FEVER AND CHANGES IN PLASMA ZINC AND IRON CONCENTRATIONS IN THE GOAT: THE ROLE OF LEUKOCYTIC PYROGEN

By

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INTRODUCTION

Infected animals characteristically exhibit, in addition to fever, a chain of non-specific host responses such as neutrophilic leukopenia followed by neutrophilic leukocytosis, hypoferraemia, hypozincaemia, hypercupraemia and changes in the concentrations of certain plasma proteins (Beisel, 1976a, 1977; Grieger and Kluger, 1978; Weinberg, 1978; Verheijden, 1979; Kluger and Rothenburg, 1980). Similar responses occur in experimental animals when sterile inflammation is produced (Verheijden, Van Miert, Schotman and Van Duin, 1980, 1982) or following intravenous injection of bacterial pyrogens (Kampschmidt and Upchurch, 1968; Blatteis, Mashburn and Ahokas, 1981; Van Miert, Van Duin, Verheijden and Schotman, 1982). Kluger and colleagues (Grieger and Kluger, 1978; Kluger and Rothenburg, 1980; Kluger and Vaughn, 1978) have suggested that the combination of fever and low plasma zinc and iron concentrations may have survival value during infection.

It is generally believed that fever is mediated by endogenous pyrogens (EP), proteins which are produced by leukocytes (LP) and other phagocytic cells and act on CNS temperature regulating centres (Dinarello, 1980; Milton, 1982). During the past decade, evidence has accumulated to indicate that the non-febrile components of the acute phase reaction (Beisel and Sobocinski, 1980) are mediated by proteins also released by phagocytic cells, which have been termed leukocytic endogenous mediator (LEM), lymphocyte activating factor (LAF) and serum amyloid A inducer (SAA-inducer). It has been suggested that these substances are similar to or may be identical with EP or LP (Merriman, Pulliam and Kampschmidt, 1977; Kampschmidt, Pulliam and Merriman, 1978; Rosenwasser, Dinarello and Rosenthal, 1979; Murphy, Simon and Willoughby, 1980; Sztein, Vogel, Sipe, Murphy, Mizel, Oppenheim and Rosenstreich, 1981). LEM is the causative agent for the decreases in the plasma concentrations of zinc and iron during both infection (Beisel, 1976a, 1977) and endotoxinaemia (Kampschmidt and Upchurch, 1962; Pekarek and Beisel, 1971). LEM revealed many similarities to the LP molecule (Beisel and Sobocinski, 1980; Kampschmidt, 1980); both molecules have similar molecular weights and require a free sulphhydryl group for activity. The relationship between LP and LEM has not been determined with

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certainty (Mapes and Sobocinski, 1977; Beisel and Sobocinski, 1980). This investigation was designed to compare the body temperature responses and the changes in plasma zinc and iron concentration in a number of febrile states to determine whether febrile reactions are closely associated with changes in the plasma concentrations of these trace metals. Accordingly, the authors measured any alteration in plasma zinc and iron concentration during fever caused by: (1) a rickettsia (*Ehrlichia phagocytophila*; tick-borne fever), (2) two trypanosomes (*Trypanosoma vivax, Trypanosoma congolense*), (3) bacterial pyrogens such as those of *Escherichia coli* (LPS) and *Staphylococcus aureus* (enterotoxin B) and (4) goat leukocytic pyrogen.

MATERIALS AND METHODS

Animals

Thirty-eight healthy dwarf goats, females and castrated males, were used; they weighed between 19 and 50 kg (mean ± S.E.: 33·4 ± 1·3 kg). Also, 9 lactating mastitis-free goats and 18 kids were selected to examine the effects of bacterial toxins and leukocytic pyrogen (LP). In the lactating goats, which weighed between 28 and 35 kg, the toxins were given by the intramammary route in 1 ml of pyrogen-free 0·9 per cent NaCl solution. The kids received both *E. coli* lipopolysaccharide (LPS) and LP with an interval of one week to avoid interactions; they weighed between 3·1 and 5·8 kg (mean ± S.E.: 4·06 ± 0·17 kg). Six of the 37 goats were infected with *E. phagocytophila*, 2 with *T. congolense* and 6 others with *T. vivax*. Staphylococcal enterotoxin B (SEB) was injected i.v. into 15 animals which, as far as was known, had had no previous exposure to this toxin. However, tests for the presence of antibodies against SEB were not performed. *Escherichia coli* LPS and pyrogen-free saline were tested in 9 of the 37 animals; different doses were given i.v. at intervals of at least 3 weeks to avoid the induction of endotoxin-tolerance.

All animals were kept indoors and fed a diet of hay and pelleted concentrate. Water was provided ad libitum.

Temperature Measurements

Rectal temperature of the infected goats was measured 2 or 3 times daily with certified mercury thermometers. The other goats were trained to stand quietly during actual recording sessions by repeatedly placing them in conventional goat stocks for several hours daily. In these animals rectal temperature was measured with a thermistor-based electronic thermometer (Scanning tele-thermometer, model 47, Yellow Springs Instrument Corp. Inc., Yellow Springs, Ohio, U.S.A.); the appropriate thermistors (probe no. 401) were inserted at least 80 mm into the rectum. Temperatures were read at 15 min intervals for a minimum of 6 h. After injection of LP/EP, temperatures were measured every 5 min for 3 h. The temperatures were monitored for at least 45 min prior to injection. After bacterial pyrogen administration, deviations of rectal temperature from baseline were tabulated. A fever index (FI) proportional to the area between the response curve and the baseline (with the response plotted so that 5 units on the ordinate represents a change of 1 °C and so that 2 units on the abscissa represent 1 h) was calculated for each response. Ten units of FI are, therefore, equivalent to a 1 °C change lasting 1 h. All experiments took place at an ambient temperature between 18 and 22 °C. No animal was febrile at the time of injection or infection.

