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STEREOTYPIC RESPONSES TO INFECTION AND INFLAMMATION
Probable Activation of Phagocytic Cells[1,2]

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Abstract—There is a sequence of stereotypic metabolic changes in rats subjected to inflammation or infection. In each stress, rats respond with increases in body temperature and plasma insulin and with decreases in plasma zinc, ketone bodies, and free fatty acids. The data suggest that infection or inflammation causes an activation of phagocytic cells and also that leukocytic endogenous mediator, when injected into rats, causes some of the same alterations. Rats doubly vagotomized respond to infection in the same fashion as intact rats.

INTRODUCTION

It is proposed that there is a sequence of stereotypic metabolic changes which accompany both inflammation and infection. The data presented attempt to describe the sequence of metabolic events occurring during infection and/or inflammation so that information can be obtained which might aid in the supportive management of disease.

Neufeld et al. (1, 2) have described host metabolic alterations during Venezuelan equine encephalitis and bacterial infections in rats starved throughout the study. Among the alterations brought about by these infections were: elevated body temperature and plasma insulin and depressed plasma zinc, free fatty acids, and ketone bodies.

In the present study phagocytic cells were activated by infection with a gram-positive organism, *Streptococcus pneumoniae*, an endotoxin con-

[1]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[2]The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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taining gram-negative organism, *Salmonella typhimurium;* a gram-negative organism in which endotoxin is minimal, *Francisella tularensis;* an induced sterile turpentine abscess; and administration of commercially standardized endotoxin. In all studies the following parameters were measured in starved rats at selected time intervals: rectal temperatures and plasma concentrations of zinc, ketone bodies, free fatty acids, and insulin. Rats were starved during both control and test experiments in an attempt to control the variable degree of anorexia which normally accompanies such infections or inflammations. Leukocytic endogenous mediator, described by Pekarek et al. (3), initiates many of the responses observed during inflammation and/or infection. Also, the effect of infection was studied on the same parameters in doubly vagotomized rats in order to determine whether or not the parasympathetic nervous system plays a role in the stereotypic effects of infection.

MATERIALS AND METHODS

Male Fisher-Dunning rats, weighing approximately 175–200 g, were purchased from Microbiological Associates (Walkersville, Maryland). The rats were maintained in light- and temperature-controlled rooms [12 h light (0600–1800 h) and 12 h dark, 25° ± 1°C] and fed Purina pellets (Ralston Purina Co., St. Louis, Missouri) ad libitum until initiation of the experiment. Twenty-four hours prior to initiating infection or inflammation, food was removed.

Rats were infected with *S. pneumoniae*, 10^4 colony-forming units (CFU), subcutaneously, as described by Neufeld et al. (2); with *F. tularensis*, 10^7 CFU, intraperitoneally, as described by Powanda et al. (4); or *S. typhimurium*, 10^8 CFU, intraperitoneally as described by Wannemacher et al. (5). Sterile turpentine abscesses were produced by the subcutaneous injection of 1 ml of turpentine (Phipps Corp., Boston, Massachusetts). Endotoxin stress was induced by the intraperitoneal injection of 1 mg of *Escherichia coli* lipopolysaccharide B (Difco Laboratories, Detroit, Michigan). Similarly, starved control rats received either an equal number of heat-killed organisms or, in studies of the effect of endotoxin or turpentine, an equal volume of saline. The number of bacterial cells and the amount of endotoxin and turpentine administered were selected to produce sequences of metabolic responses within approximately similar time limits.

For the first 24 h rats were killed every 3 h and blood was obtained from the pleural cavity after severing the vena cava. For the next 24 h, samples were collected every 12 h. Ketone bodies were determined as described by Neufeld et al. (2), free fatty acids according to the method of Dalton and Kowalski (6), zinc by the method of Pekarek et al. (7), and insulin by the method of Muller et al. (9).

Leukocytic endogenous mediator (LEM) was prepared and administered to rats intraperitoneally, as described by Pekarek et al. (3). Six hours after the administration of 1.0 ml of crude LEM, rats were killed and plasma was separated. Statistical significance was determined by one-way analysis of variance of data collected at all time periods. A P value of 0.05 was considered significant under the null hypothesis. Bilateral vagotomy was performed as described by McNamee and Vaughn (9).

