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PATHOPHYSIOLOGICAL ASPECTS OF *E. COLI* MASTITIS IN RUMINANTS

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ABSTRACT

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It is a common theory that the systemic signs in cows suffering from coliform mastitis would be caused by absorption of endotoxin from the udder into the circulation. However, definite proof to validate this hypothesis is as yet not available. Therefore, the effects on some clinical and clinical-chemical parameters of administering either intravenously or intramammarily *E. coli* endotoxin to normal and endotoxin-tolerant ruminants were comparatively examined. The absence of marked effects on rumen motility following the intramammary administration of endotoxin was striking. Moreover, in cows the intramammary administration of one fifth of the dose of endotoxin to which the animals were made tolerant produced a maximum effect on body temperature and plasma zinc concentrations. These observations suggest the release of inflammatory endogenous mediators in the udder and their subsequent absorption into the circulation rather than the absorption of endotoxin. However, attempts to validate this hypothesis failed. Further research for detection methods more sensitive than the bioassay used in this study is recommended.

INTRODUCTION

The role of endotoxin in the pathogenesis of various diseases in human and animal beings is repeatedly discussed in the literature. With regard to coliform mastitis in cattle such literature is plentiful. In cows suffering from coliform mastitis release of endotoxin from the cell-wall of coliform bacteria in the udder would account for the *local* signs of inflammation (Carroll et al., 1964; Schalm et al., 1964, 1971). The *systemic* signs in these patients would be caused by ab-

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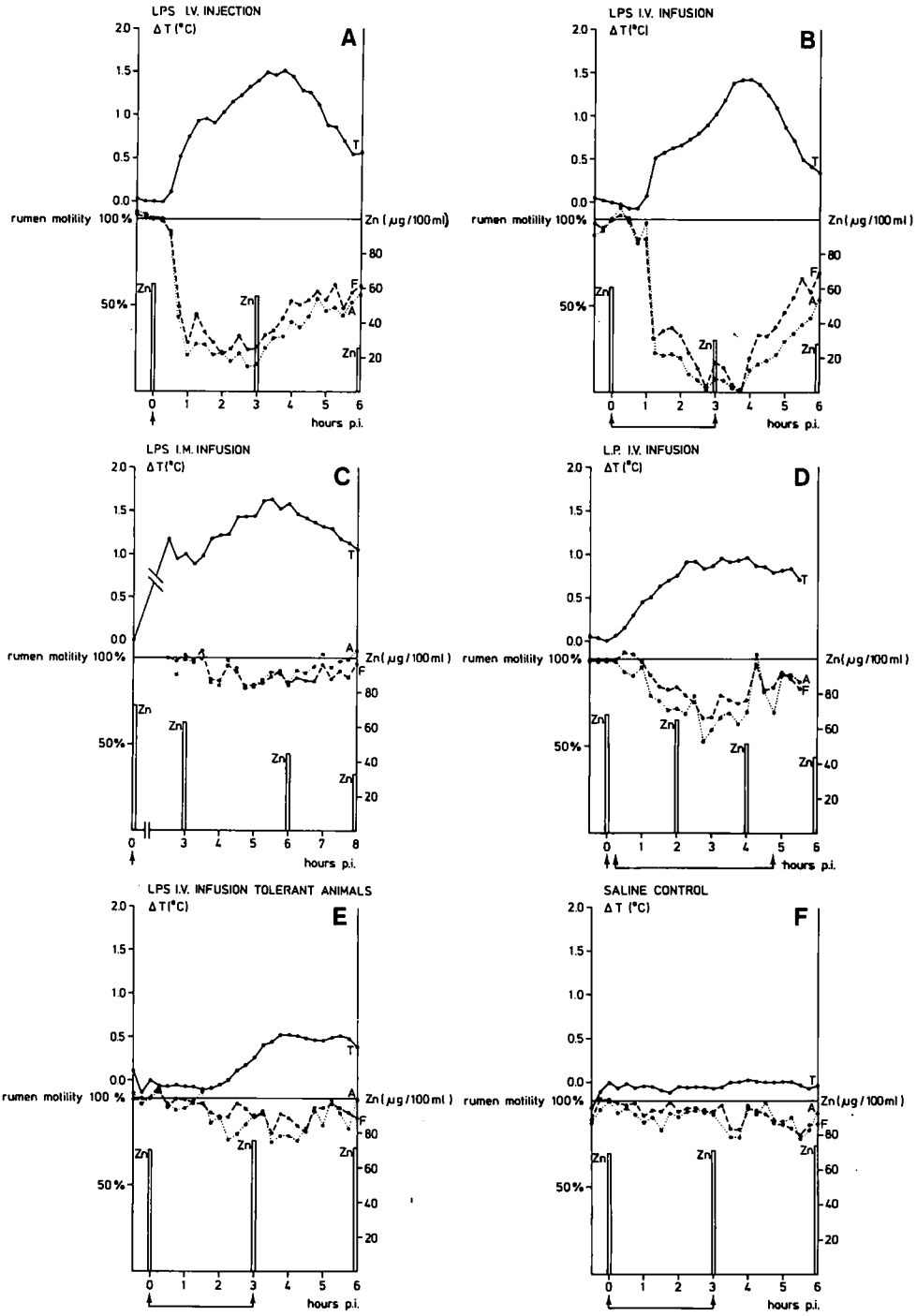
LPS I.V. INJECTION
ΔT (°C)
A
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.
LPS I.V. INFUSION
ΔT (°C)
B
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.
LPS I.M. INFUSION
ΔT (°C)
C
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.
L.P. I.V. INFUSION
ΔT (°C)
D
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.
LPS I.V. INFUSION TOLERANT ANIMALS
ΔT (°C)
E
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.
SALINE CONTROL
ΔT (°C)
F
rumen motility 100%
Zn (µg/100ml)
50%
hours p.i.