Blood Biochemical Analysis

Plasma zinc and iron concentrations were determined by methods described

previously (Van Miert *et al.*, 1982). Blood samples were obtained from the goats before, and once daily (tick-borne fever) or 2 and 3 times daily (trypanosomiasis) after infection. In the other experiments samples were obtained before, and at 3, 6, 9, 12 and 24 h after the toxin was given. In the experiments with kids the number of samples (8 ml each) was limited: before, and at 3, 6 and 9 h (zinc, group A: n = 4), at 45 min, 2 and 4 h (zinc, group B: n = 5) and at 3, 6 and 9 h (iron, group C: n = 5) after, LP was given. Samples of blood were collected in vacutainer tubes (Venoject®; Terumo) by puncture of the jugular vein in goats and the tarsal vein in kids. The blood from these samples was heparinised (143 USP units sodium heparin per tube) and, after centrifugation, the plasma was stored at −20 °C until analysed for zinc and iron concentrations.

Toxins

The toxins used were purified lipopolysaccharide (LPS) prepared by phenol extraction from *E. coli* $O^{111}B^4$, by the method of Westphal (lot 614378, Difco Laboratories, Detroit, Michigan, U.S.A.) and purified staphylococcal enterotoxin B (lot 14/30, a gift from Dr W. R. Beisel, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland, U.S.A.), which has been used extensively in other studies (see review Beisel, 1976b). The toxins were dissolved in sterile, pyrogen-free saline solution to a concentration of 100 µg per ml. These stock solutions were diluted to the proper concentration immediately before administration; they were given by injection into the jugular vein, the tarsal vein or by the intramammary route.

Ehrlichia phagocytophila was isolated from sheep at Ameland, The Netherlands. The strain was transmitted i.v. to a splenectomized sheep with 150 ml of pooled EDTA blood from 23 donor sheep. Three days after infection blood smears from this febrile animal were positive. Blood samples were taken from the jugular vein (with heparin as an anticoagulant) and deep-frozen after addition of DMSO (to a final concentration of 9 per cent). In the present experiments goats were infected with *E. phagocytophila* by i.v. inoculation with 2 ml of the stabilate. During the course of infection, blood samples were examined by Giemsa-stained smears and the morphological criteria of Woldehiwet and Scott (1982).

Trypanosoma vivax Y58, a pathogenic strain of trypanosome isolated from cattle at Yakawada, Nigeria and described by Leeflang, Buys and Blotkamp (1976), was used in the present study. Goats were infected by i.v. inoculation with 10^6 parasites. *Trypanosoma congolense* S104 was isolated from a male Grant's gazelle in Northern Tanzania in 1966. Four years after its initial isolation, the strain was cyclically transmitted and deep frozen. After 1970, the stabilate was sub-passaged a few times in mice. During the test period, trypanosomes were determined in blood by wet blood films with EDTA as an anticoagulant.

Goat Leukocytic Pyrogen

Methods for maintaining glassware and solutions free of bacterial contamination, for isolation of peritoneal exudate cells and for preparing goat LP have been described earlier by Van Miert and Atmakusuma (1970). LP was given by injection into the tarsal vein of kids at a dose rate of 5 ml per kg, equivalent to 2×10^8 leukocytes per kg.

Statistical Analysis

Results were expressed as mean ± S.E.M. In comparisons made between 2 groups, an independent *t*-test was used. In values compared from the same animal between the baseline and later values, the paired *t*-test was used.

Drugs

Diminazene aceturate (Berenil®, Hoechst Farbwerke AG, Frankfurt, West

Germany) and oxytetracycline (Engemycine®, Gist-Brocades N.V., Delft, The Netherlands) were used as chemotherapeutic agents at the end of the experiments with infected animals.

RESULTS

The mean rectal temperature in the goats (n = 47) and the kids (n = 18) before they were infected or treated with pyrogens was 38·8 ± 0·05 °C and 39·1 ± 0·15 °C, respectively. The base lines of the plasma zinc and iron concentrations in goats were 9·44 ± 0·35 μmol per l (61·67 ± 2·3 μg per 100 ml) and 23·9 ± 0·85 μmol per l (133·44 ± 4·8 μg per 100 ml). In the kids these pre-administration values were 18·64 ± 0·77 μmol per l (121·85 ± 5·04 μg per 100 ml) and 26·6 ± 4·3 μmol per l (148·47 ± 24·05 μg per 100 ml).

Trypanosomiasis

Intermittent febrile reactions were observed 4 to 5 days after infection (Fig. 1), coinciding with peaks of parasitaemia. Increased concentrations of plasma zinc were found in one of the *T. congolense* infected goats, while no marked hypozincaemia was seen in the other animals (n = 7) despite high temperature peaks. Plasma concentrations of iron on the other hand tended to undergo some decline during febrile episodes (examples are given in Fig. 1). At the end of the experiments, all infected goats were treated with a trypanocide (diminazene aceturate 7 mg per kg i.m.).