RESULTS

The effect of four of the inflammatory states on rectal temperature is shown in Figure 1. Elevation of body temperature occurred as early as 3 h postinoculation in the studies using turpentine, somewhat later for endotoxin and *F. tularensis*, and as late as 12 h for *S. pneumoniae*.

With the exception of infection with *S. pneumoniae*, depression of plasma zinc was significant at 6 h postinoculation (Figure 2). Significant depression in the concentration of plasma zinc following infection with *S. pneumoniae* did not occur until 12–15 h postinoculation. Figure 3 shows the effect of the various stresses on plasma ketone body concentration. By 15 h postinoculation, all caused a significant depression in the concentration of plasma ketone bodies, in contrast to the increase noted in the control rats starved for an equal length of time.

Generally the elevation in plasma insulin (Figure 4) coincided or preceded the depression of plasma ketone bodies for all of the inflammatory stresses. These changes were accompanied by a depression in the concentration of plasma free fatty acids (Figure 5), but for most stresses this occurred later in the inflammatory process (15–48 h).

Figure 6 shows that when rats were inoculated with *S. typhimurium*, there was fever and depression of plasma ketone bodies both in the rats

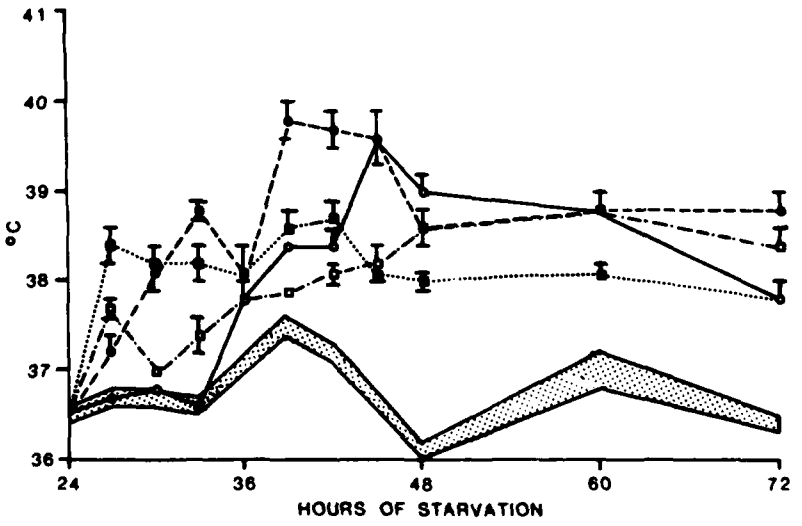

Fig. 1. The effect of stereotypic stress on rectal temperature. (○) 10^4 CFU *S. pneumoniae*, subcutaneously; (●) 10^7 CFU *F. tularensis*, intraperitoneally; (■) 1 ml turpentine, subcutaneously; (□) 1 mg *E. coli* endotoxin, intraperitoneally; (dotted band) controls (either heatkilled *S. pneumoniae* or *F. tularensis*, or saline for turpentine and endotoxin).