sorption of endotoxin from the udder into the circulation (Burvenich, 1978; Carroll et al., 1964; Griel et al., 1975; Hartman et al., 1976; Jain and Lasmanis, 1978; Pounden, 1947; Radostits, 1961; Said, 1973; Schalm et al., 1971; Ziv et al., 1976). However, definite proof to validate the latter hypothesis is still lacking. Moreover, the strong resemblance in clinical symptoms of cows with Gram positive in comparison with Gram negative infections of the udder (Verheijden, 1979; Walser et al., 1972) raised serious doubts as to the validity of the concept that coliform mastitis should be an endotoxemia in its acute phase. Further research was therefore conducted on the role of endotoxin in the pathophysiology of *E. coli* mastitis in ruminants. For this purpose the effects on some clinical and clinical-chemical parameters of administering either intravenously or intramammarily *E. coli* endotoxin to normal and endotoxin-tolerant ruminants were comparatively examined. Moreover, the possible role of endogenous mediators — in particular endogenous pyrogen (Atkins and Bodel, 1974; Dinarello, 1979; Dinarello et al., 1977; Snell, 1971) — was studied.

MATERIALS AND METHODS

The experiments were conducted using three Friesian-Holstein dairy cows, three lactating goats and nine kids. The pyrogen used was a purified lipopolysaccharide (LPS) from *Escherichia coli* 0111B4 (Difco Laboratoria, Detroit, Michigan, U.S.A.). Intramammary administration was done in one of both hind quarters, intravenous administration in the jugular vein (adults) or in the tarsal vein (kids). Sterile pyrogen-free syringes and needles were used for each infusion. Methods for maintaining glassware and solutions free of bacterial pyrogen contamination, for preparing leucocytic pyrogen and for recording body temperature and rumen motility have been described elsewhere (Van Miert and Atmakusama, 1970; Van Miert et al., 1976, 1972, 1977; Verheijden, 1979). Plasma zinc concentrations were determined by atomic absorption spectophotometry (Perkin-Elmer, model 305 B).

Fig. 1. Mean changes in body temperature (T), rumen motility (frequency-F and amplitude-A) and plasma zinc concentrations (Zn) for 3 goats following:

A, the intravenous injection of 0.1 μg/kg LPS;

B, the intravenous infusion of 0.03 μg/kg/hour LPS;

C, the intramammary administration of 0.1 mg LPS;

D, the intravenous infusion of leucocytic pyrogen (a priming injection of 5 ml/kg was given followed by a continuous infusion at the rate of 2 ml/min. — total dose equivalent to 9.10^9 leucocytes);

E, the intravenous infusion of 0.03 μg/kg/hour LPS to animals tolerant to a single intravenous injection of 0.1 μg/kg LPS;

F, saline control.

RESULTS

In goats intravenous administration of endotoxin, either by means of a single injection (Fig. 1A) or infused (Fig. 1B), induced a biphasic rise in body temperature, inhibition of forestomach motility and a lowering of plasma zinc concentrations. In contrast, intramammary administration of endotoxin produced different effects on these parameters (Fig. 1C). Striking was the absence of marked effects on forestomach motility following the intramammary administration of endotoxin. On the other hand, in goats there was a strong resemblance between the effects of intravenous infusion of leucocytic pyrogen (Fig. 1D) and the intramammary administration of endotoxin on these parameters.