Tick-borne Fever

Characteristic tick-borne fever inclusion bodies were found in the blood leukocytes at the 3rd or 4th day of infection, while blood samples were positive during 3 to 8 days (n = 6). After a temperature peak at the 3rd day of infection, there was an increase of rectal temperature which reached a plateau of 40 to 41 °C from the 4th day until the 6th to 9th day, when a rapid fall to normal body temperature occurred (Fig. 2). There was an abrupt and short-lasting decline in both plasma zinc and iron concentrations to only 37 per cent of normal (Fig. 2).

Two other goats were infected i.v. with *E. phagocytophila* and after 4 days, when the rectal temperature was 41 °C, 300 ml blood was obtained from the jugular vein in 5 IU heparin per ml blood. The heparinised blood was centrifuged immediately. The plasma obtained was passively transferred to 4 kids and assayed for EP/LEM activity. The dose of plasma was 15 ml per kg intravenously. The recipient kids showed only a small increase in rectal temperature, with maximum values (0·3 to 0·6 °C) after 40 to 45 min. No significant changes were observed in plasma zinc (16·75 ± 0·63 μmol per l) and plasma iron (23·67 ± 0·88 μmol per l) concentrations 4 h after injection of donor-plasma. However, at the 3rd day these kids showed febrile responses (40·5 to 41·9 °C), positive blood smears and decreased plasma zinc (10·0 ± 0·58 μmol per l) and iron (14·5 ± 2·9 μmol per l) values ($P < 0·01$). At

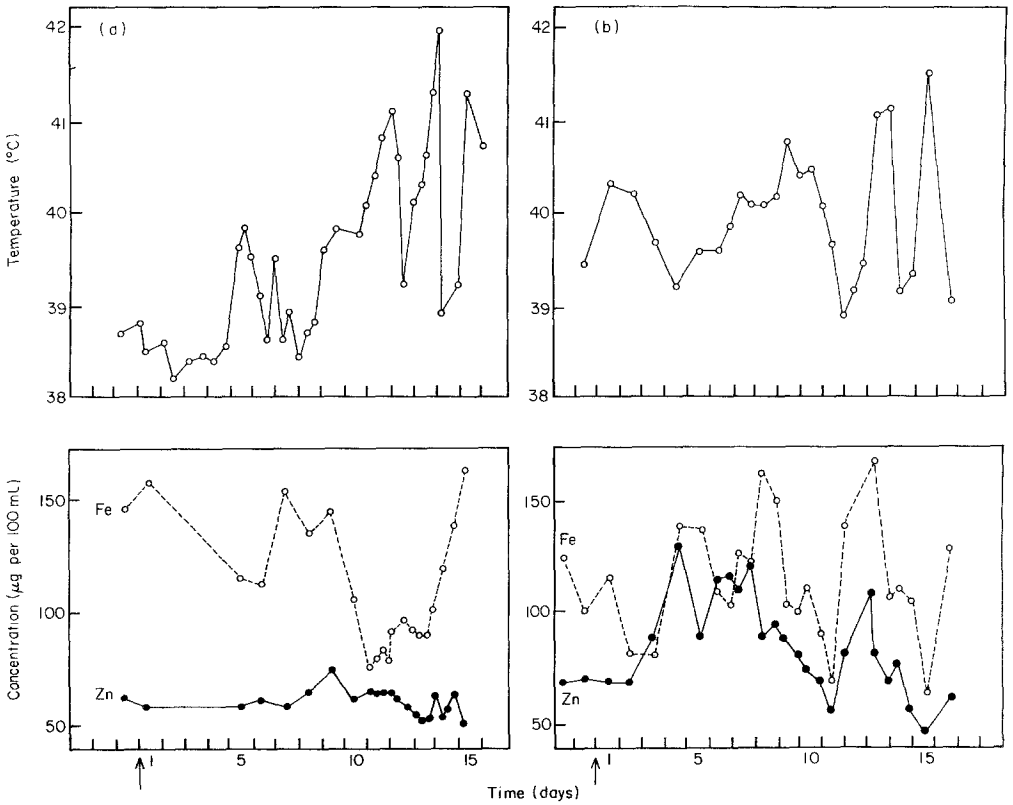

Fig. 1. Changes in rectal temperature (○——○) and plasma zinc (●——●) and iron (○- - -○) concentrations during the acute phase of trypanosomiasis after intravenous inoculation (↑) with 10^6 parasites of (a) *T. vivax*, (b) *T. congolense*.

the end of the experiments, the infected goats and kids were treated i.m. with oxytetracycline 20 mg per kg.