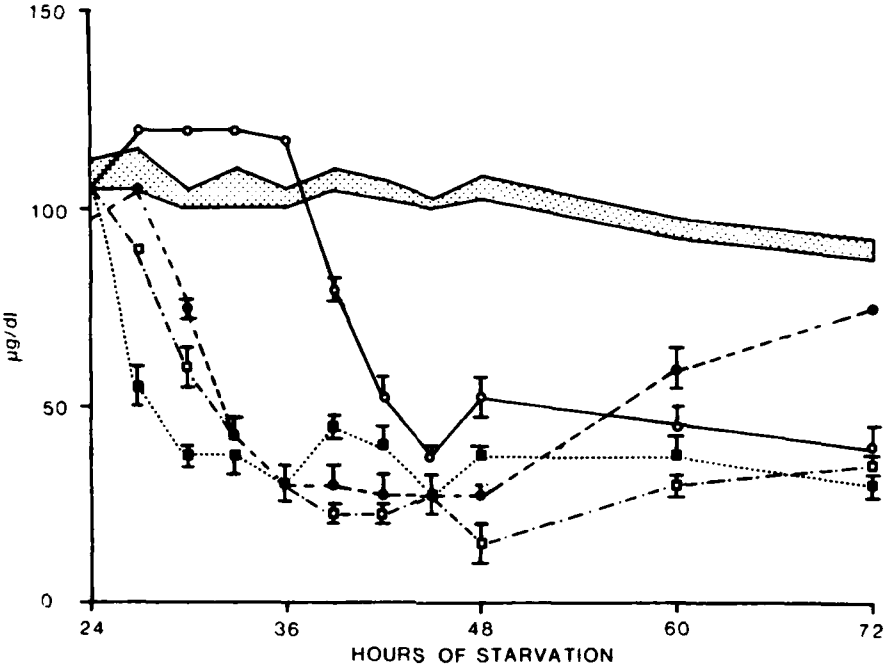

Fig. 2. *Effect of stereotypic stress on plasma zinc.*

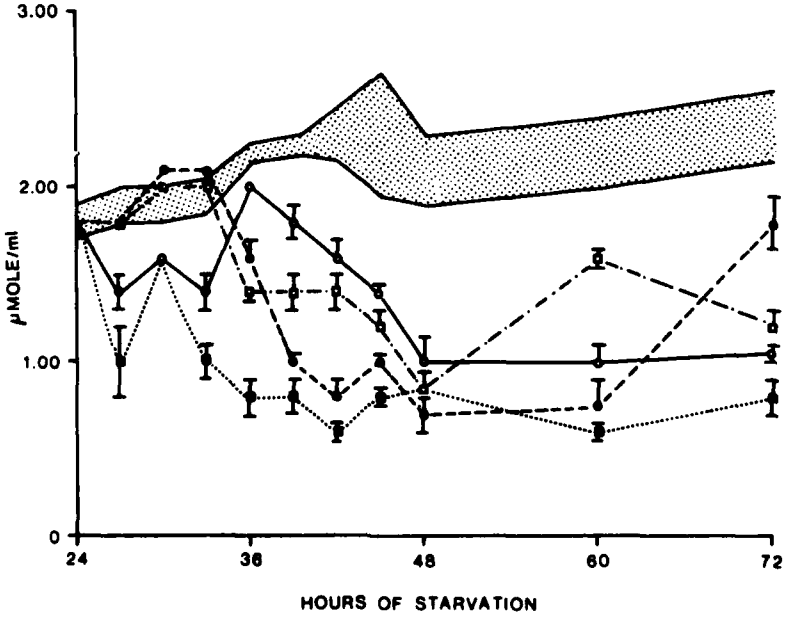

Fig. 3. Effect of stereotypic stress on plasma ketone bodies.

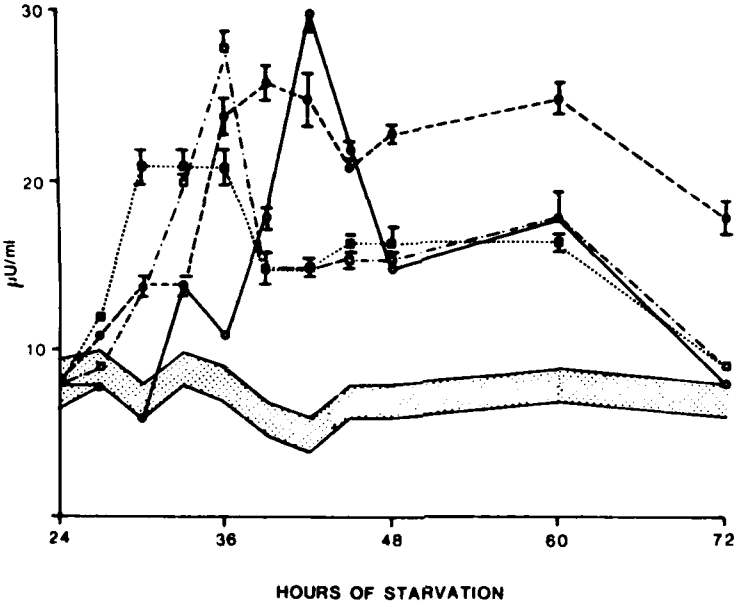

Fig. 4. Effect of stereotypic stress on plasma insulin.

challenged with viable organisms and heat-killed organisms. The early responses in each group can be attributed to the endotoxin contained in this particular organism. In the rats administered viable organisms, fever declined at 15 h but reappeared later as the organism multiplied. Plasma ketones were depressed early but increased to high levels later (30 h) in the rats which received heat-killed *S. typhimurium*. Ketone bodies in the rats which received the viable organisms remained depressed.