In goats and cows tolerance to *E. coli* endotoxin could be rapidly induced by daily intravenous injection of the pyrogen. This refractory state was characterized by a marked decrease in the magnitude of the changes observed. Goats that were tolerant to a single injection of LPS were also tolerant to an intravenous infusion of LPS (Fig. 1E). However, in cows intramammary administration of one fifth of the dose of endotoxin to which the animals were made tolerant produced a maximum effect on body temperature and plasma zinc concentrations (Fig. 2).

In kids intravenous injection of LPS induced a biphasic rise in body temperature and a lowering of plasma zinc concentrations (Fig. 3). In contrast, after the intravenous injection of leucocytic pyrogen an early monophasic febrile response was observed in these animals, while plasma zinc concentrations did not change significantly (Fig. 4). However, in kids, intravenous injection of plasma drawn from goats one hour before their peak febrile reaction to the intramammary administration of endotoxin failed to induce an early febrile reaction compatible with an endogenous pyrogen response.

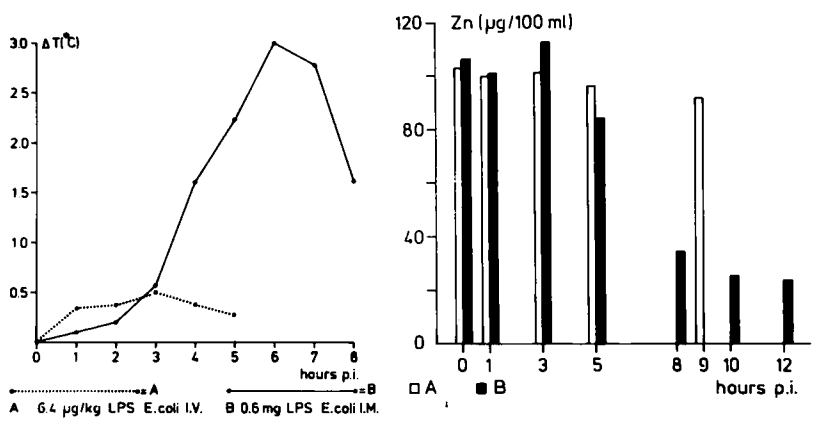

Fig. 2. Mean changes in body temperature (ΔT) and plasma zinc concentrations (Zn) for 3 cows made tolerant to endotoxin following: A, a single intravenous injection of 6.4 µg/kg LPS *E. coli*; B, the intramammary administration of 0.6 mg LPS *E. coli*.

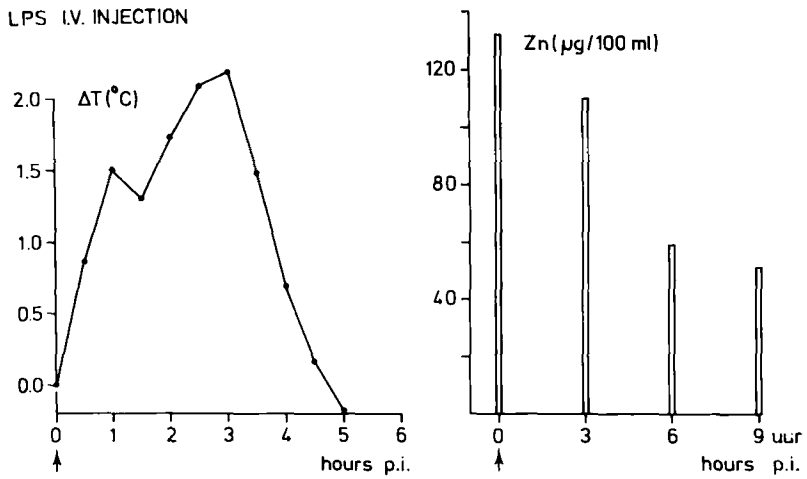

Fig. 3. Mean changes in body temperature (ΔT) and plasma zinc concentrations (Zn) for 6 kids following a single intravenous injection of 0.1 μg/kg LPS *E. coli*.

