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THE RELATIONSHIP BETWEEN ESSENTIAL FATTY ACIDS AND FEVER

Eva BENEDICT-KENEDI

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ABSTRACT

In this thesis the role of essential fatty acids (EFAs) in thermoregulation and the polyunsaturates (PUFA) in the genesis of fever is investigated. Although recognised, that metabolites of arachidonic acid are involved in the biochemical sequences leading to fever, it is also acknowledged that fever response depends on lipid mobilisation. However, the exact biochemical mechanisms involved in this event remain unknown to date. In order to investigate a relation between serum lipids and fever, rabbits were subjected to dietary manipulation (deficient, or excessive EFA diet) and their hyperthermic responses to intravenous injections of (a) human leucocyte pyrogen (HLP); (b) endotoxin (Salmonella Thyphosa); and (c) cerebroventricular injections of prostaglandin E_2, were compared with rabbits fed on a normal diet. In addition, serum triglyceride (TG), cholesterol (CHO) were measured, and serum and brain lecithin fatty acids analysed, before and during fever. Data shows that during endotoxin induced fever, serum TG, and CHO concentration (mm/l) increases parallel to the body temperature (^{o}C). The lack or excessive intake of dietary EFA affects serum and brain PUFA content. This, in turn, influence not only the febrile response to HLP or endotoxin, but also the temperature of the body. Thus, a lack of EFA led to hypothermia and an excess resulted in hyperthermia. Both events reduced the effect that fever has on the body temperature and tolerance to the febrile action of pyrogen occurred.

LIST OF ABBREVIATIONS

CHO	= Cholesterol
$CHCl_3$	= Chloroform
CO_2	= Carbon dioxide
EFA	= Essential fatty acid
FFA	= Free fatty acid
GC	= Gas chromatograph
HLP	= Human leucocytic pyrogen
IL-1	= Interleukin 1
ICV	= Intracerebroventricular
IV	= Intravenous
KDO	= Keto-3-deoxy octeonic acid
LPS	= Lipopolysaccharide
MeOH	= Methanol
Na_2SO_4	= Sodium Sulphate
ng	= nanogram
O_2	= Oxygen
PGE_1	= Prostaglandin E_1
PGE_2	= Prostaglandin E_2
PC	= Phosphotadylcholine
PE	= Phosphotadylethanolamine
PL	= Phospholipid
PUFA	= Polyunsaturated fatty acid
R.Q.	= Respiratory Quotient
TG	= Triglyceride
TLC	= Thin-layer chromatograph
VLDL	= Very low density lipoprotein
ul	= micro-liter

LIST OF ABREVIATIONS

18:1(w9)	= Oleic acid
18:2(w6)	= Linoleic acid
18:3(w3)	= Linolenic acid
18:3(6)	= Dihomo- - linolenic acid
20:3(w9)	= Eicosatetraeonic acid
20:4(w6)	= Arachidonic acid
20:5(w3)	= Pentaeonic acid

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Their commitment to serving humanity through scientific research became the guiding light on my road to the discovery of "The Role of Essential Fatty Acids in Thermoregulation and in Fever", and I humbly dedicate this thesis to their memory, in hope that it will contribute to the ideal of science in service of mankind.

DECLARATION

This thesis is the original work of Eva Benedict-Kenedi. Neither the substance or any part of this thesis has been submitted for any degree in any other university.

This dissertation has the approval of the Animal Ethics Committee and the number of certificate of approval is 85/27

Eva Benedict-Kenedi

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Is Chronic Imbalance in Polyunsaturates A Commune Feature of Hyperthermia in Rabbits?. 1989. S.A.I.M.R. Anniversery Congress.

IN THIS THESIS THE COMPLETE WORK ON THE ABOVE ABSTRACT ARE PRESENTED IN THE FOLLOWING ORDER.

The Role of Arachidonic Acid in Fever

Plasma Lipids Involvement in the Genesis of Fever.

The Role of Linolenic Acid in the Genesis of Fever

Chronic Mobilisation of Eicosonoids: A Common Feature of Hyperthermia in Rabbits.

1.0 INTRODUCTION

In this thesis "positive" thermal response is considered to be " fever" when the body temperature of rabbits exceeds the normal resting range by 0,6 ^{o}C within 60 minutes after intravenous injection of a pyrogen. A "negative" thermal response is considered to be "tolerance" when this increase cannot be met during the same period of time (as described above), even with repeated doses of pyrogen. The thesis will highlight the fact that unresponsiveness to pyrogen is dependent on the body temperature of the host. The term "hyperthermia" will be applied only when the higher temperature attained is maintained (6-7 hours) beyond the normal range, after injection of pyrogen. The average body temperature of the rabbit is 38,4±0,6^{o}C, and a drop to below baseline level is considered "hypothermia" if this occurs independently of environmental factors or intervention with pyrogen. The thesis will take into account several factors which have not been considered to date, i.e. (i) a shift in the serum lecithin fatty acids configuration during exogenous pyrogen fever, (ii) the dependence on w6 and w3 fatty acids in the maintaining of stable body temperature, and (iii) the role of triglyceride and cholesterol in the onset, peak and duration of endotoxin fever. Collectively, the inter-dependence of host-fuel-agent, in the genesis of fever will be the main object of this work.

To date, little is known concerning the effects of infectious disease induced by Gram-negative bacteria on body lipids, even though it has been recognised that malnourished patients are more prone to bacterial infection than normal, healthy individuals (Hess,1932; Scrimshaw, Kligler, Guggenheim and Herrnheiser, 1946; Schrimshaw, Taylor and Gordon, 1968; Brenton, Brown and Warton, 1967; Brook 1972). It is also accepted that excessive dietary intake of fat or the presence of obesity may be similarly deleterious. High-fat diets have been shown to depress the resistance to tuberculosis in rats (Hedgecock, 1948; Castello, Hedgecock and Hamilton, 1962) and chickens (Solotorovsky et al., 1961), and to lower resistance to malaria infection in rats (Ramakrishman, 1954). Obesity accompanies a marked increase in mortality due to distemper virus infection of dogs (Newberne , 1966). The exact mechanism whereby a deficiency or excess of dietary or tissue fat serves to impair host resistance has yet to be determined (Biesel and Fiser 1970). According to Biesel and Fiser (1970)"fever reduces dietary intake and the stress of discomforting symptoms occurs consistently during generalised infectious illness, regardless of the cause"(Table 1). The mere fact that changes in serum lipids vary widely from infection to infection in man (Nishishita, 1941; Gallin, Kay and O'Leary, 1969),

TABLE I

Basic mechanisms leading to observed effects of infection on lipid metabolism of the host

A. Effects associated with the presence of invading microorganism
   1. Direct effects
      a. Utilization of host lipids required by replicating microorganisms
      b. Disruption of host cell metabolism by intracellular microorganisms
      c. Localized destruction of fat cells at sites of an infectious process
   2. Indirect effects
      a. Alterations in host lipid metabolism caused by bacterial exotoxins, endotoxins, or enzymes
      b. Activation of lipase and other lysosomal enzymes within host phagocytes
      c. Release of mediator substances from host cells, i.e., endogenous pyrogen, interferon
B. Effects secondary to development of generalized illness due to infection
   1. Decreased dietary intake of fats
   2. Minimal interference with intestinal absorption of fats
   3. Altered lipid metabolism within host cells
      a. Altered rates of hormone-mediated lipolysis within fat depots to supply increased metabolic demands
      b. Altered rates of lipid synthesis within the liver
      c. Altered rates of fat utilization by peripheral tissues
      d. Changes related to localization of infection within specific tissues, i.e., liver, pancreas, brain
   4. Alterations in lipid transport
      a. Changing concentrations of serum lipids and their transport proteins
      b. Altered activities of nonhormone-mediated lipases
   5. Participation of specialized lipids in the coagulation mechanism or in inflammatory and allergic responses
   6. Ill-defined effects related to the prior nutritional status of the host
   7. Terminal effects associated with shock and overwhelming septicemia

in rabbits (Hirsh et al., 1964; Farshtchi and Vester 1968; Kaufman Matson and Biesel,1976) or in monkeys (Fiser, Denniston and Biesel, 1972), implies that microorganism-related specificity exists within the body for the initiation or control of tissue and serum lipid alteration in the host. Lipid response thus depends upon the aetiology of the invading microorganisms and their toxic products have the most pronounced effects on lipid metabolism (Gallin, Kay and O'Leary, 1969; Gallin, O'Leary and Kay, 1970). Further investigations show that Gram-negative septicemia affects lipid metabolism in a way that differs from fever induced by Gram-negative bacteria. During septicaemia in man there is a marked increase in total serum lipids (TL), free fatty acids (FFA), triglycerides (TG), smaller increases in phospholipids (PL), and pre-ß-lipoprotein (VLDL), and unchanged values for serum cholesterol (CHO) (Gallin, Kay and O'Leary,1969). During fever, following injection of Gram-negative endotoxin, most investigators observed an increase in the concentration of serum total lipids, lecithin, cholesterol and fatty acids (Nishishita 1941; LeQuire et al., 1959; Hutcherson, Hamilton and Gray, 1953; Steiger et al., 1953; Földvari and Kertai, 1967). As lipid response has been found to be dependent on the amount of endotoxin injected and on the nutritional state of the host

(namely starvation) (LeQuire et al. 1959), the observed changes in lipid metabolism were attributed "primarily" to fever and "secondarily" to infection (Nishishita, 1941; LeQuire et al., 1956; Gallin ,Kay and O'Leary, 1969). Fasting apparently increases the incidence of an uncontrolled rise in serum lipids (LeQuire et al.1959) and causes the overproduction or accumulation of acetone bodies, particularly in the early phase (Biesel and Fiser, 1970). Since keto acids have been recognised to stimulate bacterial growth (Ramakrishnan 1948), but at the same time counteract antibacterial activity of lactic acids (Dubos, 1955), the increased susceptibility to infection often associated with starvation can be attributed partly to ketogenesis brought about by altered lipid metabolism (Dubos, 1950, 1953, 1955). Several workers have also reported the occurrence of"hypertriglyceridaemia" in Gram-negative bacillus infections (Hirsch et al., 1964; Smith, 1965; Griffith et al., 1972 ; Levin et al., 1972). The cause of these increases has not been defined (Fiser et al., 1973; Fiser et al., 1974), although it has been suggested by Kaufmann et al. (1976) that "endotoxin produces abnormalities in lipid disposal that either produce or contribute to the elevated triglyceride values observed during Gram-negative bacterial sepsis". Published values for plasma cholesterol during infectious diseases have been reported to decline (Banerjee and Bhaduri, 1959),

increase (LeQuire et al. 1959) or remain unchanged (Farshtchi and Lewis, 1968; Biesel and Fiser, 1970; Fiser et al., 1971). These changes in cholesterol content have been regarded as a significant factor in the defence mechanism of the host against invading organisms (Gallin et al., 1969), as cholesterol combines readily with bacterial toxins, thus making them less toxic (Stroesser, 1935), or block cholesterol synthesis at the squalene step (Gallin et al.,1969; Villee, 1970). The initial fall, and subsequent late rise, in cholesterol level has been observed in scarlet fever, dysentery and typhoid fever in man (Nishishita, 1941). In rabbits,small doses of endotoxin lipopolysaccharide (LPS) do not change the concentration of cholesterol, whereas at higher doses it produces an increase in cholesterol level (LeQuire et al., 1959). As is evident from the above introduction, changes in lipid metabolism during infectious disease have been recognised; however the cause of these changes remains obscure. Information provides no insight into changes in the kinetics of lipids (Fiser et al., 1972) and available measurements define values only of its uptake or release from adipose stores, from the site of hepatic metabolism, or from the site of peripheral utilisation (Farshtchi and Lewis, 1968; Gallin et al., 1969). No data is available concerning the impact of infection on the rates of lipid uptake or release at any of these sites

nor on intracellular synthesis and hydrolysis of "specific lipids" (Biesel and Fiser, 1970). An increase in the concentration of serum lipids could be the result of a decrease in their utilisation by the liver and peripheral tissues during infectious diseases (Farshtchi and Lewis,1968). However this explanation would not account for the increased needs for energy production associated with the presence of fever, (Fiser et al., 1973) at a time when dietary intake is generally low (Nishishita, 1941; LeQuire et al., 1959). The increases in serum lipids observed during the febrile state appears to represent somewhat heightened mobilisation of fat from depots (Farshtchi and Lewis, 1968; Gallin, Kay and O'Leary, 1969; Gallin, O'Leary and Kay, 1970), with subsequent increases in the synthesis and release by the liver (Gallin, Kay and O'Leary ,1969; Kaufmann, Matson and Biesel, 1976). Since free fatty acids are known to be the principal source of fuel during reduced calorie intake (Fiser et al., 1972), a role in energy production can be ascribed to fat depots of the infected host (Fartshtchi and Lewis, 1968; Gallin et al., 1969; Fiser et al., 1972). Increased triglycerides (TG) during infection cannot, however, be attributed to the absorption of dietary fat for there is no increase in chylomicron during febrile disease (Fredericson, Levy and Lees, 1967; Gallin et al., 1969) and, furthermore, acute infectious disease

produces a surprisingly small effect on intestinal absorption of fat (Table 1) and it can even be reduced in typhoid fever or diarrheal diseases (Biesel and Fiser, 1970). A rise in TG during fever can be attributed to an increased release of VLDL (Fiser, Denniston and Biesel, 1972), as the latter is recognised to :(i) serve as a vehicle for the transport of TG from the liver to peripheral tissue (Frederickson and Gordon, 1958): (ii) increase (in man) during bacterial infection (Gallin et al., 1969; Fiser et al.,1972). An increase in triglycerides thus spares lipid oxidation, which according to Drabkin (1959) "is one of the means by which survival is lengthened during starvation". In the aggregate, all this data implies that, in order to survive, invading microorganisms exercise a direct influence on the "milieu interior" of the body. Their presence provokes metabolic disturbances (Table 1), which consequently leads to excessive mobilisation of fatty acids (Nishishita, 1941) and carbohydrates (Kun and Miller, 1948). An excess in both of these substances in the plasma interferes with proper oxidation of polyunsaturated fatty acids (PUFA). Defective PUFA oxidation could account for the "experimental diabetes" (Smith, 1965; Holman et al., 1983) observed by many workers (Nishishita, 1941; LeQuire et al., 1956; Farshtchi and Lewis 1968; Gallin et al., 1969; Kaufman et al., 1978) during the febrile state,

or in starvation (Nishishita, 1941; Frederickson and Gordon, 1958; LeQuier et al., 1956; Fritz, 1961). This diabetic state contributes to the great susceptibility to infection observed in patients with uncontrolled diabetes or with prolonged starvation (Smith, 1965). The diminished resistance of a diabetic patient can however be reversed by the administration of insulin, so that of starved individual resistance can be improved by adequate feeding (Fritz, 1961). The febrile state cannot be reversed by either adequate feeding (even though glucose administration prolongs the life of infected animals) or by administration of insulin (which is deleterious) (Smith, 1965). Thus the observed metabolic changes, even when very transient, between host and bacteria, lead to physiopathological changes which can become irreversible (Table 1). Since lipids derived from invading organisms serve as antigens stimulating an appropriate immunogenetic response by the host cells, while increasing phagocytosis by the reticuloendothelial system (RES) (Di Luzio 1960), it can be linked to the defence mechanism of host against the harmful nature of the invading organism. Increased turnover in phospholipid synthesis thus becomes a requirement for phagocytic activity during febrile disease, since the formation of a new membrane is

regarded as a prerequisite for phagocytosis (Day, 1967; Biesel and Fiser, 1970) (Table 1). This requirement has been demonstrated by Day and co-workers (1966) with lymphnode macrophage.

According to their findings "these macrophage were hydrolysing triglycerides and the resultant fatty acids have been found to be incorporated into their phospholipids. Apart from this single work of Day et al. (1966) little information has been gathered concerning phospholipid chemistry of lymphocytes.