Responses to Staphylococcal Enterotoxin B

The i.v. injection of SEB in doses of 0·02 µg per kg (n = 5), 0·1 µg per kg (n = 4) and 2·0 µg per kg (n = 6) caused a variety of clinical effects, ranging from a pyrogenic response, malaise, salivation, grinding, groaning, with stasis of the forestomach contractions to severe diarrhoea, sometimes mixed with pseudomembranes and some blood. The onset of fever was accompanied by shivering which occurred 30 to 45 min after injection; a second period of shivering was seen after 120 to 150 min. Furthermore, and most remarkable, no relationship between the dose of SEB injected and the temperature response observed (FI) could be found (Table 1). There was an abrupt decline in the concentrations of trace metals. The lowest zinc concentrations were found 9 to 12 h after SEB was given, whereas the iron depression was greatest 12 h after toxin administration (Fig. 3). The data obtained indicate that a relationship

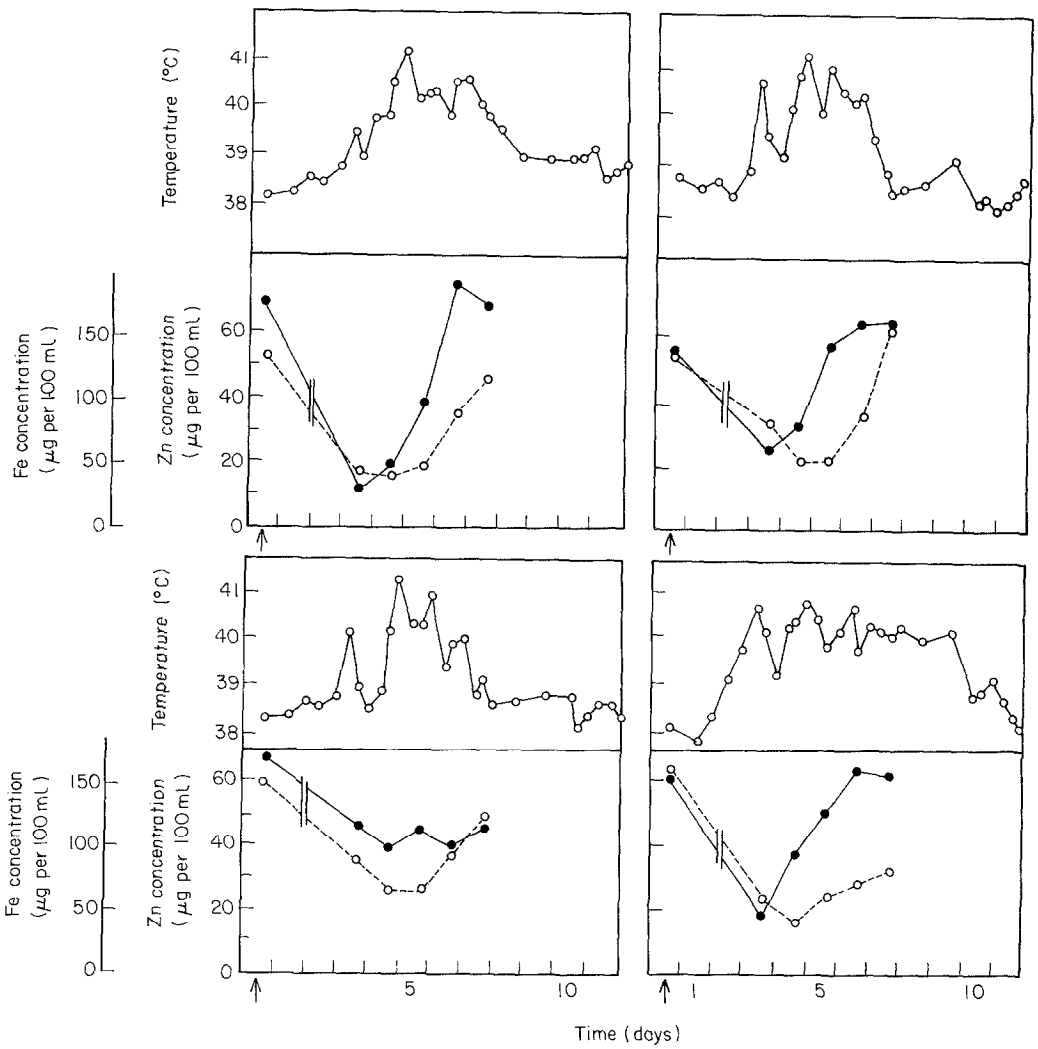

Fig. 2. Changes in rectal temperature (○——○) and plasma zinc (●——●) and iron (○- - -○) concentrations during the acute phase of tick-borne fever after intravenous inoculation (↑) with *Ehrlichia phagocytophila* (n = 4).

exists between the dose of toxin injected and the changes in concentrations of these trace metals (0·02 μg per kg and 2·0 μg per kg: $P < 0{\cdot}05$).

Intramammary infusion of SEB (1 mg ~ 30 to 35 μg per kg) was followed by a certain lag period before the onset of local signs of inflammation. Swelling and sensitivity to palpation developed in the glandular tissue within 6 h. Approximately 3 h after infusion, body temperature started to rise. Moreover, and most remarkable, the drop in plasma iron concentration developed more rapidly after intramammary administration than after i.v. injection, whereas the decline in plasma zinc concentration to intramammary SEB was more delayed (Fig. 4).