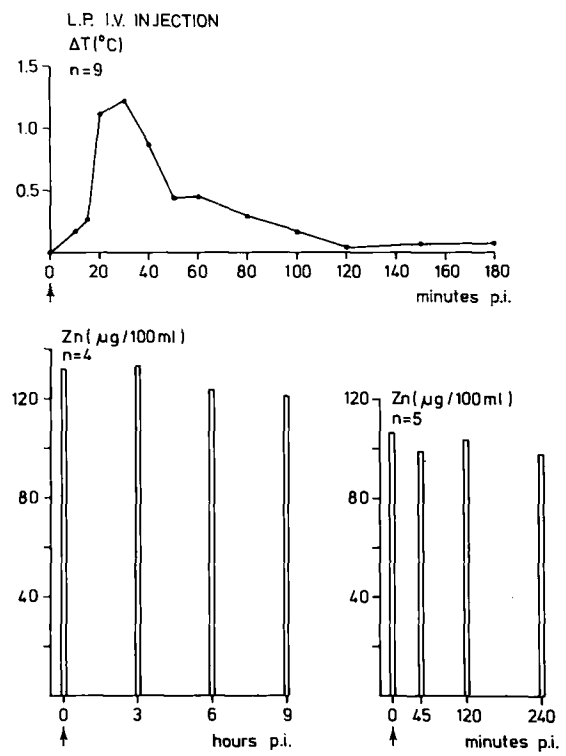

Fig. 4. Mean changes in body temperature (ΔT) and plasma zinc concentrations (Zn) for 9 kids following a single intravenous injection of leucocytic pyrogen (15 ml/kg equivalent to 2—4.10^9 leucocytes).

DISCUSSION

The findings in the present study clearly demonstrate that the endotoxin - mastitis - model is not quite comparable with the spontaneous infection. Namely, cows suffering from coliform mastitis generally show fever, a lowering of plasma zinc and iron concentrations and inhibition of forestomach motility (Verheijden, 1979; Verheijden et al., 1980). However, in cows with Gram positive infections of the udder, forestomach motility was diminished as well. Hence, it seemed unlikely that the inhibitory effect on forestomach motility in clinical cases of coliform mastitis simply should be attributable to circulating endotoxin. The febrile reaction, coupled with a fall in plasma zinc, following the intramammary administration of endotoxin to tolerant animals suggest the release of inflammatory endogenous mediators in the udder and their subsequent absorption into the circulation rather than the absorption of endotoxin. Of course, it is impossible to preclude that occasionally small quantities of endotoxin are absorbed into the circulation.

Significant decreases in plasma zinc and iron concentrations coupled with elevated body temperatures have been shown to occur during bacterial, viral and protozoal infections (Beisel, 1972; Beisel et al., 1971; Cartwright et al., 1946; Pekarek et al., 1969, 1970; Verheijden, 1979; Verheijden et al., 1980; Vikbladh, 1951), endotoxemia (Beisel, 1972; Kampschmidt and Upchurch, 1962; Kincaid et al., 1976; Pekarek and Beisel, 1969) and a variety of other stressful conditions (Halsted and Smith, 1970; Pekarek and Beisel, 1971) in man and experimental animals. These changes would be mediated by endogenous mediators released in part by activated polymorphonuclear leucocytes and monocytes (Beisel, 1972; Kampschmidt and Upchurch, 1969; Pekarek and Beisel, 1971; Snell, 1971). When stimulated in vitro, phagocytic leucocytes release proteins such as leucocytic endogenous mediators (LEM) and endogenous (leucocytic) pyrogen (LP). In the literature there is some controversy concerning the question whether LEM and LP are identical or not (Beisel, 1972; Beisel and Sobocinski, 1980; Kampschmidt et al., 1978; Mapes and Sobocinski, 1978; Merriman et al., 1977). The finding that in kids the intravenous administration of LP evoked fever but failed to induce changes in plasma zinc concentrations supports the theory that changes in body temperature and plasma zinc and iron concentrations should be mediated by different proteins.

Van Miert (1970) found that in goats intravenous injection of leucocytic pyrogen caused fever but failed to induce inhibition of forestomach motility. On the other hand, animals which were made tolerant to endotoxin by daily intravenous injections proved to be quite susceptible to the pyrogenic action of leucocytic pyrogen (Van Miert and Atmakusama, 1970). On the basis of these data and the above findings it seemed highly likely that the systemic signs in *E. coli* endotoxin induced mastitis should predominantly be due to the absorption of endogenous mediators from the udder. However, attempts to detect such mediators in the blood during fever evoked by intramammary endotoxin administration failed. This is in keeping with the fact that circulating endogen-

ous pyrogen has yet to be convincingly demonstrated with the aid of bioassays during febrile diseases in man (Dinarello et al., 1977; Greisman and Hornick, 1972; Perlow et al., 1975). A possible explanation might be the rapid clearance of endogenous pyrogen by the liver (Lorber et al., 1971). Recently, Dinarello et al. (1977) developed a radio-immunoassay for human leucocytic pyrogen. This test should be more sensitive than a bioassay. Further research for the suitability of such an immunoassay to demonstrate circulating pyrogen in the febrile animal is recommended.

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