An in vitro study by Raetz (1978) shows that catabolism and turnover of phospholipids are essential for cell-membrane adaptation to environmental changes. This adaptation to environmental stimuli affects both cell permeability and the configuration of fatty acids existing within phospholipids (Melchior et al., 1970). An increase in the saturated fatty acids has been observed to render the membrane less fluid, whereas unsaturated fatty acids has the opposite affect (Melchior et al., 1970). Thus appears that membrane properties are modulated by the kind and proportions of fatty acids they contain (Holman, 1987). Recently Reinaud et al. (1989) demonstrated (in vitro) that human lymphocytes incubated with ^{14}C linoleic acid is transformed into 13(S)-9Z,11E-HDO. The method used by these researchers allowed them to distinguish between products resulting from "external" and "endogenous" linoleic acid derivatives, which preexist

within leuckocytes. Their study shows that linoleic acid concentrations becomes lower than 50 uM, 13-HODE and 9-HODE are the only detectable products. At higher concentrations, linoleic acid conversion into 13-HPODE cannot be reduced rapidly enough, and can therefore be transformed into other products which are similar to those observed upon incubation of leukocytes with 13-HPODE (Reinaud et al. 1989). Their finding shows that "5-lipoxigenase' , the most active enzyme from human leukocytes, and arachidonic acid is not involved, since (i) formation of 13-HODE does not require the presence of Ca^{++} and (ii) it has been reported that linoleic acid is a poor substrata for this enzyme in a cell-free system" (Reinaud et al.,1989). They summarised their finding that 15-lipoxygenase from human leukocytes is mainly responsible for the formation of 13-HODE by intact cells" (Reinaud et al., 1989), as follows:

15-lipoxygenise Peroxidase

18:2——————>13(S)-HPODE——————>13(S)-HODE(w6)

Linoleic acid is an important dietary fatty acid and a major constituent of phospholipid (PL). As such it is implicated (via the 15-lipoxygenase pathway) in the activation of lymphocytes without any stimulus. The existence of this pathway could provide the answer to many important issues which have as yet remained unanswered, i.e. (i) the mechanism whereby lymphocytes are activated to produce endogenous pyrogen (IL-1),

(ii) the cause of spontaneous release of leucocytes without any stimuli in various malignancies, iii) the fall in serum linoleic acid concentration under various malignancies. Fever, which is one of the predominant feature of silent disease and is observed in patients with various malignancies such as acute leukaemia, histiocytic lymphomas, renale-cell carcinoma and Hodgkin's disease, could be attributed in some instances to spontaneous release of interleukin-1 (Bernheim, Block, Atkins, 1979; Bodel, 1974; Bodel, 1978), the mediator of endogenous pyrogen fever (Dinarello, Conti and Mier, 1986) and can be produced via the lipoxenous pathway described by Reinaud et al. 1989). It is currently held that arachidonic acid (AA) is the essential element for the formation of prostaglandins (via cyclo-oxigenise) (Bernheim, 1986) and for leukotrienes (via lipoxygenesis) (Fig. 1). This process involves phospholipase, an enzyme which can be activated by Ca^{++} (Lapenina and Cuatercasas, 1979, Stitt, 1986) (Fig. 2) or by hormones i.e. thyroxine (Shambaugh and Biesel, 1967), growth hormone (Biesel, et al. 1968), insulin (Shambugh and Biesel, 1967). However, this theory disregards the fact, that de novo synthesis in 20:4(w6) depends on the availability of 18:2(w6) (Holman, 1964; Yammamoto et al., 1965; Alden et al., 1986), with the result that any alteration in the concentration of linoleic acid affects the defence

Fig. 1.

HARRY A. BERNHEIM

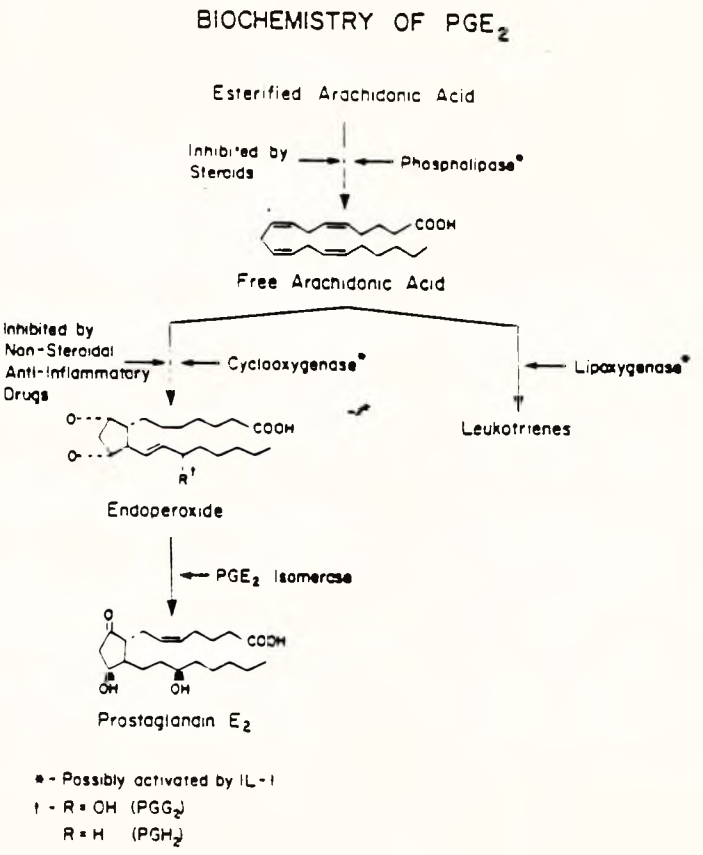


FIG. 1. Biochemistry of arachidonic acid and its derivatives.

PROSTAGLANDIN E IN FEVER

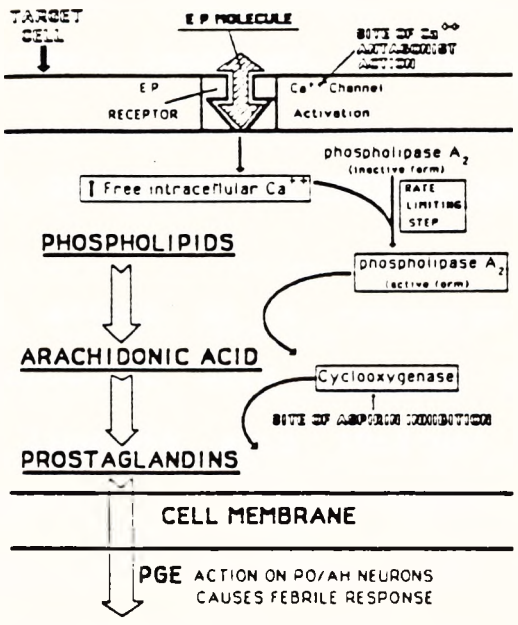


FIG. 2. A diagram illustrating the events occurring when EP stimulates the putative target cell that is thought to release PGE and the steps at which three different substances that inhibit fever are believed to act to prevent the production of PGE. The EP molecule attaches to the cell EP receptor site, activating the calcium channel, which causes unbound intracellular [Ca^{++}] to rise inside the cell. Ca^{++} channel antagonists such as verapamil and nifedipene block this step, thereby preventing the activation of the enzyme phospholipase A_2, which converts phospholipids into arachidonic acid. The steroidal anti-inflammatory drugs are also thought to block the action of phospholipase A_2 by impairing calmodulin activity. Finally, aspirin-like drugs are thought to block the activity of the enzyme cyclooxygenase which converts arachidonic acid to prostaglandins.

mechanism of the host against bacteria and, in turn, tolerance or unresponsiveness to infectious disease occurs (Kenedi et al., 1984, Kenedi and Mendelsohn, 1989, Kenedi and Mendelsohn, 1990).

Although numerous in vitro research studies demonstrate the antibactericidal effect of C_{18} fatty acids, there is no experimental evidence in support of the fact that this is also occurs in vivo; thus this extremely important finding remains to be elucidated.

2.0. OBJECT OF THE PRESENT INVESTIGATION

The effect of a reduction in the level of serum arachidonic acid 20:4(w6) by an increase or decrease in linoleic acid 18:2(w6) in a febrile condition, induced by Gram-negative bacterial endotoxin, (Salmonella Thyphosa.) is unknown. Although it is recognised that endotoxin fever in humans and in rabbits is arachidonate dependent (Splawinski et al., 1978; Coceani et al., 1986), it is also accepted that increased amounts of linolenic acid 18:3(w3) reduce the metabolites of 18:2(w6) (Mohrhauer and Holman, 1963; Rham and Holman, 1964; Anding and Hwang 1986) by acting as a competitor for the same cyclo-oxigenise (Pace-Asciak and Woolfe, 1968), thereby not only influencing the formation of arachidonic acid, but suppressing its metabolites as well (Hwang and Carrol, 1980).

The metabolites of arachidonic acid i.e. PGE_2 have been shown to be involved in the biochemical sequences leading to fever induced by bacterial endotoxin (Feldberg and Saxena, 1975; Milton, 1976; Splawinski et al., 1978; Skarnes et al., 1981). Therefore, deficiency in the synthesis of arachidonic acid induced by a lack (Kenedi, et al. 1984) or excess of 18:2(w6) (Kenedi and Mendelsohn, 1988, Kenedi and Mendelsohn, 1989), diminishes the fever response (Kenedi and Mendelsohn, 1988, Kenedi and Mendelsohn, 1989), which in the course of constant stimulation by exogenous or endogenous pyrogens leads to tolerance of the fever (Atkins, 1960; Kanoh et al., 1977). The pathological consequence of tolerance to pyrogen, induced by lack of, or excessive intake in linoleic acid (EFA) together with chronic induction of fever, have not been associated with a diseased condition, as the biochemical mechanism leading to tolerance has been unknown until the present study. The present thesis has been undertaken to demonstrate that when dietary intake of essential fatty acid is impaired in any way, this condition will affect the body's response to pyrogen and in turn will lead to tolerance instead to fever.

The recognition of this will hopefully lead to a better understanding of the nutritional aspect of the host's defence mechanism in the infectious process and possibly a more successful approach to the prevention and treatment of infectious disease.

3.0. REVIEW OF THE RELEVANT LITERATURE

Fever as a cardinal manifestation of illness has been recognised since the foundation of medicine was laid by Hyppocrates during the time of Pericles in the fifth century B.C. Since then it has been the subject of study and commentary for well over 2,000 years. Although febrile disease was alternatively praised and feared in early times (Atkins, 1984), a rise in temperature gradually came to be viewed as an attempt by the Nature to heal itself and, therefore, as being beneficial (Signal, 1978). Louis Pasteur believed fever to be the reaction of the organism to starve out bacterial infection, making it beneficial. However, one of the great difficulties in understanding the febrile process arises, from the fact that it is often confused with hyperthermia. In clinical terms "fever" is used to describe any condition in which the patient's body temperature exceeds that of the normal resting range. The term is often applied regardless of the aetiology of the temperature (Blight, 1973). On the other hand, thermal physiologists use the general term "hyperthermia" to describe any unspecified or unidentified elevation of body temperature which is above the normal resting range (Stitt, 1979). Dissociation of hyperthermia from fever is made even more difficult by the fact that, apart from calorimetric measurement (which is seldom used today),

there is no direct diagnostic tool which can differentiate the one from the other. The clinical thermometer is a physiological gauge, registering the body temperature variations against an established standard set-point. The derived fever curve does not indicate whether the elevated body temperature is hyperthermia or fever, nor does it take into account the time when the measurement is taken (morning or afternoon). Consequently, the nutritional state of the host (fed or fasted state) is not taken into account. Hyperthermia has been defined as an increase in "metabolic heat" production (Grant, 1949; Atkins, 1960) or a reduced functioning of the heat loss by radiation or evaporation (Benedict, 1916). Such changes can be induced by physical (Butkow et al., 1984), pathological (Bernheim et al., 1979) or pharmaceutical (Baumann and Bligh, 1974; Hanson et al., 1975) interventions. This demonstrates that hyperthermia is governed by the same mechanism that controls internal body temperature (Stitt, 1978), and per se could account for the metabolic alterations observed in acute infection (Biesel. et al. 1967). Fever differs markedly from all form of hyperthermia in that it is a regulated rise defended by a fully functional thermoregulatory mechanism (Stitt, 1979). Antipyretic drugs (such as aspirin) can interfere with febrile hyperthermia (McLeod, 1922; Vane, 1970, Stitt, 1986) by returning the body temperature to a normal

level. No other form of hyperthermia can be inhibited by an aspirin-like substance (Stitt, 1986). Furthermore, certain infectious or febrile diseases can be caused by abnormal cellular activity, which increases heat production. Diseases such as leukaemia, polycythemia, some types of anaemia, cardiac failure, hyper-resonance and dyspnoea all involve increased cellular activity and thus reflect uncontrolled metabolic rate (Harpers, 1967). In fever, the respiratory quotient (R.Q.) is constant (Splawinski et al., 1978), whereas in hyperthermia it fluctuate (Harpers, 1967). It is conceivable that the circulating endogenous toxin products within the host affect the metabolic , endocrinological, neurological and immunological events (Table 1) and in turn promote an increase in the body temperature (Biesel and Fiser, 1970). The full extent of this response includes a dramatic rise in the synthesis of hepatic acute-phase proteins (Dinalrello and Wolff, 1982). Further findings show that a febrile state (similar to hyperthermia) can be induced by the intervention of various substances such as Gram-negative and some, but not all, Gram-positive bacteria (Atkins and Wood, 1955; Abernaty and Spink, 1958; Atkins and Friedman, 1963; Sheagran et al., 1967), viruses (Wagner et al., 1949; Atkins and Huang, 1958; King, 1962), pathogenic fungi (Braud, 1960) and steroids (Bodel and Dillard, 1968).

Antitoxins of Gram-negative bacterial origin have been characterised as being the most active pyrogen (Westphal, 1957; Lüderitz et al., 1966, Galanos et al., 1969). They are non-dialysable and markedly heat-resistant, being inactivated within only two hours at 160 ^{o}C (Westphale, 1957; Atkins, 1960), and can be neutralised by hot acid or alkalis (Lüderitz et al., 1966). The pyrogenic activity of this endotoxin is very high: in rabbits intravenous doses of about 0,001 ug/kg body mass produce a pyrogenic response and in humans a dose of 0,10 ug/kg (Favorite and Morgan , 1942; Wolff, 1973; Wolff et al., 1976) has the same effect. The main toxic and pyrogenic principal that endotoxin in the outer cell wall is composed of lipopolysaccharide has been well reviewed (Westphal, 1957; Lüderitz, Staub and Westphale, 1966; Galanos, Rietchel et al., 1969; Nicado and Vaara, 1985). Structurally it has been shown to consist of three separate region. Region (I) comprises O-specific-chain, which is specific to each organism and is responsible for its immunological properties (Hellerquist et al., 1968; Robins and Wright, 1971; Mäkela, Voltonen and Voltonen, 1973). Region (II) comprises a basal core which is also polysaccharide by nature and includes 2-keto-3-deoxy octeonic acid (KDO), as well as a phosphate group and ethanolamine (Lüderitz, Jann and Wheat, 1964).

