could also lead to cell damage. *Utili et al.* (1976) showed that LPS reduced the bile flow in vitro in the rat.

Early-phase ET tolerance has been attributed to a reduced responsiveness of the reticuloendothelial system – especially the Kupffer cells – to the synthesis and/or release of cytokines, e.g. interleukin-1 and tumour necrosis factor (TNF) which have been shown to induce many of the physiological responses that are observed after administration of ET *(Vogel et al.* 1987; *Bibby & Grimble* 1991). These 2 cytokines are probably differentially regulated in vivo during ET tolerance *(Zuckerman et al.* 1991). It has also been shown that ET hepatotoxicity and Kupffer cell production of TNF-α in the rat are greatly diminished when rats previously pretreated with ET are again challenged with ET *(Hartung & Wendel* 1992). The authors concluded that ET tolerance is dependent on both impaired Kupffer cell function and a functional change in the hepatocyte.

A lack of response in S-BA and S-GLDH was seen here following the 2nd to 5th LPS injection in both groups. This suggests that these 2 parameters are objects for and a reflection of the induction of hyporesponsiveness to ET. It is noteworthy that the most obvious difference between groups found here concerned the response in S-BA and S-GLDH. Animals fed ET had a smaller response in these 2 parameters following the first LPS injection, than animals fed standard feed. This implies that the previous exposure to oral ET regulated the response to the injected LPS, probably as a consequense of a minor gastrointestinal uptake of ET from the feed and a subsequent detoxification in the liver. This interpretation finds support in an investigation by *Fujiki et al.* (1988) where it was shown that oral administration of ET to mice for 5 consecutive days, without inducing any toxic effects, protected the animals against the lethal effects of iv injection with *P. aeruginosa* or *L. monocytogenes* and also reduced the number of bacteria in the liver. In a previous study we observed increases in S-BA and S-GLDH in pigs exposed to ET- contaminated feed. Taken together, this suggests S-BA and S-GLDH to be 2 biochemical parameters suitable to reflect ET influence in the live pig.

The alterations in B-WBC and differential count in connection with repeated ET administration in the pig have been studied by *Yagoda et al.* (1988) and our results are consistent with their findings. Most probably, the lack of increase in PMN counts after the first iv LPS injection in the ET-fed group was due to the previous oral ET challenge which had made the cells mediating the bone marrow mobilization of neutrophils less responsive to LPS, an interpretation that finds support in earlier investigations in the mice *(Fruhman,* 1972). This could also explain why a larger increase in PMN count was seen in these animals after the 4th injection, since their bone marrow compartment of neutrophils was not to the same extent recruited initially. Endotoxin activation of PMN has been shown to be unaffected by cyclooxygenase inhibitors, indicating that prostaglandins are unlikely to mediate the stimulation *(Burrel* 1990, rev.). This finding is in agreement with our study as there were no difference in P-PG between the ET- and standard-fed group in response to the iv ET challenge.

Effects of repeated iv LPS

The ET-induced changes in S-Fe and S-Zn are

thought to be mediated by interleukin-1 produced by monocytes, neutrophils and Kupffer cells *(Dinarello* 1984, rev.). The changes in the patterns of S-Fe, S-Zn and S-Ca following the repeated LPS injections indicate that a certain degree of hyporesponsiveness to LPS had developed as a sequel to the first injection.

The pattern of decreased P-PG concentrations in connection with repeated LPS injections is consistent with earlier observations *(Yagoda et al.* 1988). The results from an experiment by *Rodriguez de Turco & Spitzer* (1990) suggest that prostaglandins released by non-parenchymal cells and polymorphonuclear phagocytes in the liver of LPS-infused rats may help impair liver function and metabolism during endotoxemia. This could give an additional explanation for the increases in S-BA and S-GLDH following iv LPS, as discussed above.

The decreased B-PLT counts following the first LPS injection could be attributable to any of several mechanisms, such as a direct action of LPS on the platelet membranes, thereby inducing release of procoagulants and platelet aggregation. *Dobrowsky et al.* (1991) could by administering a platelet-activating factor receptor antagonist block ET induced thrombocytopenia in the pig. The refractoriness to the repeated injections is most probably a further reflection of the desensitizing effect that the first LPS injection had.

The histopathological changes of the lungs in all the gilts do probably reflect endotoxin induced microvascular lesions with leakage of erythrocytes *(Goodman et al.* 1979). *Deitch et al.* (1992) has shown that nonlethal intraperitoneal endotoxin injections promote bacterial translocation from the gut to the mesenteric lymph nodes and this phenomenon did probably cause the paracortical hyperplasia of the mesenteric lymph nodes seen as a consistent change in this study. The follicular hyperplasia of the spleen is probably due to B-lymphocyte proliferation caused by the LPS injections.

Conclusions

The early phase endotoxin tolerance has been defined as a transient period after an initial sublethal exposure to ET during which a normally responsive individual is rendered hyporesponsive. Even if reduced response is a more proper description in this case, the oral ET exposure preceding the experimental endotoxemia did probably induce such a condition. Supporting this assessment are the differences between the ET- and standard-fed groups in clinical response, S-BA, S-GLDH, B-WBC and PMN following iv LPS.

It is likely that different pathological conditions would render the animal more sensitive to ET in the feed. One such condition could be an impaired liver function with a reduced detoxifying capacity or reduced bile secretion. It has been shown that bile deficiency in obstructive jaundice may lead to endotoxemia and that oral bile salt administration can prevent this *(Nolan* 1988, rev.). Gastrointestinal disorder might also be an important factor. These are aspects that we intend to study in future investigations.

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Sammanfattning

Reducerad effekt av intravenösa endotoxininjektioner efter peroral endotoxintillförsel hos svin

Permanenta venkatetrar inopererades på 6 icke konsmogna gyltor (parvis kullsyskon; ca 30 kg kroppsvikt) en vecka före försökets början. Tre gyltor utfodrades 2 gånger dagligen i 6 dagar med foder innehållande 200 mg endotoxin per kg foder. De andra 3 gyltorna erholl foder utan tillsats av endotoxin. Darefter injicerades samtliga djur intravenöst med endotoxin (1.0 µg/kg kroppsvikt) en gång om dagen i 5 dagar. Blodprover analyserades avseende totalantal och differentialräkning av vita blodkroppar, hematokrit, trombocyter, gallsyror, glutamatdehydrogenas, järn, zink, calcium samt en prostaglandin $F_{\alpha 2}$ metabolit.

Djuren uppvisade inga tydliga kliniska symptom eller förändringar i de undersökta blodparametrarna i samband med den perorala endotoxintillförseln. Då endotoxin administrerades intravenost uppvisade de djur som ej endotoxinutfodrats större forekomst av kliniska symptom och mer uttalade ökningar i serumkoncentration av gallsyror och aktivitet av glutamatdehydrogenas jämfort med djuren som endotoxinutfodrats. Detta talar for att endotoxin tagits upp från mag-tarmkanalen i samband endotoxinutfodring – visserligen endast i sådan mangd att leverns detoxifierande fomåga ej overskreds, men tillräckligt for framkalla ett visst mått av tolerans mot intravenost injicerat endotoxin.

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