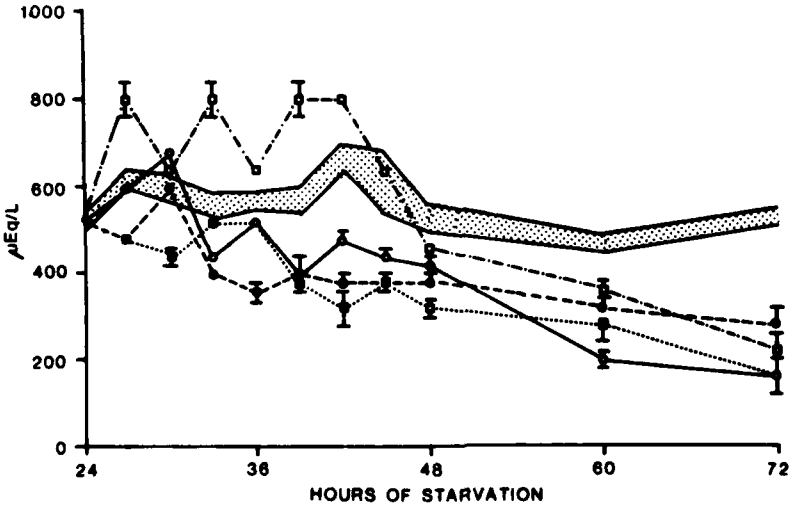

Fig. 5. Effect of stereotypic stress on plasma free fatty acids.

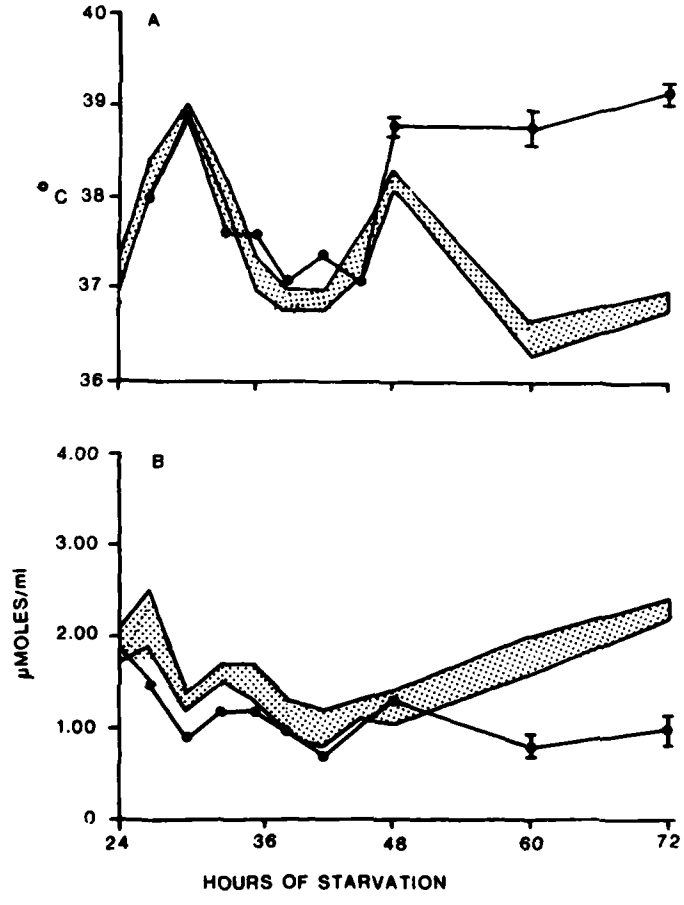

Fig. 6. Effect of *S. typhimurium* infection on rectal temperature and plasma ketones.

In most of the data presented, the time of appearance of the variations in the measured parameters was delayed in the rats infected with *S. pneumoniae*. This phenomenon is apparently due to the smaller relative dose of the original inoculation. Figure 7 shows the time of temperature elevation and ketone body depression with three different doses of *S. pneumoniae* (10^2–10^4 CFU/rat).

Pekarek et al. (3) described a factor called leukocytic endogenous mediator (LEM) which, when injected into the intraperitoneal cavity of rats, caused some of the same changes as those which have been described for the infectious or inflammatory agents. Data from rats injected with LEM are shown in Figure 8.