Region (III) is known as Lipid A (Kim and Watson, 1964; Burton and Carter, 1964; Gmeine, Lüderitz and Westphale, 1969; Adams and Singh, 1970; Galanos et al., 1971; Yin et al., 1972, Lüderitz et al.,1972; Rietschel and Galanos, 1977) which is the toxic moiety of this complex polymer (Atkins, 1960; Kenedi et al., 1982). In addition, LPS has been recognised to play an active role in the physiology of Gram-negative bacteria, by making them resistant to host defence factors such as lysozyme, ß-lysin (Donalson et al., 1974, Nikaido and Vaara, 1985) and various leucocyte proteins, which are toxic to Gram-positive bacilli (Patterson-Delafield et al., 1980). It also provides an effective barrier, protecting Gram-negative bacteria against the inhibition of medium C_{10} and long-chain fatty C_{18} acids and prevents their accumulation on the inner cell membrane. This protection is essential for the survival of bacteria in the intestinal tract, where such fatty acids are produced by the digestion of fats (Sheu and Freese, 1973). Recently it has been shown by Freese et al., 1973, that fatty acids inhibit "in vitro" growth and oxygen consumption of Gram-negative bacteria by inhibiting the transportation of amino and keto acids through the cellular membrane. Another importent function of the LPS membrane is to endow the bacterial surface with strong hydrophilicity, which is importent in evading phagocytosis as well as avoiding specific

immune attack, by altering the surface antigen constitution (Mäkela et al., 1980). The antimicrobial effects of fatty acids (soaps) have have been known for many years (Kodicek, 1949; Nieman, 1954, Kabara, 1978), but despite recent advances in describing this phenomenon, there has been little progress towards understanding the mechanisms involved (Kabara, 1978, Kanai and Kondo 1979). Fatty acids have been found to inhibit the growth of fungi (Wyss, Ludwing and Joiner, 1945), protozoan (Lees and Korn, 1966), viruses (Sands, 1977) and numerous types of bacteria (Kanai and Kondo, 1979). Most investigations into the sensitivity of bacteria to fatty acids suggest that long-chain fatty acids (C_{18}) are the most common fatty acids with strong growth-inhibitory activity (Gailbrath et al., 1971; Miller et al., 1977: Kanai, 1977). Short-chain fatty acids are recognised to be toxic in high concentrations in pH-dependent fashion, while polyunsaturated fatty acids affect only certain types of bacteria and are toxic at much lower concentrations in a more pH-independent fashion (Nieman, 1954; Kanai and Kondo, 1979). The toxicity of polyunsaturated fatty acids has been attributed to their detergent effect, which disrupts bacterial membranes (Kanai and Kondo, 1979; Coonrod, Lester and Hsu, 1984). It has also been suggested that linoleic acid toxicity for variety of bacteria is due to an increase in the concentration of short-chain aldehyde

compound formed by auto-oxidation (Gutterige, Lamport and Dormandy, 1976). Recently Knapp and Melly (1986) found that several species of Gram-negative bacteria were equally susceptible to the bactericidal effect of arachidonic acid as Gram-positive organism. Gailbrath et al. (1971) compared the antibacterial activities of various fatty acids with regard to their chain length, degree of unsaturation and isomerism. Up to C_5 no inhibition was noted, but saturated fatty acids with a longer chain showed activity in the following order: $C_8<C_{10}<C_{12}<C_{14}>C_{16}>C_{18}$. As regards to the activity of C_{18} fatty acids, the following order has been obtained: $C_{18:0}<C_{18:1}<C_{18:2}<C_{18:3}$ Kodieck (1949) pointed out the necessity of a polar-end group for bacterial activity. Esterification of C_{14} and $C_{20:4}$ to methyl or cholesteryl esters completely destroyed their strong antibacterial activity against mycobacteria, suggesting the need for the carboxyl group (Kondo and Kanai, 1977). From these findings it appears that the longer the fatty acid chain, the more double bonds are required for their antibacterial activity (Laser, 1952; Kondo and Kanai 1979). Consequently, inhibitory capacity increases with their degree of unsaturation, so that linolenic acid (containing three double bonds) is more inhibitory, and linoleic acid (two double bonds) less so, whilst oleic acid (one double bond) and stearic acid

(saturated) have insignificant properties (Butcher et al., 1969; Ko et al., 1978; Heczko et al., 1979). Nevertheless, linoleic acid can be more inhibitory to bacteria than linolenic acid (Bayliss, 1939; Fuller and Moor, 1967; Kabara et al., 1972) as the amount of free linoleic acid exceeds that of linolenic acid (Wilkinson, 1972). The work of Lacey and Lord (1981) demonstrates that bacteria become more resistant to linoleic acid in the presence of serum, than in its absence. In addition, lipids, such as lecithin, sphingomyelin and cholesterol neutralise the toxicity of long-chain fatty acids (Kodicek and Worden, 1945; Dubos 1954; Wynne and Foster 1950; Kabara, 1978). Although a satisfactory explenation of the mode of action of these compounds has not yet been presented (Kabara, 1978), evidence seems to indicate that the antibacterial effect of fatty acids is due to an alteration of cell permeability (Kodicek and Worden, 1945) or its effect on enzyme protein itself (Hardesty and Mitchell, 1963). This antibacterial activities can be reversed by lecithin or cholesterol, as both are acknowledged as the most potent surface-active agents, with the ability to reverse the blocking action of the unsaturated fatty acids (Kabara, 1978). The serum bactericidal effect on Gram-negative bacteria and on the formation of an antibody-antigen complement system has also been implied by Skarnes and Watson (1969).

These investigators suggested that the bactericidal activity of serum against the bacterial cell wall could be due to hydrolysis of membrane phospholipids induced by the activation of bacterial or serum phospholipase (Bleden et al., 1967; Slein and Logan, 1967). Further investigations implicated that, serum-mediated killing of E. coli is independent of free fatty acid (FFA) release or activation of bacterial phospholipase, as a rise in both could be the result, rather than the cause, of these events (Kreutzer et al., 1972; Nojima et al., 1971). The nature of the interaction between linoleic acids and serum has also remained unclear, although it has been established that the neutralising capacity of serum correlates with the ability to dissolve emulsions of linoleic acid in water, i.e. the detergent effect (Lacey and Lord, 1981). In the aggregate, these findings demonstrate that polyunsaturated fatty acids (PUFA) directly or indirectly affect the sensitivity of the bactericidal activity of serum. The mere fact that long-chain fatty acids have selective toxicity against bacteria suggests that fatty acids released from dietary lipids influence the population of intestinal flora. Anaerobic intestinal bacteria have strict requirements for long-chain fatty acids, and biohydrogenation of linoleic acid into octadecaonic acid was not only demonstrated by Moratomi et al. (1976), but it has also been suggested that

"long-chain fatty acids in the intestine are the factors controlling the localisation and population level of indigenous bacteria". However, most investigations were concerned with the idea, that since long-chain fatty acids have in vitro bactericidal activities, these compounds may have the same therapeutic effects in vivo, as topical application of pure linoleic acid to the skin surface results in dramatic encompassment of bactericidal effects (Kabara, 1978; Lacey and Lord, 1981). In spite of the in vitro evidence, difficulties were encountered with the therapeutic administration of free long-chain fatty acids via parenteral routes, due to their haemolytic and cytotoxic properties (Lambert, Miller and Frost, 1956), which can deprive the organism of calcium (Kodicek, 1949; Laser, 1952; Nieman, 1954). On the other hand, Cooper and West (1962) and Cooper (1964) demonstrated that "triglycerides" possess specific fatty acid radicals, which stimulate the activities of the reticulo-endothelial system (RES), both in vitro and in vivo. By means of histological methods and chromatographic analysis Gaugas et al. (1970) demonstrated an uneven spread of triglycerides (TG), phospholipids (PL) and unsaturated fatty acids in the macrophages present in the lymphoid system of mice infected with M. lepraemurium. They postulated that "if lipid material(s) exist in infected cells which

produce either growth-promoting or growth-inhibiting effects, the substance responsible is an unsaturated fatty acid".

Although it has been postulated that long-chain fatty acids have a cytotoxic effect, it is generally believed that this event could not occur in living bodies containing an abundance of neutralising agents such as serum. However, Hax et al. (1974) found that free fatty acids have the opportunity to act as cytotoxic agents, as they have the the opportunity to increase the population of Acanthamoeba castellani in a growth medium through increased activity of phospholipase A. The latter accelerated the release of long-chain fatty acids into the environmental fluid and became insusceptible to the neutralising effect of the medium, thus generating cytotoxic fatty acids. Similarly, Frye and Friou (1975) found cytotoxicity to be the result of increased activity of phospholipase A, which alters the molecular structure of the target cell membrane, resulting in "lysis" of the bacterial cell membrane (Gailbrath and Miller, 1974). Cytotoxicity induced by long-chain fatty acids affects various cellular metabolic functions, including inhibition of respiratory activity (Sheu and Freese, 1973), uncoupling of phosphorylation (Stadie, 1945; Pressman and Lardy, 1956; Wojtaczk and Wojtaczak, 1960; Borst et al., 1962) and altering mitochondrial function by increasing their susceptibility for

peroxidation (Marco et al., 1961, Knapp and Melly, 1986). Increased peroxidation involves H_2O and bacterial iron (Repin, Fox and Berger, 1981; Knapp and Melly, 1986) which contribute further to the toxicity of polyunsaturated fatty acids to other cells, such as erythrocytes (Weiss, 1980), tumour cells (Nathan et al., 1979) and lung cells (Shasby et al. 1981). The above findings collectively support the existence of a sensitive balance between unsaturated fatty acids and resistance to infectious disease (Burr and Burr, 1929, 1930). In the higher organism, dietary fatty acids are incorporated into two types of lipid molecules: glycerides (Fig. 3, arrow 2) and phospholipids (Fig. 3, arrow 3). In glycerides, fatty acids serve as an energy reservoir, while PL represent the component of all biological membranes (Fig 3, arrow 3). Hence, dietary fatty acids could affect the assemblage processes concerned with growth, maintenance and repair of the living body as a whole, or its constituent parts or organs. Thus malnutrition could be defined in the same terms as a disease: "the failure of the adaptive mechanism of an organism to counteract adequately the stimuli or stresses to which it is subjected, resulting in disturbances of function or structure of any part or organ or system of the body" (Hoerr and Osol, 1956). In his unifying concept of the pathogenesis of disease, Seleye (1957) defined stress as "the state manifested by a nonspecific syndrome which affects all biological systems".

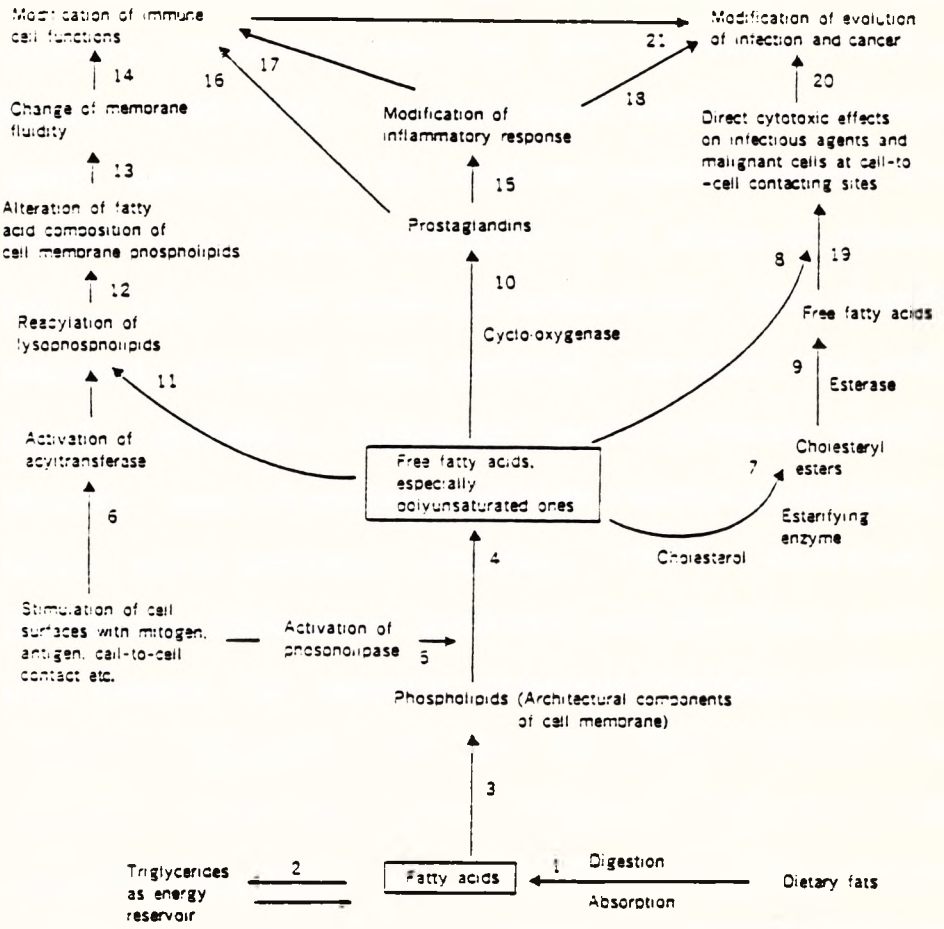


Fig. 3. A proposed scheme of fatty acid participation in infection and immunity at cellular level.

" A stressor produces stress". Shortage of food is included by Seleye amongst the several environmental factors conditioning the response to a stressor: "dietary disorder may be the stressor" (Seleye and Hauer, 1955-1956). Another such factor is an infective agent manifesting its local or general effect in three stages: (i) alarm reaction; (ii) stage of resistance; and (iii) the state of exhaustion. This changes are mediated through the central nervous and endocrine gland systems. Although each disease profile is unique, most diseases exercise identical effect on the fatty acid pattern (Holman, 1987). Deficiencies in 20:3(26), 20:4(w6), 20:5(w6) and 22:5(w6) fatty acids are common to several diseases (Holman, 1987), which further supports Seleye unifying concept of the relation between the disease process and the nutritional state of the host. C_{18}-C_{22} fatty acids have thus been recognised as being involved either directly or indirectly in disease processes affecting immunological reactions. Mead and Martin (1976) found that linoleic acid 18:2(w6) prolongs the survival of skin allograft in rats by reducing cytotoxic responses of isolated spleen cells. It has been established that the spleen is the major action site of linoleic acid 18:2(w6) activity affecting the lymphoreticular system (Mertin et al., 1977). In addition Mertin and Hunt (1976) demonstrated increased immuno-potentiation and accelerated skin allograft rejection in mice fed on

a diet deficient in PUFA. As a whole, this implies that PUFA participate actively in immunoregulatory mechanisms. This includes $C_{18:2(w6)}$ stimulated suppressor-cell generation and excess biosynthesis of immuno-inhibitory prostaglandins. Polyunsaturated fatty acids thus has been recognised to be involved either directly or indirectly in disease processes as they are both the precursors of prostanoids and eicosonoids and are basic importance to the essential structure of living cell (Holman, 1986). Although the present work investigate the role of essential fatty acids in thermoregulation and in the genesis of fever, we cannot disregard the physiological and biochemical role of PG's (Fig. 3). Bergström, Carlson and Weeks (1961) recognise them as biologically active lipids which are synthesised by endoperoxidases via cyclo-oxigenation (Fig. 3, arrow 10) of unsaturated fatty acids (Fig. 3 arrow 4) (van Evert, Nugtern, Van Dorp, 1978), released from phospholipids (Fig. 3 arrow 3) (Samuelsson, 1970; Seyberth et al., 1975; Friedman, 1979; Bernheim, 1986), cholesterol esters (Fig. 3, arrow 8) (Dobaskovà, 1983) and triglycerides (Friedman, 1979). For fatty acids such as linoleic, linolenic or arachidonic acids to become available for prostaglandin synthesis, they have to be released by membrane-associated phospholipase A ((Fig 3. arrow 5) (Kunz and Vogt, 1971) or by triglyceride lipase (Friedman, 1979). The control of prostaglandin

synthesis is thought to be localised at this stage, when the precursors are released following the activation of acyl hydrolase (Seyberth et al., 1975; Van Evert, Nugtereen and Van Dorp, 1978; Friedman, 1979). Availability in the substrata of precursor acids therefore affects the quality and quantity of the PG formed (Samuelsson, 1970). The direct precursors of PG's are FFA and PUFA (Fig. 3, arrow 4) (Friedman, 1979). The metabolic formation of PUFA's and their conversion into prostaglandins involves acyl activation, chain elongation, acyl-transferase and oxidation (Lands and Samuelsson,1968). Hence, the ester linkage in the ß-position has to be hydrolysed by phospholipase A before initiating the biosynthesis of prostaglandins in animal tissue (Huwang and Carrol, 1980). Although PG's are present in a minute quantity in almost all mammalian tissue (Dray, Charbonnel and McClouf, 1976), they are not stored, but synthesised (following an appropriate stimulus), rapidly released and metabolised (Ramwell and Snow, 1966). This suggests that they are regulators of physiological and biochemical activities, either stimulatory or inhibitory, as sympathetic nerve stimulation of adipose tissue induces both lipolysis and release of prostaglandins (Bergström, Carlson and Weeks, 1968). Against this background, the release of FFA from adipose tissue by PGE_1 has been recognised (Fig. 4). An in vitro study by Steinberg and

co-workers (1964) indicates that " PGE_1 reduces the release of glycerol into the medium during incubation of epididymal fat of the rat". Since glycerol release indicates the rate of lipolysis independently from reesterification (Fig. 4), it is acknowledged that PGE_1 inhibits the breakdown of triglycerides (Bergström, Carlson and Orr, 1963; Steinberg, Vaughan, Nestel and Strand, 1964; Bergström and Carlson 1965; Carlson, 1965; Berti, et al., 1967; Paoletti, Lentati and Kobolkiewitcz, 1967), depending on the nutritional state of the host. In the fed condition PGE_1 becomes the most potent inhibitors of lipolysis known: in low concentration (1.0 ng/ml) reduces the release of glycerol, in the higher doses (100 ng/ml) inhibits it maximally (Berti et al., 1967; Steinberg and Vaughan, 1967). Conversely, in the fasted state it favours FFA release (from adipose tissue) to meet energy requirements (Kupieczki, 1967). It is further acknowledged that PGE_1 acts on glucose metabolism, similar to the action of insulin (Vaughan, 1967; Haessler and Crawford 1967), although it is not clear whether this effect on glucose metabolism reflects primary action of PGE_1 or whether it occurs secondarily as a result of inhibited lipolysis and lowered intracellular fatty acid pool (Bergström, Carlson and Weeks 1968). In any event,it illustrates that fluctuation in the concentration of PGE_1, regulates the release of FFA which is stored in the