TABLE 1

FEVER AND CHANGES IN PLASMA ZINC AND IRON CONCENTRATIONS DUE TO *E. coli* LPS AND SEB

Pyrogen	*Dose (μg per kg) intravenously*	*Fever Index* (FI) (mean ± S.E.M.)*	*Mean maximal reduction in percent from baseline values*		*Number of goats*
			Plasma Zn	*Plasma Fe*	
SEB	0·02	30·8 ± 10·4	61·2 ± 10·7	68·0 ± 2·7	5
	0·1	42·9 ± 8·6	73·8 ± 2·4	81·5 ± 4·7	4
	2·0	40·3 ± 5·1	78·5 ± 1·5	84·6 ± 4·8	6
	30–35†	48·0 ± 7·1‡	68·8 ± 6·2	86·3 ± 2·0	4
E. coli LPS	30–35†	85·2 ± 7·5‡	59·1 ± 4·7	56·2 ± 7·6	5
	0·1	79·2 ± 9·6	77·8 ± 3·6	77·5 ± 2·1	6
	0·03 per h for 3 h	38·8 ± 3·7	55·6 ± 2·4	ND	5
	0.01 per h for 3 h	42·0 ± 6·0	40·2 ± 15·6	ND	3
Saline	0·2 ml per min for 3 h	−2·6 ± 4·1	5·1 ± 5·7	ND	4

*Fever Index (FI) for a 6 h period.
†One milligramme by intramammary route, equivalent to 30 to 35 μg per kg.
‡Fever Index (FI) for an 8 h period after administration into the udder.

Responses to Escherichia coli *Endotoxin*

The i.v. injection of *E. coli* LPS (0·1 μg per kg; n = 6) was followed by a certain lag period before the onset of an abrupt increase in rectal temperature. Shivering accompanied the onset of fever, occurring after approximately 25 min and lasting 30 to 40 min. A second period of shivering was observed after 100 to 115 min and lasted approximately 45 min. The febrile responses were characteristically biphasic, with peaks occurring at 75 (60 to 90) min and 190 (180 to 225) min and a rapid return to normal values (Fig. 3). Within 6 h after *E. coli* LPS was given, significant decreases were seen in both plasma zinc and iron concentrations, with the lowest values occurring after 9 to 12 h. The pattern of the initial changes in plasma concentrations of these trace metals was comparable with the pattern observed after SEB, although the effect of the latter upon zinc and iron was more persistent (Fig. 3).

Escherichia coli LPS given as an intravenous infusion at dose rates of 0·00017 μg per kg per min (n = 3) and 0·0005 μg per kg per min (n = 5) during 3 h caused similar but less marked effects upon body temperature and plasma zinc concentration (Table 1; $P < 0·01$). These data suggest that a relationship exists between the dose of *E. coli* LPS injected and the changes in plasma zinc values. The shapes of the temperature curves were different for *E. coli* LPS and SEB (Fig. 3). Moreover, the febrile reactions after *E. coli* LPS were more pronounced (Fig. 3, Table 1), which indicates that in goats SEB is not a potent pyrogenic agent.

When kids were injected i.v. with *E. coli* LPS 0·1 μg per kg, biphasic temperature responses were seen (n = 11), with significant decreases in plasma zinc (n = 6) and iron (n = 5) concentrations (Fig. 5). Shivering, which occurred after 20 to 30 min, was in most cases biphasic. The temperature curves showed a first peak after 1 h and a second and higher peak after 2·5 to 3 h. There were significant differences in heights and contours of the curves in kids and adult

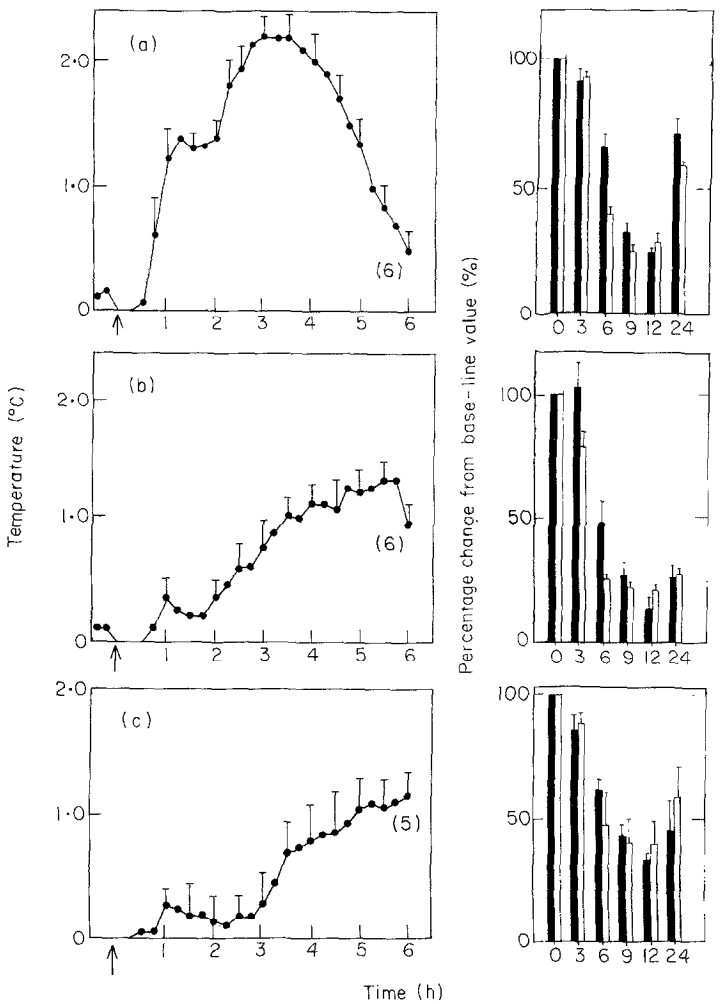

Fig. 3. The effect of *Escherichia coli* LPS and staphylococcal enterotoxin B (SEB) on rectal temperature and plasma zinc and iron concentrations. (a) *Escherichia coli* LPS 0·1 μg per kg i.v.; (b) SEB 2·0 μg per kg i.v.; (c) SEB 0·02 μg per kg i.v. ■ Plasma iron values, given as a percentage from base-line values (24·5 ± 1·8 μmol per l = 100 per cent). □ Plasma zinc values given as a percentage from base-line values (8·7 ± 0·5 μmol per l = 100 per cent). Number of goats given in parentheses. Values are given as mean ± S.E.M.

animals, respectively—especially after 3 h (Figs 3 and 5)—which explains the lower FI in kids (69·1 ± 9·2). Moreover, the magnitude of zinc and iron depression tended to be less in the kids.