To partially settle the question as to whether the relationship between high insulin and depressed ketone body concentration in infected or in-

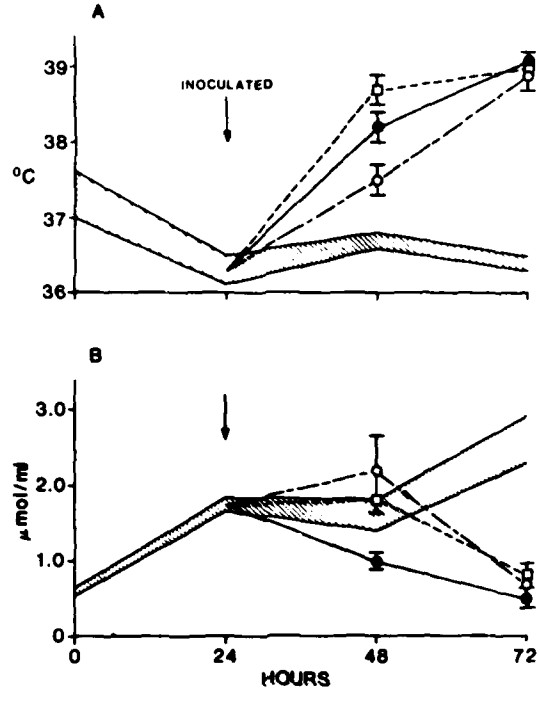

Fig. 7. Effect of graded doses of *S. pneumoniae* infection on rectal temperature and plasma ketones. (●) 10^4 CFU; (○) 10^3 CFU; (□) 10^2 CFU; (dotted band) controls.

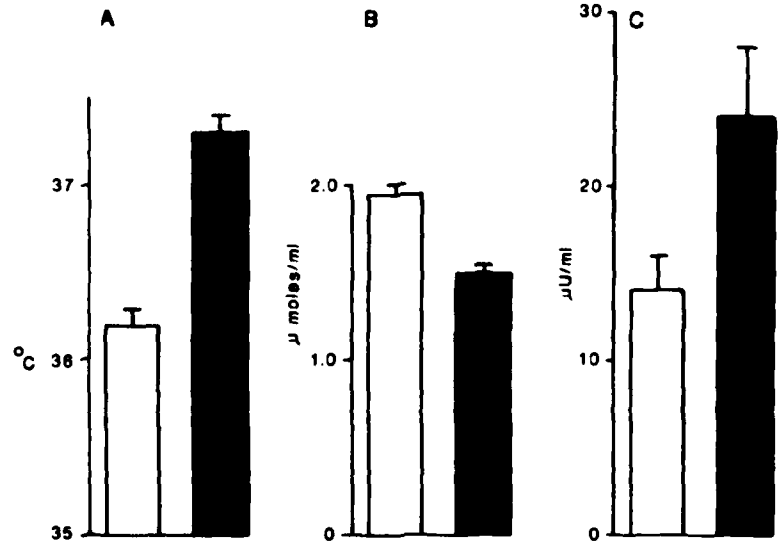

Fig. 8. Effect of crude LEM on body temperature (A), plasma ketone bodies (B), and plasma insulin (C) 6 h after administration. (□) 1 ml LEM, heat-inactivated; (■) 1 ml LEM.