PROSTAGLANDINS

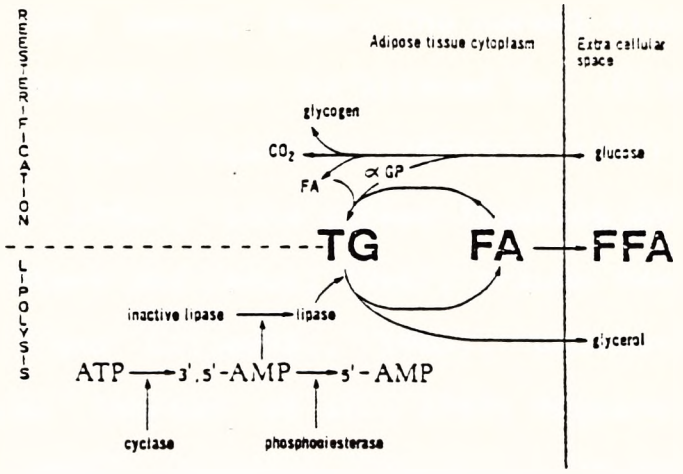


FIG. 4. Schematic illustration of lipolysis and re-esterification in adipose tissue, the two major processes that regulate the mobilization of free fatty acids. The effect of PGE_1 on these processes is reviewed in the text. FA, fatty acid; FFA, free fatty acid; TG, triglyceride; α-GP, α-glycerophosphate; 3′,5′-AMP, 3′,5′-adenosine monophosphate.

fat depot as triglycerides (TG) (Fig. 4) (Bergström Carlson and Weeks, 1968). The normal flux in FFA from adipose tissue, can be altered by (i) physiological stimuli, i.e. fasting (Fritz, 1961), starvation (Laurell, 1956), cold stress (Dawkins and Hull, 1964; Shiff, Stern and Leduc, 1966), trauma (Wadström, 1964; Carlson and Liljedhal, 1963), emotionally charged discussion (Morton et al., 1959; Bogdanoff, Estes and Trout, 1959), strenuous exercise (Fridberg et al., 1960; Havel Nimar and Borchgervink, 1963; Chouverakis and Harris, 1969), or (ii) pathological stimuli, i.e. carnitine deficiency (Fritz, 1961; Chapnoy, Angelin and Brown, 1980), diabetes melitis and ketoacidosis (Fritz, 1961; Yue et al., 1981), acute pancreatitis (Smith-Morgan and Bogner, 1981; Port and Halter, 1983) myocardial infarction (Vick-Mo and Mjos, 1981), Reye's syndrome (Pollack et al., 1975, Ansevin, 1980; Ogburn et al. 1982), hyperthyroidism (Boyd and Connel, 1963), in such a way that hydrolysis of TG is either accelerates, or slowed down, so that the concentration of FFA in the fat depot or in the plasma rises. Under such conditions, a rise in FFA can be interpreted as an adjustment of the organism to meet an increased calorie demand due to a stressful or fasted state (Frederickson and Gordon, 1958; Fritz, 1961), resulting in its uptake from the the blood by the tissue, or its release from the tissue into the blood being blocked. In this event the elevated FFA within

the blood is "recycled", before being oxidised. Thus Frederickson and Gordon (1958) showed in human subject that intravenously injected C^{14} FFA after been cleared from the plasma within 1-3 min, it reappears again in the lipid fractions. This "recycling was especially prominent with linoleic acid" (Frederickson and Gordon, 1968). The mere presence of increased TG, in serum during trauma (Wadström, 1961) and in fasting (Laurel, 1956) suggests that auxiliary mechanisms have to be activated when FFA can not supply all non-carbohydrate calories (Frederickson and Gordon, 1958) and have to be provided by increased transport in fatty acids (Frederickson and Gordon, 1958). Consequently, a rise or a drop in the concentration of PGE_1 depends on the availability of energy-generated substrata (Fig. 4) (Bergström, Carlson and Weeks, 1968). These findings may be grouped together under the concept of "caloric homoeostasis", as dissimilar substrata (Frederikcson and Gordon, 1958, Fritz, 1961) must be available for the production of energy at all times, for the organism depends for its survival on the means to mobilise a reserve supply when exogenous foodstuffs are in short supply (Fritz, 1961). On the other hand, such mobilisation can be halted when exogenous foodstuff becomes available (Frederickson and Gordon, 1958), implying that PGE_1 has the ability to release FFA and glycerides in times of decreased intra-cellular carbohydrate supply and to

store it at a time of positive caloric balance. The decrease of tissue fat during a disease condition is the result of FFA mobilisation exceeding deposition. However, it should be borne that the direct precursor of prostaglandin Es (Fig. 3, arrow 10), is FFA, and also polyunsaturated fatty acids, found in lecithin phospholipid. Various studies have shown that temperature is one of the most important factor influencing the unsaturation of PL, thus affecting not only the composition of cell membrane PL (Fig. 3, arrow 12), and membrane fluidity (Fig. 3, arrow 14) but also influencing the growth and metabolism of microorganisms (Cronan and Vagelos, 1972; Verma and Khüller, 1973; Simiensky, 1974). This change in fatty acid composition has to occur over a wide range of temperatures in order to help the cells to survive altered conditions (Verma and Khüller, 1973). These findings have been substantiated by the in vivo study of Noble et al. (1981). Their experiment demonstrated that an increased unsaturation of plasma phospholipids by 18:2(w6) allowed the new-born lamb to withstand thermal stress, while with a deficiency of 18:2(w6) showed serious signs of discomfort (lassitude, lethargy, severe panting), combined with an inability to cope with thermal stress. Further in vivo investigations by Kenedi et al. (1984) showed that rabbits kept at a normal ambient temperature (20±1^{o}C) while subjected to a diet deficient in

18:2(w6) (for 3 months) led to a drop in body temperature. The hypothermia thus produced prevented the response of the fever to endogenous pyrogen. Recently Malak et al. (1989) demonstrated in rainbow trout that lecithin biosynthesis is temperature-dependent in all tissues and in all organs. Their findings indicates i) that compensation against temperature requires an external sources of essential elements such as w3, and w6 fatty acids, ii) temperature changes result in an overall rearrangement of fatty acids within lecithin molecules. In the final analysis, this raises the point whether stability of body temperature is controlled by consistency of serum lecithin configuration. If so, a shift in the configuration of lecithin fatty acids induced by various stimuli could affect the core temperature as well as the febrile response to pyrogen, as 20:3(w6), 20:4(w6) and 20:5(w3) fatty acids are transformed into PG's in osmoregulatory organs under pathological conditions, thus affecting the heat gain or loss mechanism (Weindlandt and Milton, 1970; Milton, 1976). The fact that the pathogenesis of fever remains unresolved (Skarnes et al., 1981) gave rise to the present investigation into the biochemical mechanisms governing thermoregulation and producing fever. This has been undertaken in order to assess the importence of the energy demands of the host during infectious febrile disease and to establish the effect of the nutritional status of the host during these events.

4. ROLE OF ARACHIDONIC ACID IN THE GENESIS FEVER

MATERIALS AND METHOD

4.1. Materials

4.1.1. Chemicals, Reagents and Standards

The source of chemicals, reagents and standards employed in this study are listed in Table 4.1. All chemicals used were of analytical reagent grade.

Table 4.1. Source of chemicals, reagents and standards used

Chemicals, reagents and standards	Source
Salmonella Thyphosa Lipopolysaccharid	Difco
Prostaglandin E2	Upjhon
Pyrogen free saline	Baxter
Methanol	Merck
Chloroform	"
Petroleum-Ether (40-60)	"
Diethylthylether	"
Glacial Acetic Acid	"
Boron trifluoride	"
Sodium hydroxide	Analar
Hexane	Merck
Sodium chloride	Analar

Table 4.1. continued

Chemicals,reagents and standards	Source
Sodium Sulphate	Analar
Potassium Hydroxide	"
Hydrochloric acid (conc)	Merck
Phospholipid standard	(Sigma)
Fatty acid methyl esters	(Suppelco Inc.)
(Standards Pufa-2, NHIF, RM_3)	
Sodium methylalal	
Sublimed iodine	

4.1.2. Methods

In this study, the following techniques have been applied: (1) preparation of essential fatty acid deficient diet, (2) lymulus lysate method for sterility; (3) anaesthesia; (4) head-plating of rabbits; (5) preparation of human leucocyte pyrogen; (6) preparation of PGE_2, (7) intravenous and cerebrointraventicular injection; (8) measurement of rectal temperature; and (8) lyophilisation of brain.

4.1.2.1. Experimental animals

20 young white New Zealand rabbits, weighing between 2 and 3 kg,exhibiting a body temperature of 38,4±0,6°C were head-plated before the experiment was started for the administration of cerebroventricular injections of prostaglandin E_2. Injection procedures were carried out on conscious animals, minimally restrained in conventional rabbit stock, at an ambient temperature of 20±1°C. Each animal served as its own control.

4.1.2.2. Anaesthesia

For crebroventricular injections of prostaglandin E_2 (PGE_2), implantation of cerebral canulae was performed under general anaesthesia. Intravenous pentobarbitone sodium was administered slowly into the marginal ear vein at a dose of 30-60 mg/kg (depending of the weight of the rabbits). This provided satisfactory relaxation, while the animal continued to breathe freely. No oxygen administration was required during the operating procedure.

4.1.2.3. Insertion of cerebral canulae for ventricular injection of PGE_2.

Using aseptic procedures, a midline incision was made on the rabbit skull. This opening was enlarged by Ranger forceps, while the underlying fascia was

cleared. Thereafter the sterilised stainless steel headplate was placed in position as described by Monnier and Gangloff (1961). At the marked positions holes were drilled into the skull with a 2,0 mm electric drill and the headplates securely fixed with stainless steal screws. This was followed by the implantation of two sterile 26 gauge cannula guide tubes into each lateral cerebral ventricle, through head plate, in the same manner as described by Cooper, Cranston and Honour (1967). The cannulae were sealed by means of a cap and the skin around the template was closed up with absorbable sutures (catgut) (Kaplan and Timmons, 1979)

4.1.2.4. Post-operative management

Post-operatively, the animals received intra-muscular antibiotic injections (Ampicillin) for five days. After the recovery period of seven days, the head-plated rabbits were divided into two groups. Group A (control) received the standard rabbit pellet diet containing five per cent corn oil. Group B was fed a similar diet, but 5 per cent coconut oil was substituted for corn oil (Tables 2, 3 and 4). This diet was prepared by the author according to the method described by Infante and Kinsella (1978). The diet of group A contained 50 per cent linoleic acid and that of Group B 0,02 per cent. 100 gr/kg food was measured daily and the unconsummated food was weighed

Table 2. COMPOSITION OF NORMAL AND DEFICIENT DIET.

Composition	Normal Diet	Deficient Diet
Fat	4-5% Corn oil	5% Coconut oil
Protein	20 % Milk powder	20% Casein (Vitamin-free)
Fibre	4% Cellulose	4% Cellulose
Carbohydrate	64% Sucrose	64% Sucrose
Salt	4% Salt mix*	4% Salt mix*
	2% Choline chloride	2% Choline chloride
Vitamin mix	2% ICN Pharmaceutic	2% ICN Pharmaceutic.

* The cellulose used in the making up the diet was obtained from Sappi (Johannesburg R.S.A) and contained 88.5 % cellulose, 0.2 % Ash, 0,3 % hot water soluble and 10 % Hemi cellulose. The Ash and hot water soluble were mostly Carbonates, Chlorides, Sulphates of Sodium Calcium and Magnesium.

Table 3. COMPOSITION OF SALT MIXTURE USED IN THE DIET

Salt	Mols	Grams	Element	Amount of element in 4,0 g mixture
NaCl	5	292,5	Na	0,206
			Cl	0,319
KH_2PO_4	6	816,60	K	0,421
			P	0,334
$MgSO_4$	1	120,30	Mg	0,043
$CaCO_3$	8	800,80	Ca	0,575
$FeSO_4$	0,2	56,60	Fe	0,020
KI	0,01	1,66	I	0,0228
$MnSO_4$	0,05	9,35	Mn	0,00494
$ZnCl_2$	0,004	0,5452	Zn	0,00046
$CuSO_4$	0,004	0,9988	Cu	0,00046
$CoCl_2$	0,002	0,0476	Co	0,00002

The salts used in the make up of the mixture were analytical reagent grade. With the exception of Potassium iodide (KI) , they were ground together, then passed through a 40mesh sieve . KI was added separately to preserve iodine from degradation. (Jones and Foster, J. Nutri. 1942, 242-255).

Table 4.

COMPOSITION OF VITAMIN MIXTURE USED IN THE DIET

Name	Quantity used/kg feed
Vitamin A	90,200 i.u./g
Vitamin D	100,000 i.u./g
α-Tocopherol	1,5 g
Ascorbic Acid	45,00 g
Choline chloride	75,00 g
Methadione	2,20 g
P-aminobenzoic Ac.	5,00 g
Niacin	4,40 g
Riboflavine	1,00 g
Pyridoxin-hydrochloride	1,00 g
Thiamine-hydrochloride	1,00 g
Calcium-pentonthenate	3,00 g
Vitamin B12	1,40 g
Biotin	20 mg
Folic Acid	90 mg

Vitamin mix has been prepared according to the recipe of ICN Pharmaceuticals Life Science Group Cleveland (Ohio, U.S.A.)

again after 24 hours and subtracted from the known food weight. Thus food intake was monitored in each group throughout the experiment. The weight of each animal was measured daily prior to injections of human leucocyte pyrogen (HLP) or prostaglandin E_2. Experimental animals were housed separately in stainless steel cages and water was given ad libitum.

4.1.3. Fever Experiment

4.1.3.1. Experimental protocol

After 30 days on the essential fatty acid (EFA) deficient diet, each group was subjected on alternate days to either intravenous injections of human leucocyte pyrogen or to intra-cerebroventricular injections of PGE_2. Intravenous injections were given into the marginal ear vein by means of an indwelling butterfly needle (G-21) attached to a polyvinyl cannula that was inserted prior to injection of HLP. Prior to PGE_2 injections, the protective cap was removed, together with the stylet from the guide tube (Barney and Elisando 1978). This was followed by intraventricular injections through a sterile cannula attached to a sterile polyethylene tube,

which had been flushed with pyrogen-free saline and then filled with PGE_2. The fact that injections had been administered correctly into the cerebro-ventricle was evident from the presence of cerebrospinal fluid in the guide cannula (Cooper, Cranston and Honour, 1967). The vehicle (pyrogen-free saline) was administered to each animal in the same manner as HLP or PGE_2 in order to eliminate any interference by the carrier solvent. Endogenous pyrogen used in the experiments was prepared in a single batch, while PGE_2 was prepared freshly from stock solution before use. All glassware, tubing, and chemicals used in the preparation of HLP or PGE_2 were rendered pyrogen-free first by wet sterilisation and subsequent baking at 180^{o}C for 5 hours. This procedure was concluded by testing with lymulus lysate assay (Butkow et al., 1984).

Three experimental protocols were performed in this study. In the first series of experiments, the effect of intravenously injected endogenous pyrogen on rabbits fed the EFA deficient was compared with the effect on control rabbits. The main parameters compared were the latency to the onset of fever (i.e., the time that elapsed from the end of the injection procedure until rectal temperature rose by >0,2^{o}C), the time until peak fever was attained, and the intensity of febrile episode indicated by the rate of the rise of rectal temperature.