The intramammary infusion of *E. coli* LPS (1 mg ~ 30 to 35 μg per kg) was followed by a certain lag period before the onset of local signs of inflammation. Approximately 1·5 h after infusion, the gland started to swell, becoming firm, warm to the touch and painful. There was shivering and, after approximately 2 h, rectal temperature started to rise, with peak values 5 to 6 h after infusion (Fig. 4). Plasma zinc and iron values gradually fell; the decrease in plasma iron concentration developed more rapidly in goats given SEB than in those given

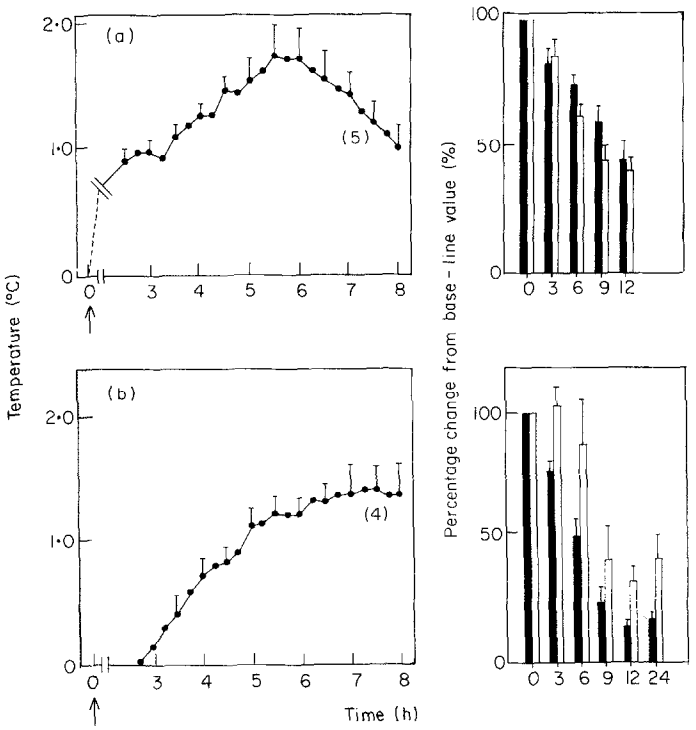

Fig. 4. The effect of *Escherichia coli* LPS and SEB on rectal temperature and plasma zinc and iron concentration. (a) *Escherichia coli* LPS 1 mg intra-mammarily per animal; (b) SEB 1 mg intra-mammarily per animal. Number of animals in parentheses. ■ Plasma iron values given as a percentage from base-line values (22·8 ± 0·7 μmol per l = 100 per cent). □ Plasma zinc values given as a percentage from baseline values (9·6 ± 0·3 μmol per l = 100 per cent).

E. coli LPS, whereas the decrease in plasma zinc concentrations in the former was more delayed (Fig. 4). Although the FI after intramammary infusion of *E. coli* LPS was significantly higher than in those given SEB (Table 1; $P<0{\cdot}01$), the magnitude of iron depression in plasma was significantly less after *E. coli* LPS (Table 1; $P<0{\cdot}01$).

Responses to Leukocytic Pyrogen

The i.v. injection of LP (5 ml per kg ~ 2×10^8 leukocytes per kg) in 3 groups of kids (A: n = 4, B: n = 5 and C: n = 5) evoked febrile responses characterized by a shorter lag period, a more rapid rise to peak height (at 25 to 30 min), a monophasic temperature curve and a quicker return to the baseline than those produced by *E. coli* LPS (Fig. 5). Intense shivering was observed after 9·7 ± 1·3 min and lasted 17·1 ± 1·9 min. Despite these characteristic febrile responses, no significant changes in plasma concentrations of trace metals were found (Fig. 5).