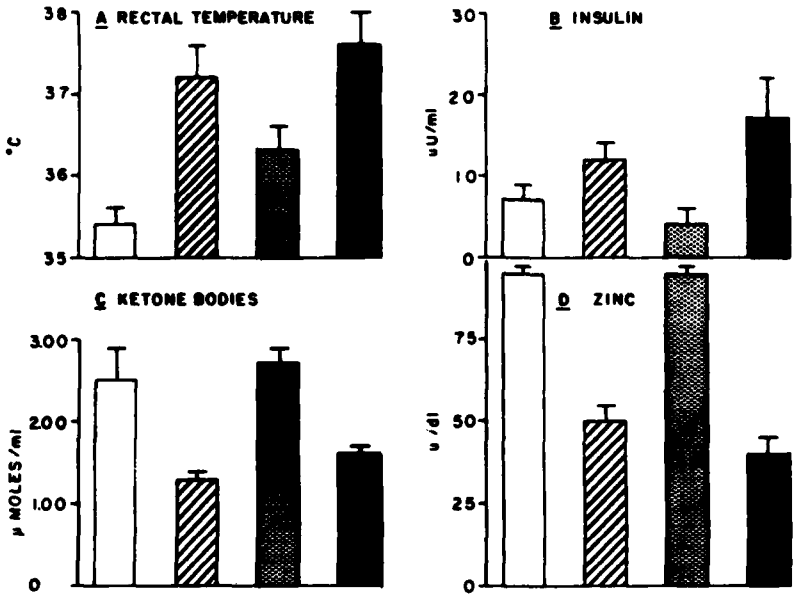

Fig. 9. Effect of vagotomy and sham vagotomy in infected and control rats on temperature, plasma insulin, ketones, and zinc. Rats infected with 10^7 cells, *F. tularensis*, LVS Strain, intraperitoneally. (A) Rectal temperature; (B) plasma insulin; (C) plasma ketones; (D) plasma zinc; (□) vagotomized, uninfected; (■) vagotomized, infected; (■) sham-operated, uninfected; (■) sham-operated, infected.

flamed rats was under neural control, rats which were doubly vagotomized were infected with *F. tularensis*. The data in Figure 9 show the elevation in rectal temperature and insulin and the depression in plasma ketone bodies and zinc seen with infected intact rats.

DISCUSSION

The appearance of an infectious or inflammatory stress in a host is marked by characteristic metabolic changes. To illustrate that host response is largely independent of the cause of inflammatory stress, four stresses were imposed upon rats and five responses were measured over a 48-h period.

Elevated body temperature, insulin elevation, and ketone body depression appeared to be related phenomena. The hypophysis may be involved in the effect of inflammation on these parameters since it had been shown by Neufeld et al. (10) that, when hypophysectomized rats were infected with *S. pneumoniae*, there was no alteration of body temperature, no depression in plasma ketones, and no elevation of plasma insulin. The uninfected hypophysectomized rat is characterized by low body temperatures, very low

insulin, and high plasma ketones in the fasted state. Of the parameters discussed in this paper, when the infected hypophysectomized rat was studied during infection, only plasma zinc responded as in the intact rat.

The similar responses evoked by these infectious and noninfectious inflammatory stresses suggest that these physiological alterations may be set into motion by common pathways. The elevation in plasma and portal insulin described by Neufeld et al. (11) and Kaminski et al. (12) during the anorectic state may represent an important step in the process, particularly since, with insulin being either absent or very low, in both the diabetic and the hypophysectomized rat (11), there was no depression in plasma ketones during the inflamed state.

Although elevated plasma insulin, depressed plasma ketone bodies, and depressed plasma zinc seem to be clear indications of inflammatory stress, they do not appear to be instigated by the same physiological mechanisms.

Work by Pekarek et al. (3) has shown that inflammatory stress results in the release of a factor from leukocytes called LEM, while Turchik and Bornstein (13) and Kampschmidt et al. (14) refer to this factor as endogenous or leukocytic pyrogen (EP). Two of the more identifiable responses to EP/LEM are the production of fever and a reduction in plasma zinc. Data presented in this paper show that LEM, in a 6-h period, also causes a depression in plasma ketone bodies and an elevation in plasma insulin.

These data suggest that there may be common causes in the inception of the stereotypic responses to the infections and inflammatory stresses tested and that LEM might be a candidate initiator for some of them. The fact that the response in infected vagotomized rats is essentially the same as in infected intact rats indicates that the parasympathetic nervous system is not directly involved.

The data now suggest that inflammatory stress evokes the interplay of several distinct responses. One of these appears to involve some signal which calls into play the hypophysis and the pancreas resulting in elevated plasma insulin and depressed plasma ketones. Another response results in depressed plasma zinc; this appears to be distinct from the mechanism controlling elevated insulin and depressed ketones because it occurs in hypophysectomized rats when the insulin, ketone, and fever effects are absent.

The nature of the proposed relationship between the hypophysis and the pancreas remains uncertain. All of the data obtained so far show a close relationship between high insulin and low plasma ketones. Moreover, the data obtained by Neufeld et al. (11) suggested that the pancreas during infection seems to be influenced by a properly functioning pituitary gland. Therefore, since the alterations in certain key metabolic parameters seem to be common to inflammatory stress, we suggest that common supportive therapy may be feasible.

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