In the second series of experiments febrile response induced by PGE_2 intra-cerboventricular injection was compared between controls and EFA deficient rabbits. Since fever by each route was induced in each rabbit, and animals were exposed to both series of protocol, statistical differences between the fever curve points were established using paired t-test ($P<0,05$ was regarded as indicating a significant difference). Finally, the animals were sacrificed after 3 months, the brain carefully removed, washed three times in cold saline, freeze-dried in liquid N_2, and lyophilised before storage at -70 ^{o}C. The brains thus preserved were used to compare the effects of diet on brain lipid composition and also to correlate the changes occurring with febrile response induced by intravenous HLP or intraventricular PGE_2.

4.1.3.2. Preparation of Human Leucocytic Pyrogen

The endogenous pyrogen used in this experiment was made according to the method described by Borsook et al. (1977). 100 ml of human buffy coat cells, were stimulated for 18 hours with 3,0 ng/ml of purified Salmonella Thyphosa (Difco Laboratories, Michigan U.S.A.) at 37^{o}C in a water bath. After phagocytosis, the synthesised buffy coat cells were centrifuged at 2000 rpm for 30 minutes and the supernatant fluid containing leucocyte pyrogen was stored in a sealed pyrogen-free container at 4^{o}C. The endogenous

pyrogen released from the buffy coat cells was diluted (1:10) in pyrogen-free saline. The standard dose chosen was 0,5 ml /kg body weight.

4.1.3.3. Preparation of Prostaglandin E_2

1,0 ml PGE_2 dissolved in 95 per cent ethanol was provided by Upjhon Laboratories (Kalamazoo, Michigan, U.S.A.) and stored in a freezer at $-15^{o}C$. The PGE_2 solution was made up each day by dissolving 100 ul in 1,0 ml of 0,9 per cent pyrogen-free saline. From the stock solution 15 ul containing ± 2,80 ng PGE_2 was injected into the lateral ventricle of each rabbit regardless of body weight.

4.1.3.4. Temperature Measurements

Rectal temperature was measured by means of copper constant thermistor probes covered by polyethylene tubing and inserted 100 mm into the rectum of each rabbit. All thermocouples were calibrated by immersion in water against a certified mercury thermometer. The output was monitored at 10 minute intervals on an Easter-Line Angus data logger. Animals were permitted a period of >60 minutes to settle down and reach a thermal steady state before any injections were given. Recording thus consisted of 1 hour before and 3-4 hours after injections.

4.2. CHEMICAL METHODS

4.2.1. Brain lipid extraction

Using the Folsh et al. (1957) method, 100 mg dried brain powder was extracted for 2 hours with 5,0 ml $CHCl_3$:MeOH (2:1, v/v) by means of a magnetic stirrer. After centrifugation, the residue was re-extracted twice with 5,0 ml $CHCl_3$:MeOH (2:1), following the same procedure. To the pooled extract (15 ml) 3,0 ml 0,1 M NaCl was added, (to separate the two phases), and the solution gently mixed and centrifuged for 10 minutes. The upper phase was discarded, and the wall of the centrifuge tube was carefully rinsed with 2x1,5 ml MeOH, whereafter the centrifugation and discarding process was repeated. Next, the lower phase was transferred to a clean tube. The extracted total lipid was dried under N_2 in a water bath at 60 ^{o}C.

4.2.2. Thin-layer chromatographic separation of total lipid into Phospholipid.

Precoated silica gel G plastic backed plates, 200x200 mm, 0,25 mm layer (Machery-Nagel) were pencil-marked. The line of origin was drawn 15 mm from the bottom edge of the plate. A 10 mm margin was drawn at each lateral side of the plate, and vertical lines 30 mm apart. The marked plates were washed with $CHCl_3$:MeOH (1/1, v/v), air-dried and activated for 1 hour at 100^{o}C before use. The weighed brain lipid of ±10 mg, dissolved in 50 ul $CHCl_3$, was spotted on the line of

origin on the TLC plate, with a Hamilton syringe. Throughout the application of lipid solution a hair-drier was used to speed up the drying of applied spots. Phospholipid standard was applied with each sample on the same plates. The TLC plate was placed in a Camag tank, lined with chromatographic paper (Whatman N^{o}1) and saturated with a solvent system, Petroleum-ether(40-50^{o}):Diethylether:Glacial Acetic Acid (80:20:1, v/v) and developed for 45 min.
After development, the TLC plates were removed from the tank and dried in an oven at 160 ^{o}C for 3 min. Thereafter, the dried plates were exposed to iodine vapour in a closed plastic box for 1 minute. The total phospholipid (which remained at the origin in this solvent system) was scraped off into a clean tube and extracted from the silica gel with 2x5,0 ml $CHCl_3$:MeOH:H_2O (5:5:1, v/v). The lower phase of the pooled extract was dried over Na_2SO_4, transferred to a clean tube, evaporated under N_2 and finally dissolved in 100 ul $CHCl_3$.

4.2.3. Thin-layer separation of phospholipid classes

Separation of brain phospholipid (PL) classes was done by dissolving the extracted total PL in 100 ul $CHCL_3$ and chromatographing as described above, but using a different solvent system, $CHCl_3$:MeOH:Glacial Acetic Acid:H_2O (50:25:7:3 v/v). Phospholipid standards (Sigma) applied on the same plate were used to identify the various PL fractions.

4.2.4. Saponification and methylation of individual phospholipids

Fatty acid methylation was performed according to the method of Metcalfe and Smith (1961). After elution of brain posphatidylcholine and phosphatidylethanolamine from TLC plates, both fractions were saponified for 10 minutes at 90°C under reflux with 0,6 ml methanolic NaOH. Boron trifluoride (1,5 ml) was added carefully, while the mixture continued to boil under reflux for a further 5 minutes. The methylesters were extracted with the addition of 0,8 ml hexane under continuous boiling for another 1 minute. After cooling, saturated NaCl was added and the glass-stoppered tubes centrifuged for 2-3 minutes. The separated hexane layer was transferred to a clean tube dried with Na_2SO_4, and finally transferred to a vial, in which the extract was evaporated to dryness under N_2 at 60 °C. The brain phospholipid methyl esters were dissolved in 200 ul hexane and stored in capped vials until they were analysed.

4.2.5. Gas chromatograph

For gas chromatographic (GC) analysis, the brain samples were evaporated until dry under N_2 and redissolved in 200 ul hexane. Using a Hamilton microsyringe, 0,5 ul of brain methyl esters were injected through a septum into a Packard 427 gas chromatograph fitted with a coiled glass column (lenght 1,8 m and 4 mm in internal diameter).

The column was packed with 10% SP 2330 on 100/100 chromasorb WAW, through which passed an inert gas (mobile phase), such as nitrogen, at a flow rate of 40-80 cm^3/min. The column was kept in an oven at 220°C, thus volatilising the compound to be analysed. As the compound left the column, it passed through a flame ionization detector containing a mixture of hydrogen and air. The detector was kept at 220 °C and was linked to an amplifier and to a chart recorder (SP 4100 Spectro-physics integrator) which recorded the peaks as the compound passed through the detector. It is common practice to maintain the injector region of the column at slightly higher T° than the column itself, to ensure rapid and complete vocalisation of the sample. The fatty acid methylesters peaks were identified using retention volumes of known GC standards: Pufa-2, RM_3, NHIF, animals source (Supelco Inc. USA).

4.3. RESULTS

4.3.1. Introduction

This study investigate the effect of a dietary induced essential fatty acid deficiency state on brain fatty acid composition, and its consequences on febrile response to human leucocyte pyrogen and to prostaglandin E_2. The data is presented as follows;

(a) Details of body and brain weight of experimental animals used.

(b) Number of HLP, PGE_2 and saline injection preformed

(c) Data of fever response to HLP in control and EFA deficient rabbits.

(d) Data of fever response to PGE_2 in control and EFA deficient rabbits.

(e) Data on body temperature between control and EFA deficient animals.

(f) Data on brain total lipid content in control and EFA deficient rabbits.

(g) Data of brain phosphotadylcholine in control and EFA deficient rabbits.

(h) Data of phosphatadylethanolamine in control and EFA deficient rabbits.

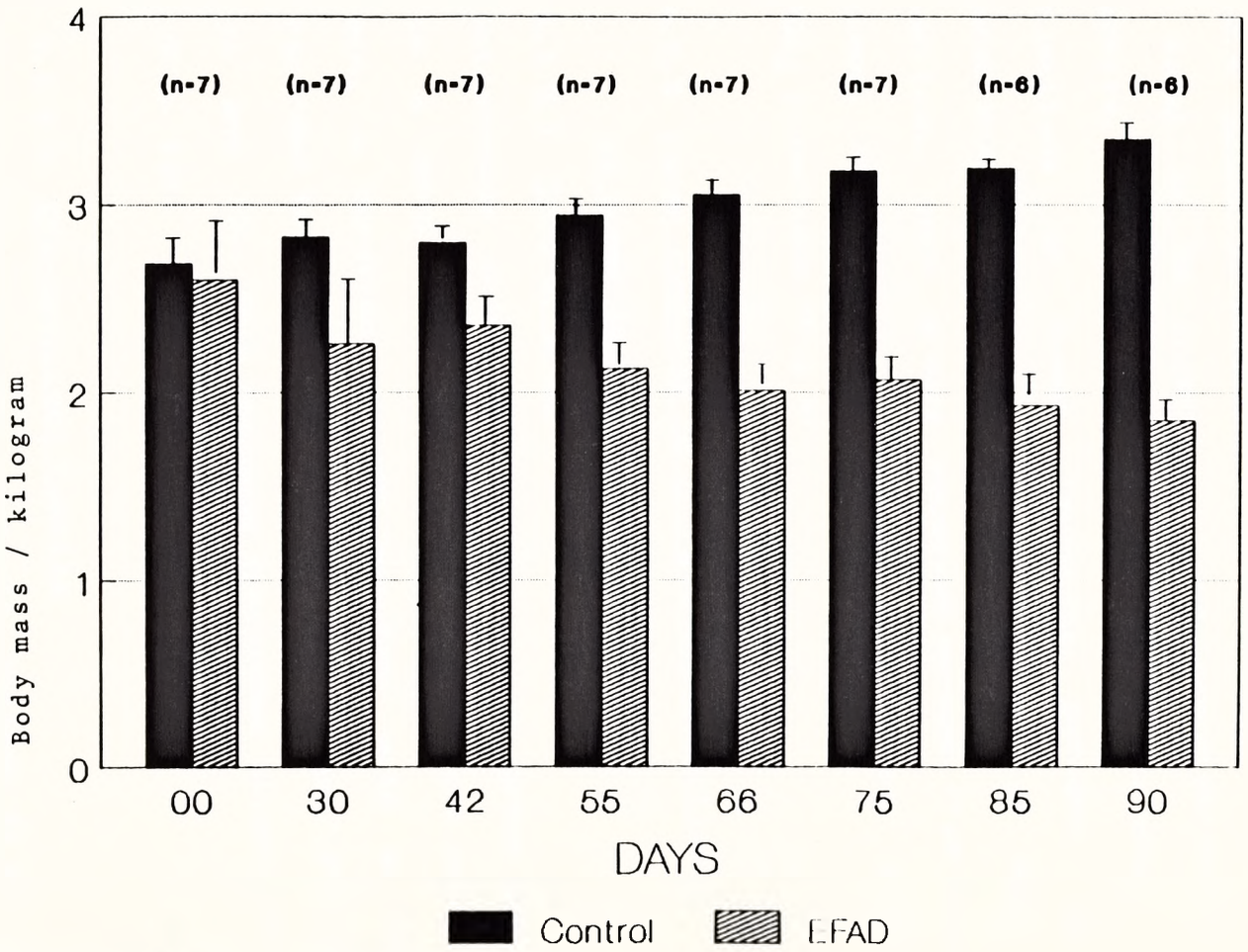
4.3.2 Presentation of the data

Results of fever response to HLP and PGE_2 were pooled together from each rabbit of each group and are presented as a series of graphs. All data was subjected to a student t-test and values of $P<0,01$ were considered significant. The data on brain total lipid, phosphatadylcholine (PC) and phosphatidylethanolamine (PE) is presented in a series of tables. Results were analysed statistically, as mentioned above.

4.3.3 Weight of body and brain

After 30 days on the experimental diet, rabbits exhibited all the symptoms associated with EFA deficiency (Burr and Burr, 1929, 1930), i.e. loss of body weight, diminished growth, accompanying scaly dry skin, loss of body and facial hair, loose stools and ataxia. During this period 3 rabbits died from pulmonary infection (Hopkins, Witter and Nesheim, 1963). Consequently, the data is presented on seven EFA-deficient rabbits instead of ten. In comparison, the control rabbits exhibited none of these symptoms. Significant differences in body weight between the two groups were recorded (Fig. 5) after 30 days on the EFAD diet. The loss in body weight of EFA deficient rabbits cannot however be attributed to anorexia, as a difference in food intake only occurred during the initial stage of the feeding experiment. The mean food intake measured over the period of 4 days after 2 weeks on the EFA-deficient diet was 343,0±68,0 gr/ 24 hours / 7 rabbits, compared to 589±81,0 gr/ 24 hours/7 control rabbits. After 30 days however, the food consumption increased to 474±60 gr/24 hours/7 rabbits. During the latter stage EFA-deficient animals consumed more food than control rabbits, although this food was burnt up to meet the caloric requirements in their diseased state, and none was used for growth or fat synthesis (Burr and Burr, 1930). As opposed to body weight, there was no significant difference in

Fig. 5. Comparison in body weights between rabbits fed the standard or essential fatty acid deficient diet.



Values are means ± SE. of 7 or 6 rabbits.

brain weight between the two groups of animals. The mean weight at the time of being put down was 9,08±0,09 gr for the rabbits on normal diet and 8,67±0,07 gr for those on deficient diet.

4.3.4 Number of experiments carried out

A total of 94 injections were administrated to 14 rabbits. Both groups received 17 intravenous (IV) injections of human leucocyte pyrogen and 17 cerebroventricular injections ((CV)) of PGE_2. In addition, both groups received pyrogen free-saline 4 times, administrated in the same manner as HLP or PGE_2 injections (Fig 6).

4.3.5 Fever response to intravenous injections of human leucocyte pyrogen (HLP).

4.3.5.1 Control group

Intravenous injections in the control group gave rise to a monophasic fever with a delay in the onset of 20 min, a duration of 180 minutes, with an average recorded fever height of 0,9-1,0 ^{o}C (Figs.7,8,9,10, 11 and 12). This response diminished slightly 90 days (Fig. 12).