DISCUSSION

There is considerable evidence to suggest that fever due to inflammatory

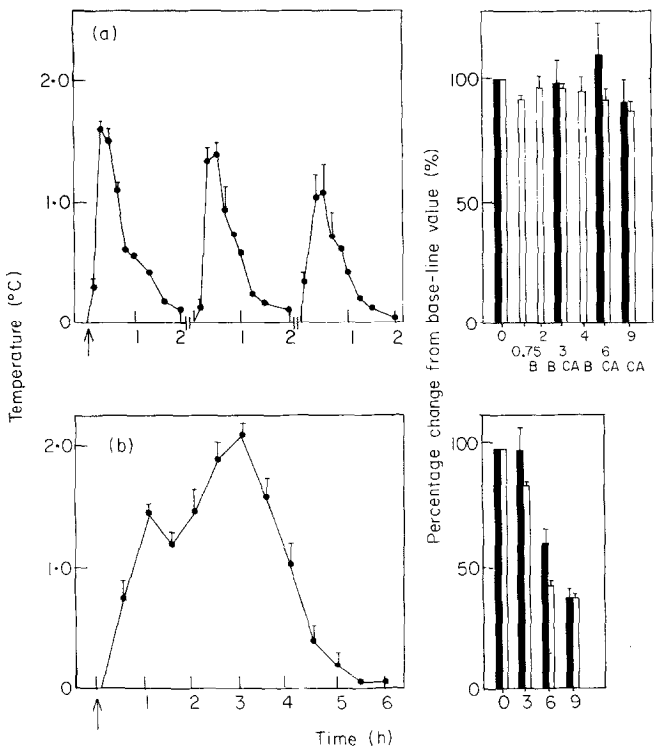

Fig. 5. The effect of (a) leukocytic pyrogen and (b) *Escherichia coli* LPS on rectal temperature and plasma zinc and iron concentrations in kids. The dose of LP = 5 ml per kg i.v. (equivalent to 2×10^8 leukocytes per kg). A: h = 4; B: h = 5; C: h = 5. The dose of *E. coli* = 0·1 μg per kg i.v. (rectal temperature h = 11; plasma zinc h = 6; plasma iron h = 5). ■ Plasma iron values given as a percentage from base-line values (26·6 ± 4·3 μmol per l = 100 per cent). □ Plasma zinc values given as a percentage from base-line values (18·6 ± 0·8 μmol per l = 100 per cent). Values are given as mean ± S.E.M.

diseases is mediated by circulating endogenous pyrogens. A pyrogen with similar properties can be produced in vitro by various cells in response to bacterial pyrogens, phagocytosis or appropriate antigenic challenge (Atkins and Bodel, 1979; Gander, 1982), and is known as leukocytic pyrogen (LP). Serum samples from patients with many different kinds of infection were shown to contain a substance characteristic of leukocytic endogenous mediator (Wannemacher, Pekarek, Klainer, Bartelloni, Dupont, Hornick and Beisel, 1975). Changes in iron and zinc concentrations during fever are thought to be mediated by this protein (LEM), which causes a redistribution of these trace metals within the body (Beisel, 1976a, 1977). The ascribed similarities and differences between LP and LEM have not provided a definitive answer to the question of whether these molecules are identical (Mapes and Sobocinski, 1977; Kampschmidt *et al.*, 1978). This report presents experimental data which support the theory that febrile reactions and the changes in plasma zinc and iron levels are mediated by different proteins. In the present study different fever models were compared using bacterial pyrogens (SEB and *E. coli* LPS), endogenous pyrogen (LP) and pathogenic organisms such as *Trypanosoma vivax*, *T. congolense* and *Ehrlichia phagocytophila*.

Infections caused by *E. phagocytophila* are characterized by higher fever assoeiated with leukopenia, which is due first to a decrease in the number of circulating lymphocytes and later (7 days after inoculation) to a neutropenia (Batungbacal, Scott and Burrells, 1982). In the early stage of *T. vivax* infection, white blood cells, especially neutrophil leukocytes, show a short-lasting decline but once the disease is established (10 days after inoculation) white blood cells gradually increase due to an increase in the number of circulating lymphocytes, although neutropenia remains a feature (Mulligan, 1970). In goats, *E. coli* LPS causes fever and a short-lasting neutrophilic leukopenia, which is followed, at the 12th and 24th hour by a significant increase in the number of circulating neutrophil leukocytes; a long-lasting lymphopenia can also be observed, causing significant changes in the lymphocyte: neutrophil ratios from 3 to 12 h after dosing (Van Miert *et al.*, 1982). SEB induces fever and a longer-lasting decrease in the number of white blood cells caused by a decrease in circulating lymphocytes and neutrophils (Beisel, 1976b).

Circulating EP has repeatedly been demonstrated in plasma from goats (Van Miert and Atmakusuma, 1970; Verheijden, Van Miert and Van Duin, 1983) and other species (Clark and Cantu, 1971; Gander, 1982) during fever induced by intravenous injection of bacterial pyrogens. However, attempts to demonstrate EP in goats during marked febrile responses due to intravenous infusion of *E. coli* LPS (0·05 μg per kg per hour during 2 h) were not successful, although the same bioassay technique was used (Verheijden *et al.*, 1983). In the present experiments, circulating EP/LEM could not be detected in plasma from febrile goats infected with *E. phagocytophila*. The most likely explanation for the discrepancy in these results involves the hypothesis of a low but constant release of EP/LEM into plasma from infected animals (or after infusion of *E. coli* LPS) in contrast to peak concentrations of EP/LEM after a single injection of bacterial pyrogens. The value of the bioassay technique used is limited due to volume limitations of the dose to be administered. Moreover, EP is cleared rapidly from the blood (Dinarello, Weiner and Wolff, 1978; Townsend and Cranston, 1979). This explains why circulating EP has yet to be convincingly demonstrated during febrile diseases (Dinarello, Renfer and Wolff, 1977).