4.3.5.2. EFA-deficient rabbits

In contrast to the controls, between 42 and 52 days the malnourished rabbits exhibited a dramatic increase (Figs. 7,8 and 9), followed by a progressive fall by

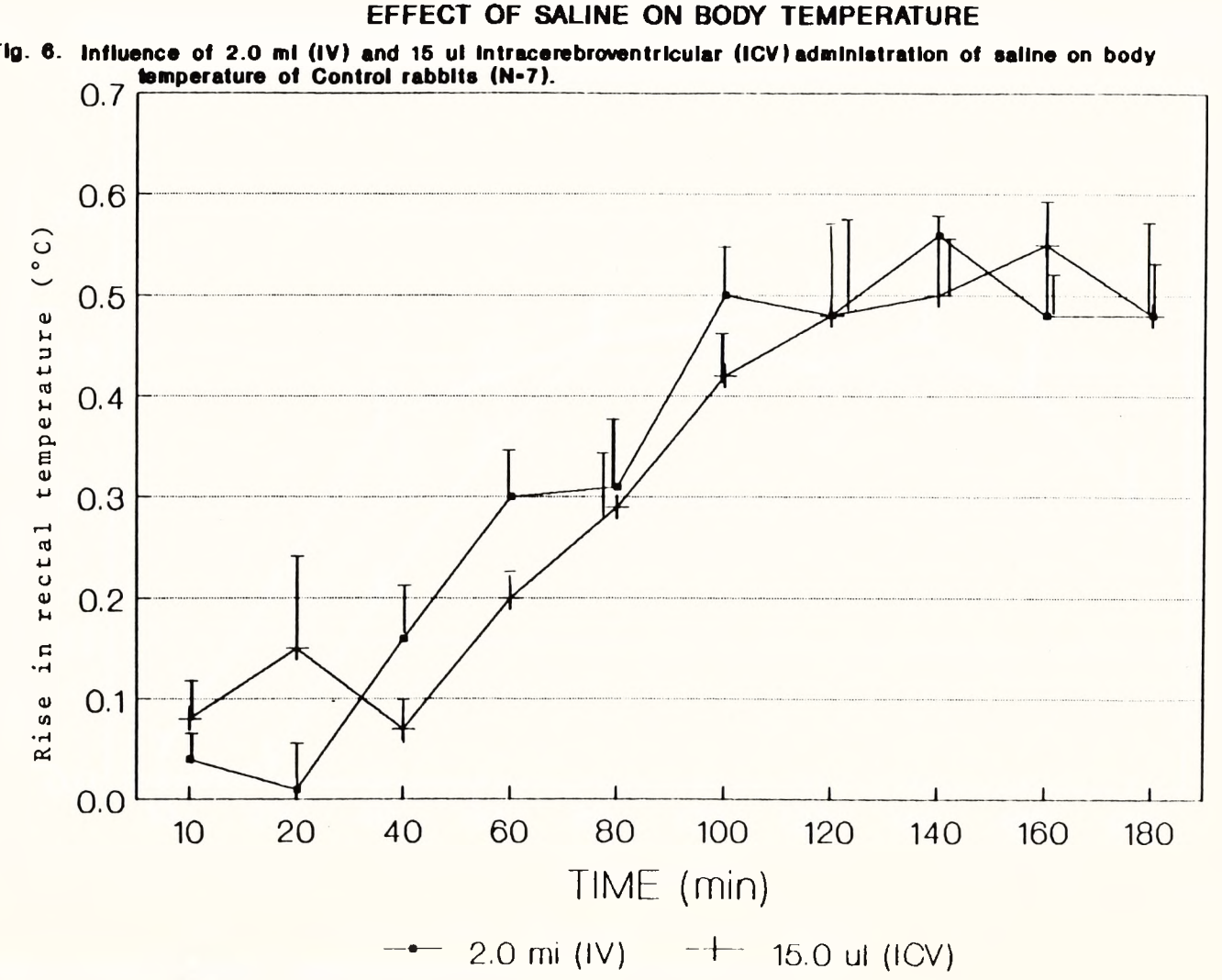


Fig. 6. Influence of 2.0 ml (IV) and 15 ul intracerebroventricular (ICV) administration of saline on body temperature of Control rabbits (N-7).

Values are means ± SE . Saline injected at time 0.

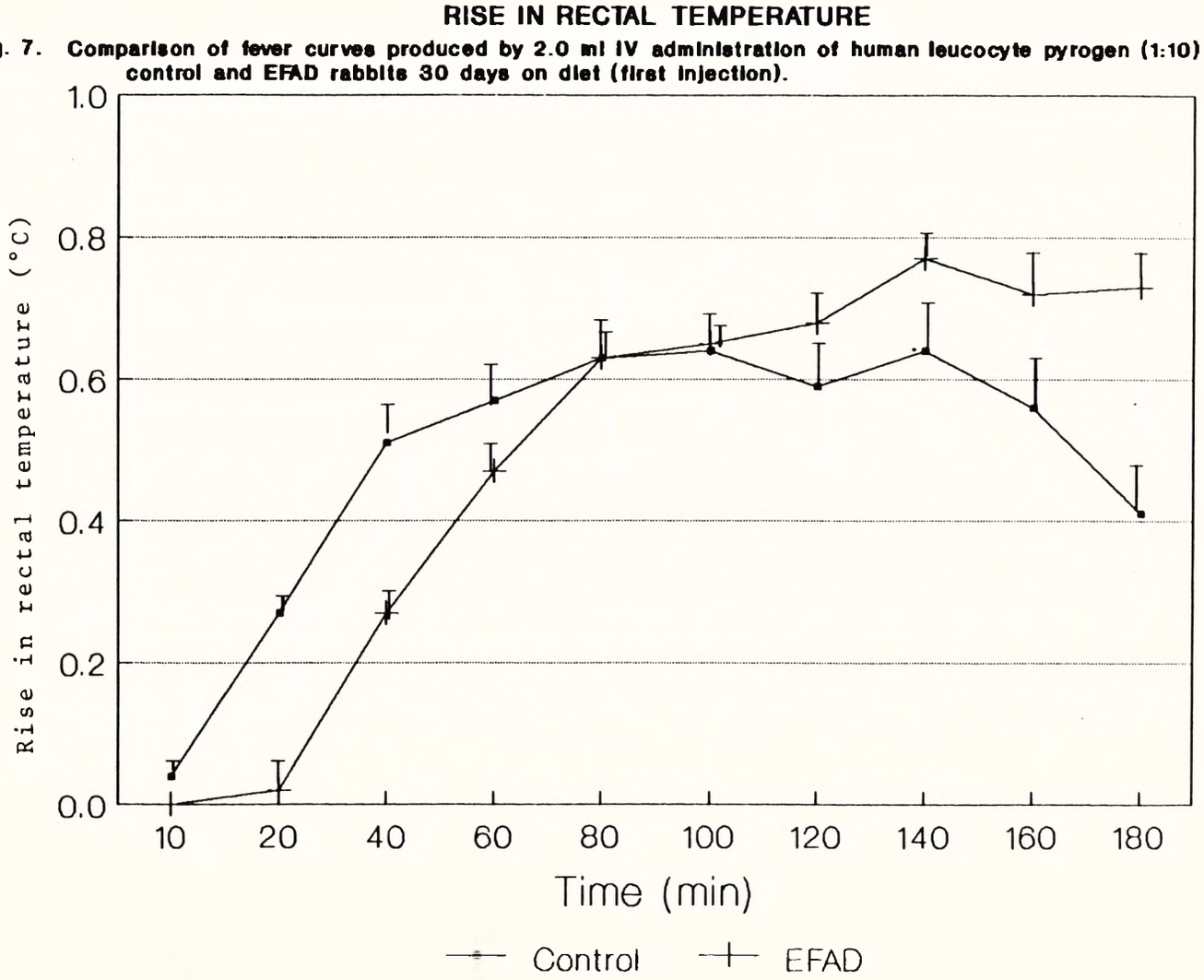


Fig. 7. Comparison of fever curves produced by 2.0 ml IV administration of human leucocyte pyrogen (1:10) in control and EFAD rabbits 30 days on diet (first injection).

Values are means ± SE of 7 rabbits (each group). Pyrogen injected at time 0.

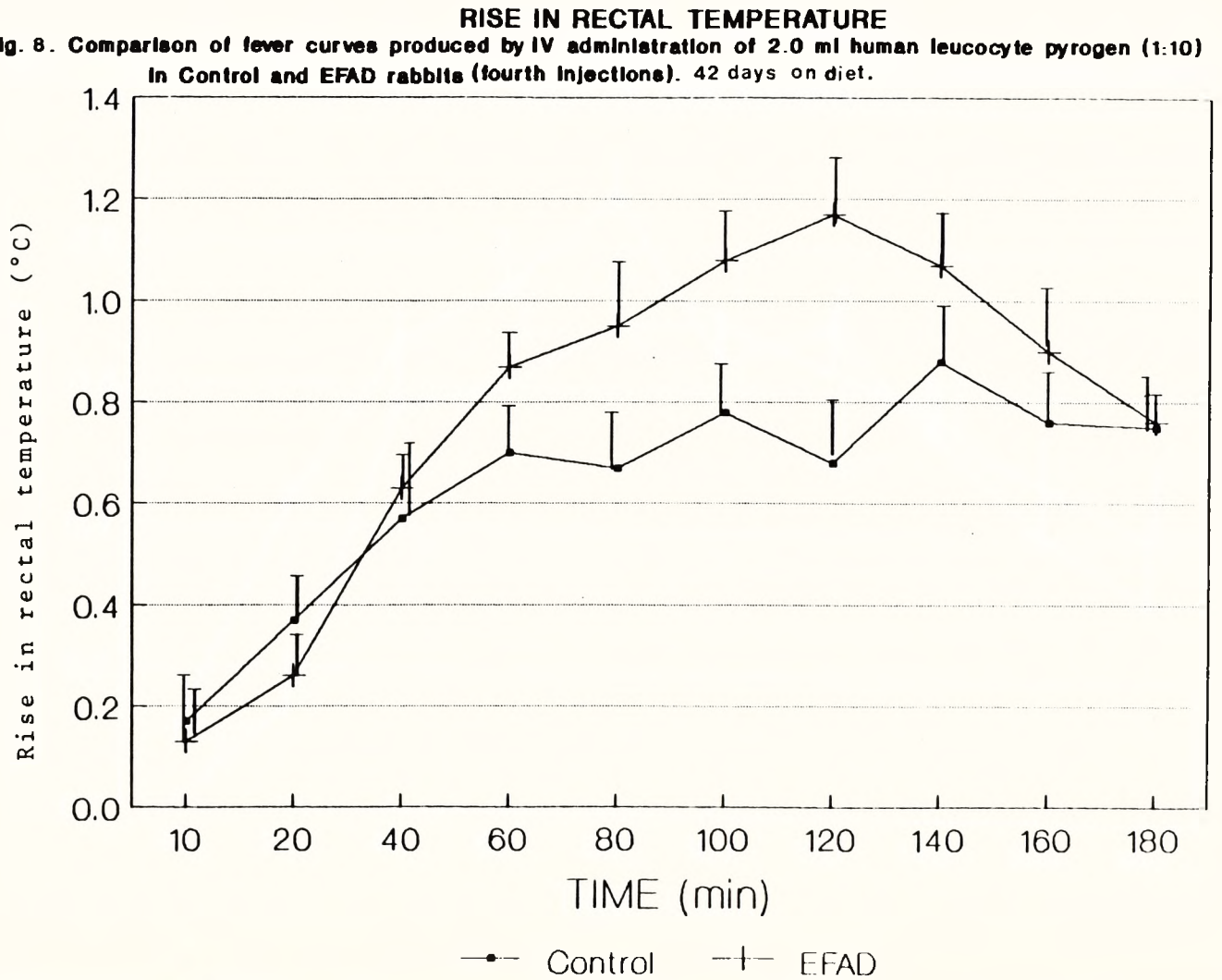


Fig. 8. Comparison of fever curves produced by IV administration of 2.0 ml human leucocyte pyrogen (1:10) in Control and EFAD rabbits (fourth injections). 42 days on diet.

Values are means ± SE of 7 rabbits (each group). Pyrogen injected at time 0.

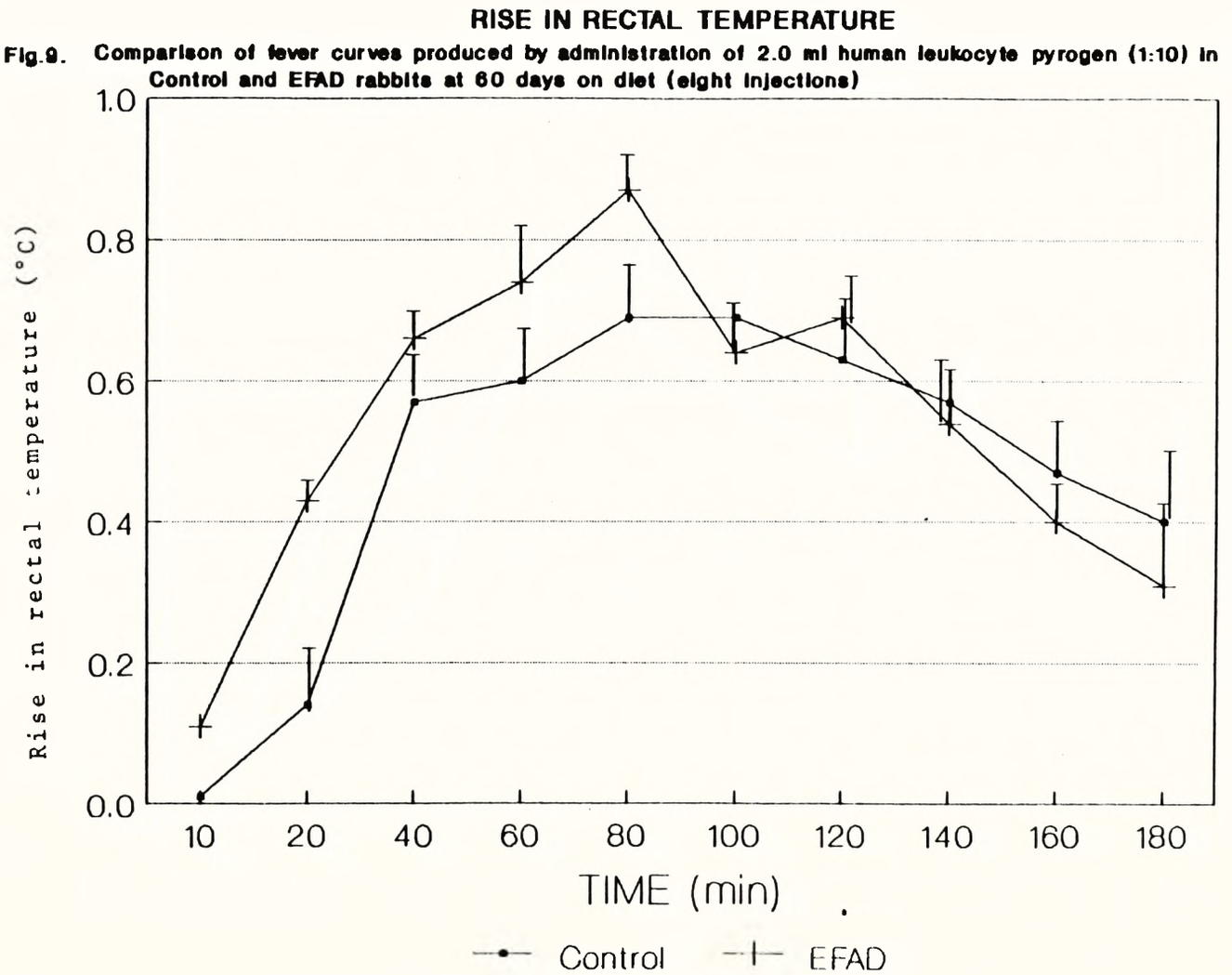


Fig.9. Comparison of fever curves produced by administration of 2.0 ml human leukocyte pyrogen (1:10) in Control and EFAD rabbits at 60 days on diet (eight injections)

Values are means ± SE of 7 rabbits (each group). Pyrogen injected at time 0.

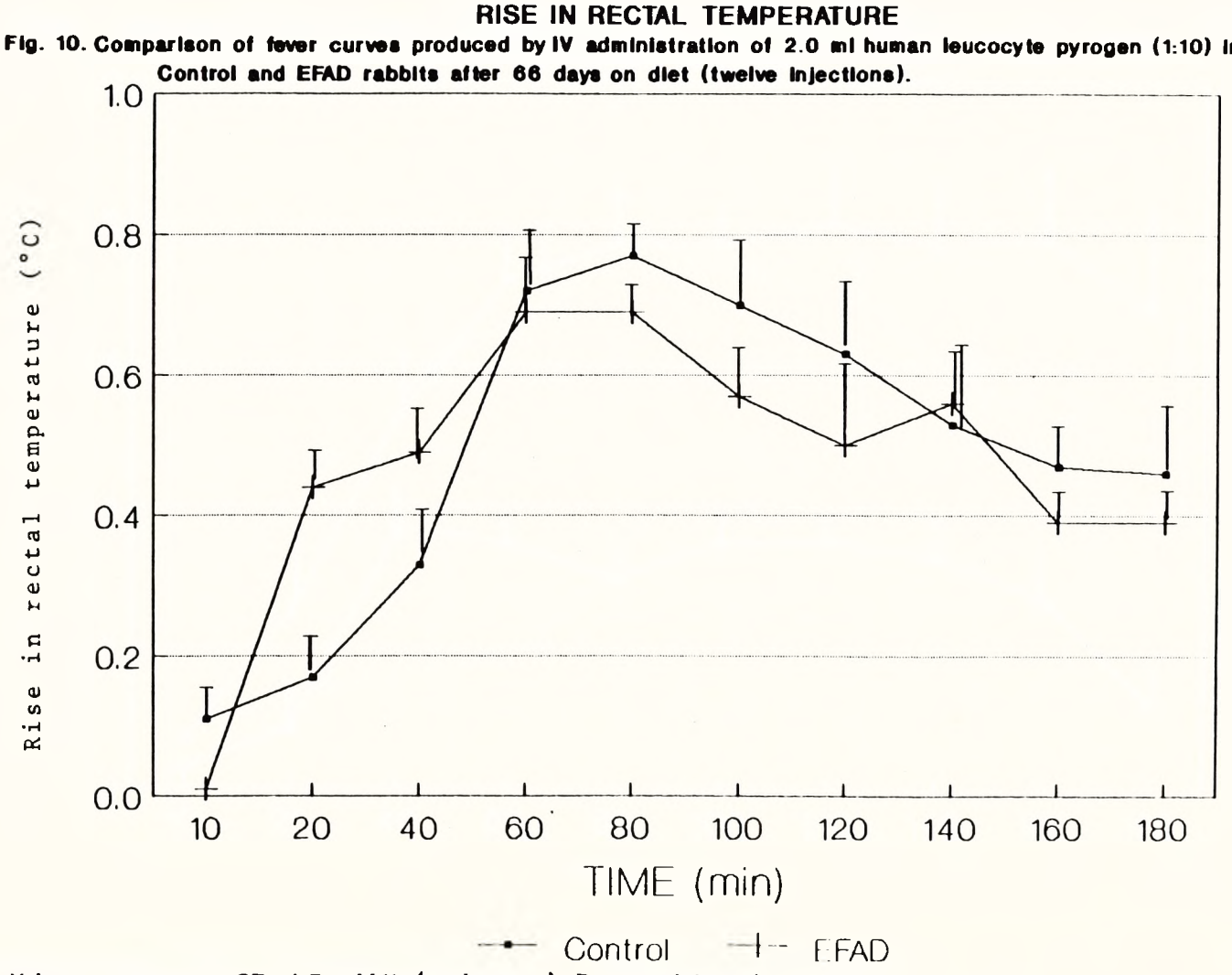


Fig. 10. Comparison of fever curves produced by IV administration of 2.0 ml human leucocyte pyrogen (1:10) in Control and EFAD rabbits after 66 days on diet (twelve injections).

Values are means ± SE of 7 rabbits (each group). Pyrogen injected at time 0.

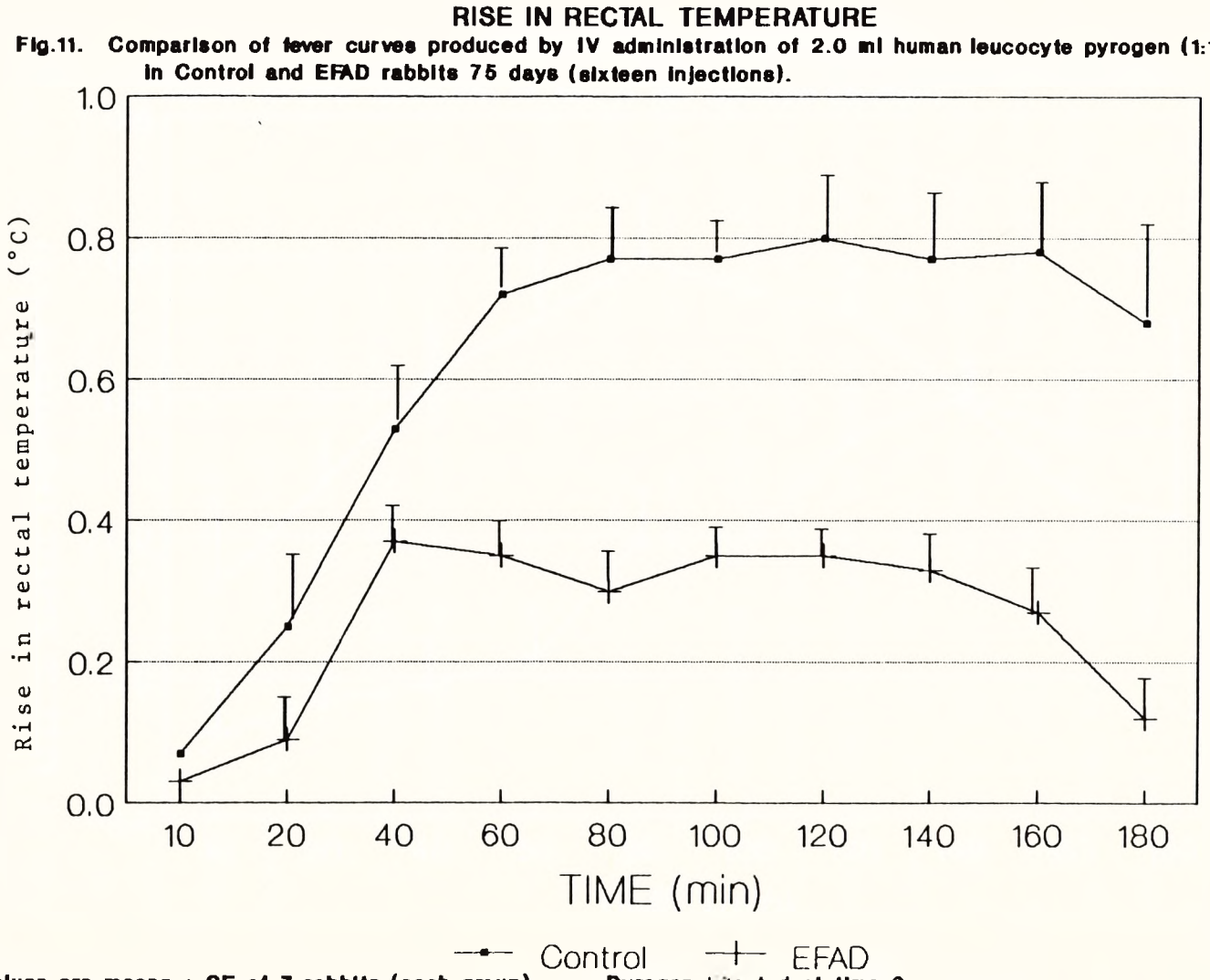


Fig.11. Comparison of fever curves produced by IV administration of 2.0 ml human leucocyte pyrogen (1:10) in Control and EFAD rabbits 75 days (sixteen injections).

Values are means ± SE of 7 rabbits (each group). Pyrogen injected at time 0.

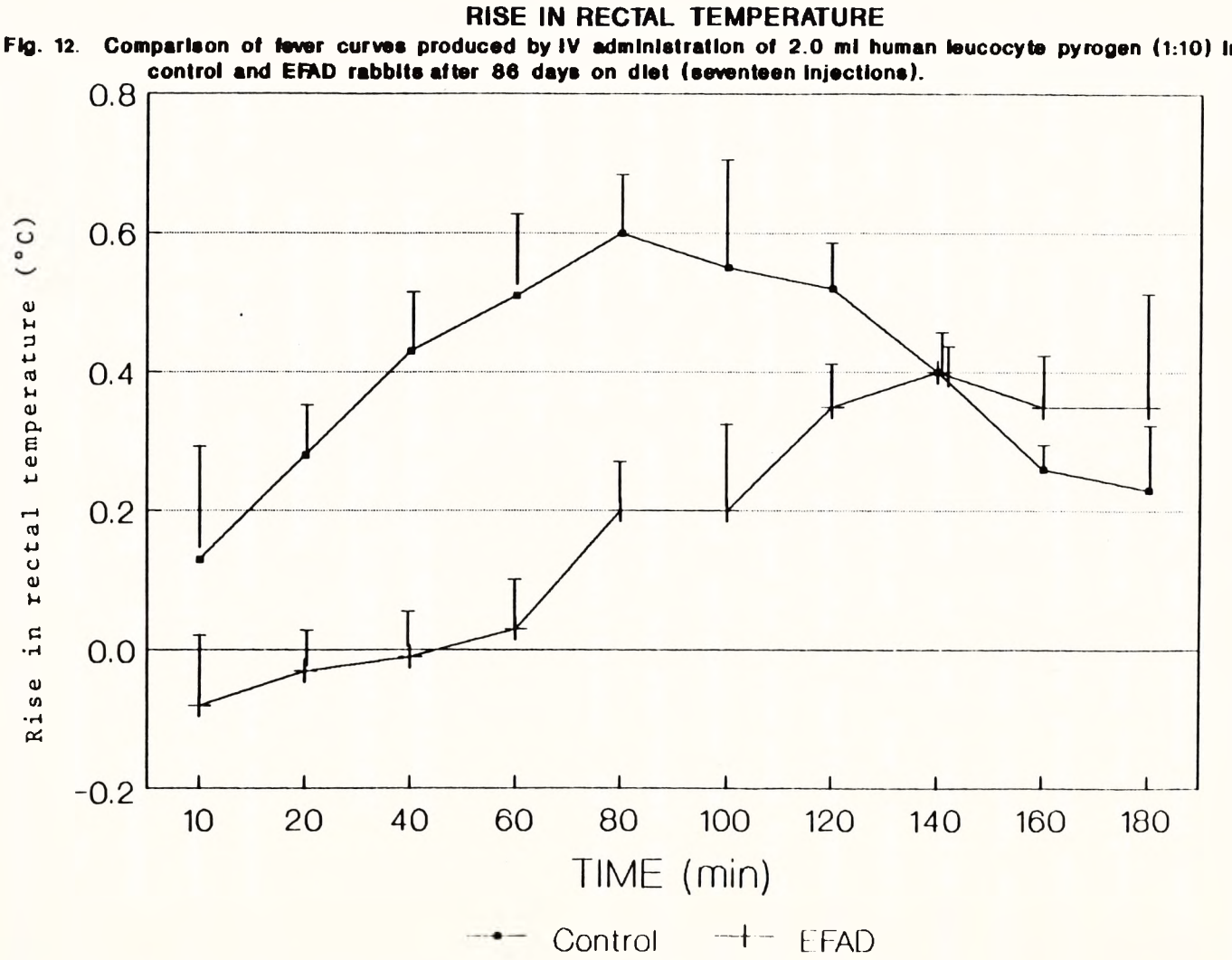


Fig. 12. Comparison of fever curves produced by IV administration of 2.0 ml human leucocyte pyrogen (1:10) in control and EFAD rabbits after 86 days on diet (seventeen injections).

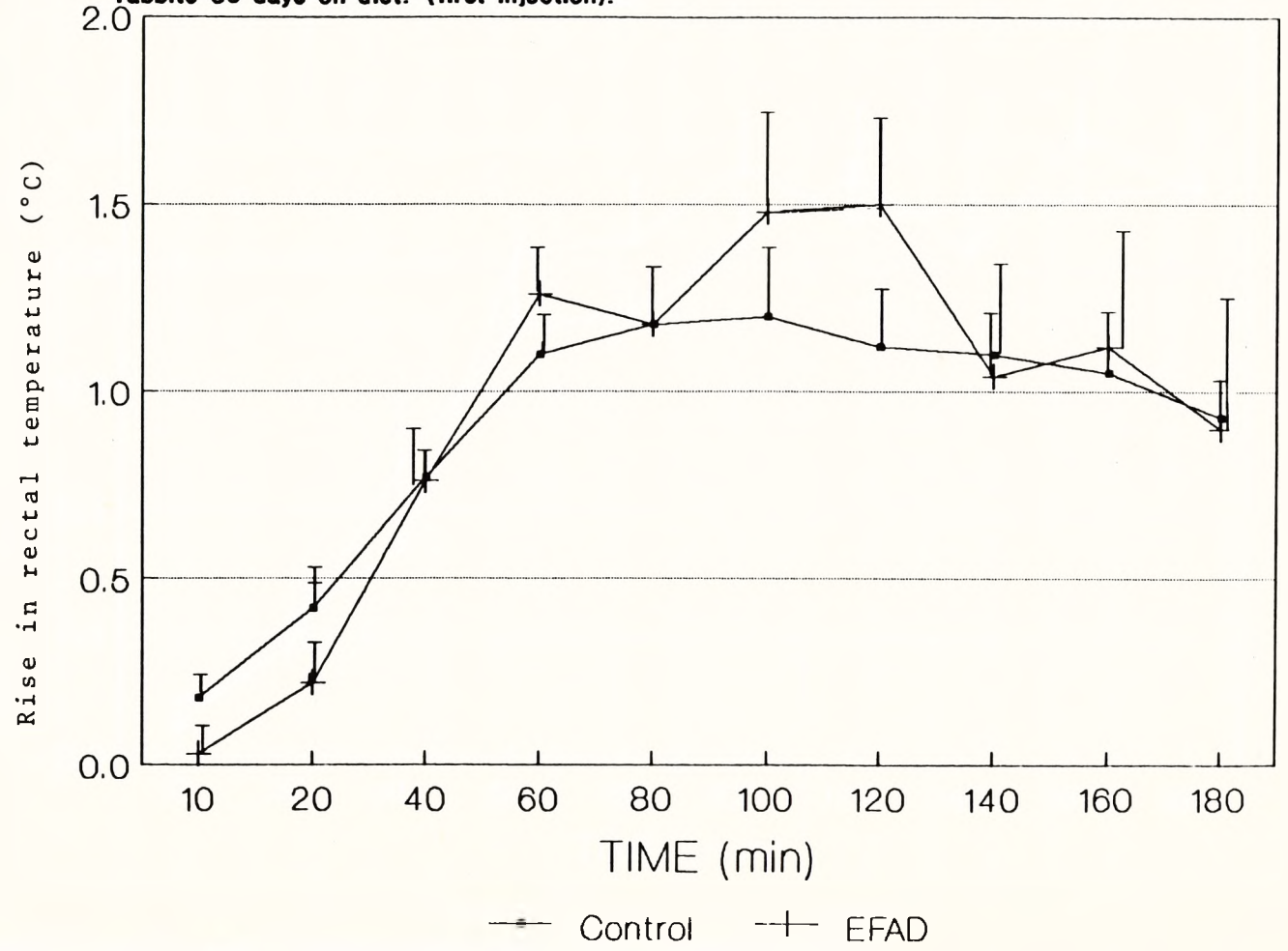
Values are means ± SE of 7 control and 4 EFAD rabbits Pyrogen injected at time 0.

75 days (Fig. 11) in the onset (20 minutes) and height (90 minutes) of the fever in response to HLP, which led to "negative fever" response by 86 days (Fig. 12). This indicated that a lack in the acyl chain of linoleic acid affected the assemblage process, concerned with the growth, maintenance and repair of the living body as a whole. Thus the organism was unable to counteract adequately the stimuli which had been subjected. Deficiency in food intake, combined with progressive injections of an infective agent, affected the alarm reaction of the rabbits and rendered them unable to generate a febrile response to blood-borne pyrogen. This led to tolerance by 79 days, followed by a state of exhaustion at 86 days. Tolerance was thus achieved by decreased phagocytic activity resulting from nutritional deficiency in essential fatty acids.