Contrary to the previously documented data for neonatal and adult guinea pigs (Blatteis *et al.* 1981), kids have significantly higher plasma zinc concentrations than adult goats ($P < 0{\cdot}001$). The intravenous injection of LP in these kids caused characteristic febrile reactions, whereas no significant changes in plasma zinc and iron concentrations were found. When these kids were injected with *E. coli* LPS, biphasic febrile reactions were observed accompanied by significant decreases in plasma zinc and iron concentrations (Fig. 5). While fever could not be evoked in neonatal guinea pigs by endotoxin injection, their plasma zinc and iron were lowered significantly (Blatteis *et al.*, 1981). Hence, the coupling of fever and plasma trace metal changes which usually occurs would not seem to be an absolute one. Moreover, no marked hypozincaemia was seen during the course of *T. vivax* infection, despite high temperature peaks, while plasma iron concentrations tended to undergo some decline. In goats infected with *E. phagocytophila* there was a marked decline in plasma zinc

to low values on the 3rd day; plasma concentrations then rose to become as high as those in the baseline pre-infection period, although the febrile reactions in these animals persisted. The decrease in plasma iron concentrations was more persistent in these goats. These results show that plasma zinc and iron do not behave in the same way.

After injection of *E. coli* LPS, kids showed febrile curves of the type seen in the adult goats, although the young animals cooled off more rapidly (Fig. 5). The rate of heat loss depends on the surface area which is proportional to two-thirds of the body weight, while heat capacity is proportional to the body weight itself (Kleiber, 1961). The ratio of the calculated surface area between the kids and the adult goats was 1 to 5, while the body weight of the kids was approximately 12 per cent of the adult weight. It is likely, therefore, that the difference in ratio of surface to weight may have been responsible for the differences in fever index in response to 0·1 μg per kg *E. coli* LPS (69·1 ± 9·2 compared with 79·2 ± 9·6).

Intravenous injection of *E. coli* LPS or SEB induced fever and lowering of plasma zinc and iron concentrations in adult goats. The decrease in these trace metal values was more persistent in goats given SEB than in those given *E. coli* LPS. No clear relationship could be found between the FI and the alterations in zinc and iron values (Fig. 3; Table 1). However, the data obtained may indicate that a relationship exists between the dose of bacterial pyrogen injected and the changes in plasma zinc and iron values (Table 1). In other species, pyrogenic doses of endotoxin lead to significantly depressed plasma zinc and iron values with lowest concentrations being observed 9 to 12 h after administration (Beisel, 1976a, 1977; Verheijden *et al.*, 1982). In these studies the magnitude of depression of these trace metals had linear relationships with the logarithm of the dose of endotoxin administered. In man and other species, plasma zinc values decline as rapidly as those of plasma iron, although the magnitude of depression in plasma zinc is not generally as great inasmuch as plasma zinc concentrations fall to approximately 70 per cent of normal values, whereas plasma iron values typically decrease by 50 per cent or more (Beisel, 1976a). Contrary to these observations, the present results obtained in goats after intravenous administration of SEB and *E. coli* LPS show no significant differences in the magnitude of depression in plasma zinc and iron concentrations.

After intramammary infusion of SEB or *E. coli* LPS, fever and significant decreases in plasma zinc and iron concentrations were observed (Fig. 4). Again, no clear relationship was found between the temperature responses (Fever Index) and the alterations in plasma zinc and iron values (Table 1). Moreover, after infusion of SEB, the depression of zinc was less pronounced than in those given *E. coli* LPS. In a previous study we found that pretreatment with flurbiprofen, a potent non-steroidal anti-inflammatory and antipyretic agent, completely abolished the febrile reactions to *E. coli* LPS in goats. However, the drug failed to modify the endotoxin-induced decrease in plasma zinc concentrations, whereas the decline in plasma iron concentrations was delayed (Van Miert *et al.*, 1982). Thus, all these data support the theory that (1) plasma zinc and iron concentrations are regulated by different mechanisms

and (2) febrile reactions and the changes in plasma trace metals are mediated by different proteins.

SUMMARY

In goats with trypanosomiasis (*T. vivax* or *T. congolense*) no marked fall in plasma zinc concentration was seen despite high temperature peaks, whereas plasma concentrations of iron tended to undergo some decline. In goats infected with *Ehrlichia phagocytophila*, there was a marked decline in plasma zinc and iron to low values on the 3rd and 4th day, respectively. Circulating endogenous pyrogen (EP) or leukocytic endogenous mediator (LEM) could not be detected in plasma from febrile goats with tick-borne fever. The intravenous injection of leukocytic pyrogen (LP) in kids caused characteristic monophasic febrile reactions, whereas no significant changes in plasma trace metals were found. So, previous evidence purporting to show that LP is similar to or may be identical with LEM is demonstrably inconclusive. Intravenous injection of *E. coli* lipopolysaccharide (LPS) or staphylococcal enterotoxin B (SEB) induced fever and lowering of plasma zinc and iron concentrations. The decrease in those trace metal values was more persistent in goats given SEB than in those given *E. coli* LPS. After intramammary infusion of SEB or *E. coli* LPS, fever and significant decreases in plasma zinc and iron concentrations were observed but no clear relationship was found between the temperature responses and the alterations in plasma trace metal concentrations. Furthermore, the decrease in plasma iron concentration developed more rapidly in goats given SEB than in those given *E. coli* LPS, whereas the decrease in plasma zinc concentrations in the former was more delayed. These data support the theory that the concentrations of zinc and iron in plasma are regulated by different mechanisms, whereas febrile reactions are mediated by another type of endogenous protein.

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