4.3.6 Fever response to cerebroventricular injections of PGE_2 in EFA-deficient and control rabbits.

There was little difference in the response of the control and EFA deficient rabbits, to intraventricular injections of 2,80 ng PGE_2, throughout the experiment. The fever height increased to 0,9 ^{o}C, with a delay of onset of 30 min and a duration of 180 min (Figs 13 and 14). Since PGE_2 is injected directly into the lateral ventricle of the brain,

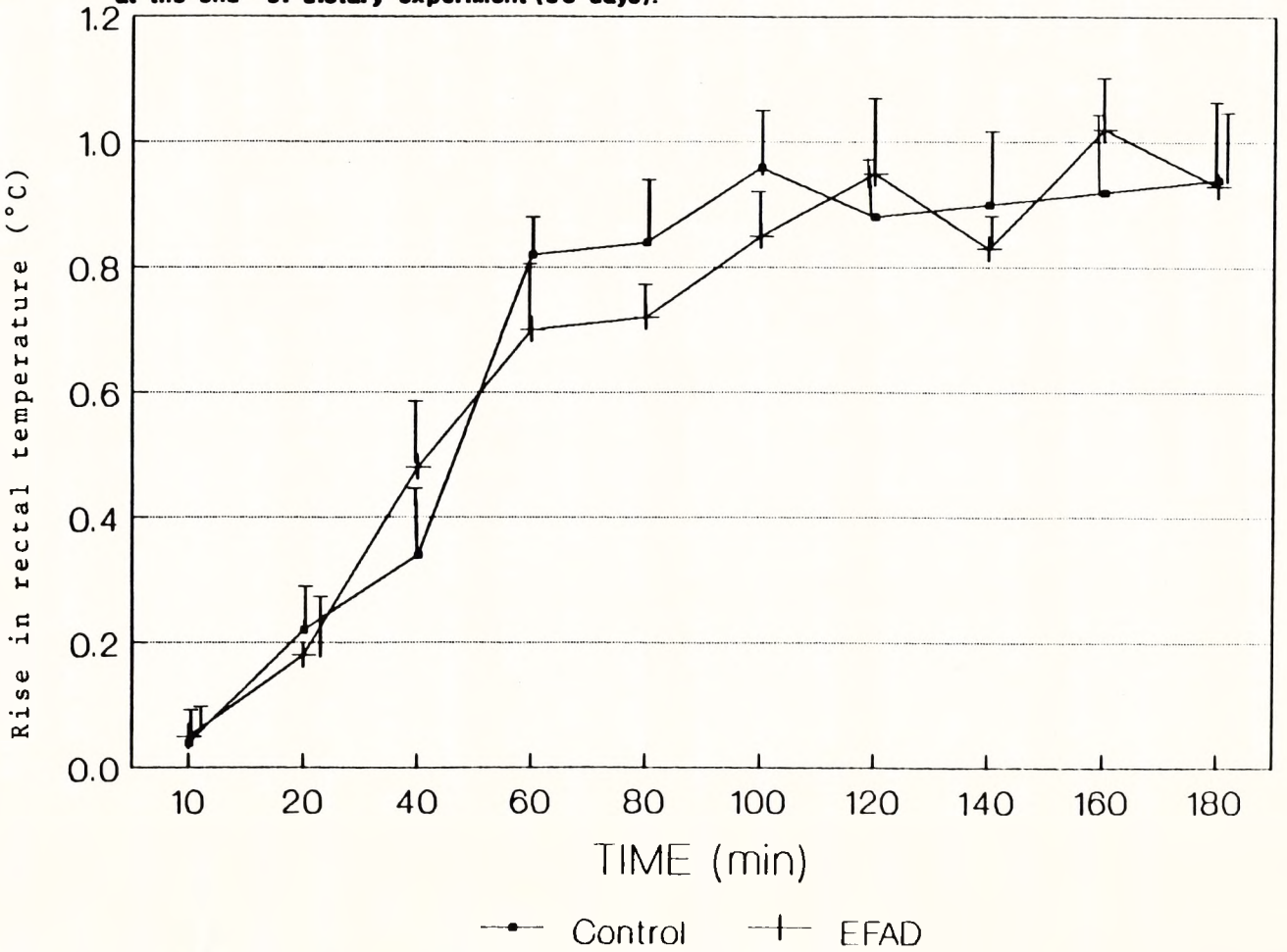
Fig. 13. Comparison of fever curves induced by ICV administration of 15 µl PGE_2 in control and EFAD rabbits 35 days on diet. (first injection).



Values are means ± SE of 7 rabbits (each group). PGE_2 injected at time 0.

EFFECT OF PGE_2 ON FEVER

Fig. 14. Comparison of fever curves produced by ICV administration of 15 µl PGE_2 in control and EFAD rabbits at the end of dietary experiment (90 days).



Values are means ± SE of 6 rabbits (each group). PGE2 injected at time 0.

The unchanged fever response implies that the EFA deficient diet was unable to affect the thermoregulatory centre, owing to the protective effect of the blood-brain barrier.

4.3.7 The effect on body temperature of EFA-deficient diet

Despite the normal environmental temperature of 20±1 ^{o}C, a progressive drop in the body temperature of EFA deficient rabbits was recorded from 52 days after introduction of the diet (Fig. 15). No changes in the body temperature occurred in rabbits fed the normal diet (Fig. 15). This finding suggests that prolonged malnutrition increases lipid oxidation, while oxidation of other substrata diminishes. Under these conditions glucose utilisation became negligible in the face of the decreased rate of desaturation of TG->18:2(w6)->18:3(w6). Inhibition in this process curtailed heat production and consequently, increased thermal loss. The thermal stability of the body appears to be closely connected to the presence of linoleic acid, and therefore absence in the compound influenced metabolic control, such as respiratory quotient (R.Q.). A drop in thermolysis (inhibition of muscle glycogenesis) combined with a a fall in thermogenesis (inhibition of lipolysis from adipose tissue) provoked by lack of dietary linoleic acid resulted in drop in body temperature (Fig. 15).

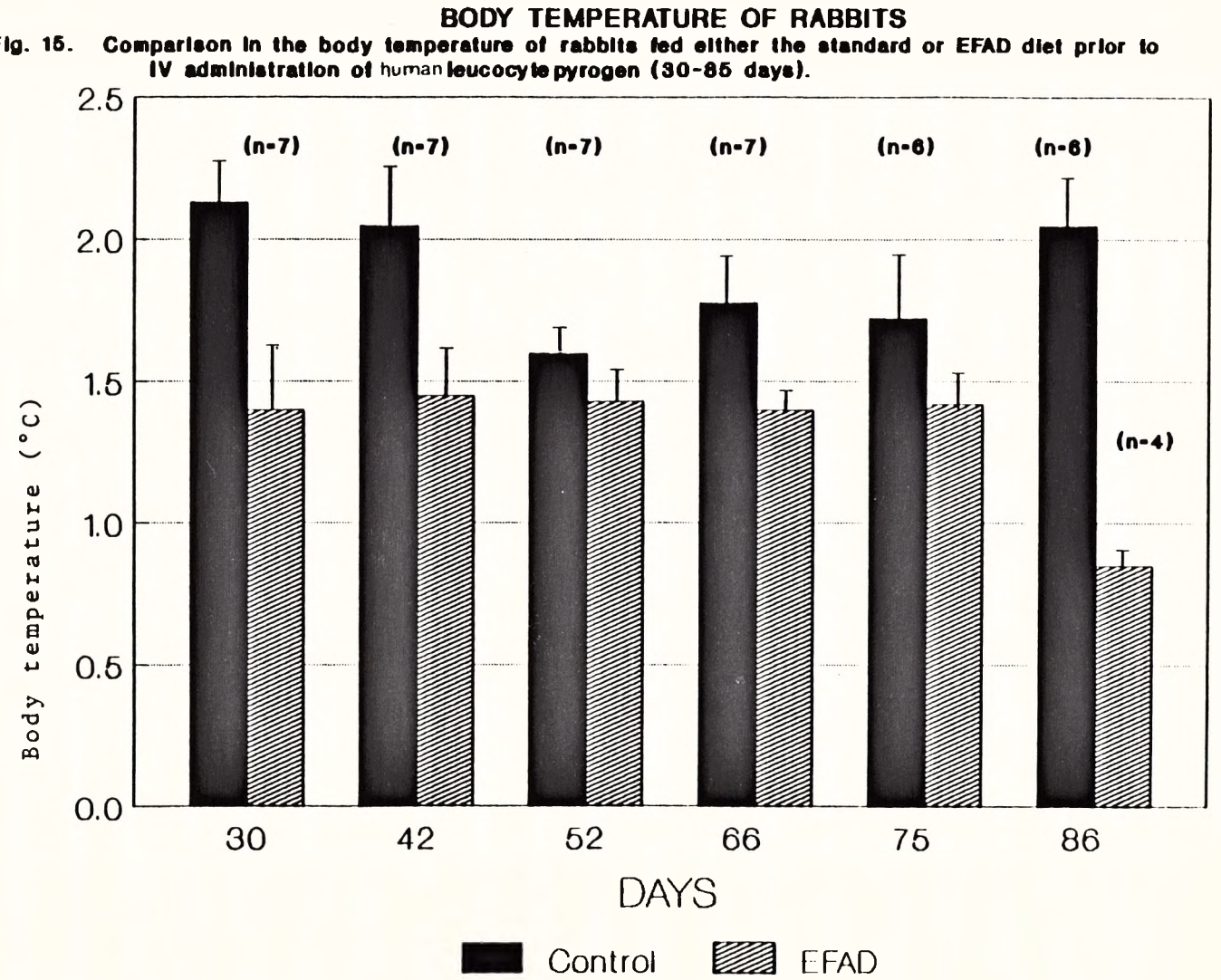


Fig. 15. Comparison in the body temperature of rabbits fed either the standard or EFAD diet prior to IV administration of human leucocyte pyrogen (30-85 days).

Errore bars are means ± SE. Body temperature expressed as 37 °C at time 0.

The endogenous "hypothermia" consequently prevented the development of fever in response to endogenous pyrogen.

4.3.8 Fatty acid composition of total brain lipid in control and EFA-deficient rabbits

No difference in the composition of total brain fatty acid was found between the two groups of rabbits (Table 5), due to the fact that brain lipids are largely formed before and immediately after birth (Galli, White and Paoletti, 1970) and are less readily affected by environmental factors, such as diet. The mere fact that the brain is permeable to the passage of linoleic acid and its derivatives and incorporation into cerebral lipid occurs more easily than acetate (Mead and Dopeshwarkar, 1972), implies that essential fatty acid is required for the maintenance of brain integrity and function. If this requirement cannot be met, from the periphery due to lack of dietary 18:2(w6) the brain will ensure its own integrity by synthesising the required fatty acids within the brain phospholipids. Since the latter contains a high level of linoleic acid, EFA-deficiency symptoms might shift to phospholipid classes, i.e. PC or PE rather than to total brain fatty acids. This interrelation between phospholipid classesess led to an investigation of phosphatadylcholine (PC) and phosphatadylethanolamine (PE) in an induced disease condition,

Table 5.

FATTY ACID COMPOSITION OF TOTAL BRAIN LIPIDS IN CONTROL AND EFA DEFICIENT RABBITS SUBJECTED TO MULTIPLE INTRAVENOUS (HLP) AND INTRAVENTRICULAR (PGE_2) INJECTIONS.

Fatty acid	Control (n=5) SE	EFAD (n=5) SE	Statistical Significance (t-test)
14:0	1,37±0,05	1,86±0.05	p < 0,005
16:0	24,41±0,60	24,15±0.25	
18:0	20,84±0,70	25,20±1,21	p < 0,005
18:1n9	24,49±0,54	26,13±0,39	
18:2n6	1,66±0,05	1,82±0,28	
18:3n6	0,22±0,04	0,22±0,02	
18:3n3	2,14±0,24	2,50±0,02	
20:3n6	0,49±0,08	0,45±0,02	
20:4n6	8,24±0,55	8,62±0,06	
20:5n3	3,67±0,23	3,84±0,06	
22:4n6	3,51±0,39	4,52±0,19	
22:5n6	4,00±1,00	4,83±0,16	
Unid.poly	2,59±0,44	1,52±0,20	

Values represents the relative percentage of fatty acids measured on GLC and the average of five determinations.
Test SE±standard error of the means

using human leucocyte pyrogen (HLP) in the presence of EFA- deficiency state.

4.3.9 Phosphotadylcholine status (PC)

The most obvious change observed in PC induced by EFA deficiency (Table 6), were the rapid synthesis of eicosatrieonic acid 20:3(w9) in order to replenish the diminishing store of arachidonic acid 20:4(w6). Consequently, inhibition occurred of the higher polyunsaturates 22:4(6) and 22:5(w6), which are dependent for their formation on 20:4(w6). The competitive inhibition induced by the elevated content of 20:3(w9) also affected dietary and endogenously produced linolenic acids, i.e. 18:3(w6) and 18:3(w3), as both had been suppressed (Table 6). However, 20:3(w9) could not affect linoleic acid content nor saturated fatty acids and therefore they remained unchanged.

4.3.10 Phosphatadylethanolamine status (PE)

As opposed to PC, PE (the major unsaturated phospholipid fatty acid in the brain) showed a substantial increase in the saturated fatty acids (16:0, 18:0, 18:1), combined with a fall in linoleic acid 18:2(w6). However, no change in the 18:3(w6), 18:3(w3) and PUFA occurred (Table 7). As a whole, these findings demonstrate that active transfer of fatty acids between PC and PE secures the stability

Table 6.

FATTY ACID COMPOSITION OF PHOSPHATADYLCHOLINE (PC) IN CONTROL AND IN EFA DEFICIENT RABBITS, SUBJECTED TO MULTIPLE INTRAVENOUS (HLP) AND INTRAVENTRICULAR (PGE_2) INJECTIONS.
(After 3 months experiments.)

Fatty acid	Control (n=6) SE	EFA (n=6) SE	Statistical Significance (t-test)
14:0	1,85±0,31	2,35±0,27	
16:0	42,25±0,75	45,15±1,90	
18:0	13,80±0,63	13,70±0,20	
18:1n9	33,10±1,03	29,75±1,19	
18:2n6	2,09±0,09	2,20±0,28	
18:3n6	0,90±0,09	0,75±0,11	
18:3n3	0,70±0,20	1,00±0,12	
20:3	0,80±0,11	1,60±0,35	
20:4n6	2,65±0,17	1,45±0,29	$p < 0,025$
20:4n6	0,78±0,10	-	
22:5n6	1,50±0,14	1,80±0,22	

Values represents the relative percentage of fatty acids measured on GLC and the average of six determinations.
Test SE±standard error of the means.

Table 7.

FATTY ACID COMPOSITION OF PHOSPHATADYLETHANOLAMINE (PE) IN CONTROL AND IN EFA DEFICIENT RABBITS,SUBJECTED TO MULTIPLE INTRAVENOUS (HLP) AND INTRAVENTRICULAR (PGE_2) INJECTIONS (After 3 month experiments).

Fatty acid	Control (n=6) SE	EFAD (n=6) SE	Statistical Significance (t-test)
14:0	1,30±0,24	1,65±0,30	ns
16:0	11,00±0,93	14,55±0,57	ns
18:0	28,60±1,15	25,45±1,15	p < 0,005
18:1n9	26,30±0,71	27,15±0,71	ns
18:2n6	6,60±1,06	0,80±0,20	p < 0,005
18:3n6	0,75±0,11	0,80±0,10	ns
18:3n3	2,80±0,18	2,80±0,28	ns
20:3	0,45±0,03	1,60±0,29	p < 0,005
20:4n6	9,35±0,78	9,00±0,44	ns
20:5n3	5,60±0,16	-	
22:4n6	5,50±0,20	5,30±0,21	ns
22:5n6	4,85±0,30	5,85±0,32	ns
Unid.poly.	3,15±0,65	5,05±0,44	

Values represents the relative percentage of fatty acids measured on GLC and the average of six determination.
Test SE±standard error of the means.

of brain linoleic acid content of PC during an EFA-deficiency state. Thus securing complete brain integrity and function.

4.4. DISCUSSION

While the influence of nutritional state on the course of the infectious process has been emphasised repeatedly, less attention has been paid to the influence of infection on nutritional requirements. The nutritional factors which have been shown to play a decisive and specific role in resistance to the infectious process leading to physiological disturbances, have been attributed to: vitamin deficiency (Heiss, 1932), endocrine disturbences (Schneider, 1946; Lurie, 1950; Kempeneers, Nizet and Dumont, 1953), essential amino acid deficiency (Dubos et al., 1954). and protein deficiency (Guggenheim and Buechler, 1947). However, nutritional factors (as mentioned above) which are essential for growth and reproduction, rarely, if ever, play a decisive role in the resistance to infection. Contrary to common belief that increased susceptibility to infection can be induced by protein depletion (Cannon, 1950) as it results in decreased phagocytic activity (Milles and Cottingham, 1943) and diminished output of leucocytes in response to injection of bacteria (Wissler, 1947; Asirvadham, 1948), the protein content of the diet does not have a direct bearing on resistance to

infectious disease (Howie, 1949; Schneider, 1945, 1946; Radcliff, 1954; Gugenheim and Buechler, 1947; Hoffman-Goetz and Klüger, 1979). This is further supported by the present study. The diet used in this experiment was rich in protein, carbohydrate, vitamins and salt, but poor in unsaturated fatty acid 18:2(w6) and rich in saturated fatty acid (14:0). These changes in the dietary fat composition affected the host/fuel relationship in such a way as, in the first place increase the susceptibility to infection by blood-born pyrogen, and in the second place to play a decisive role in resistance to the same pyrogen. The loss of body weights throughout the feeding experiment, without impairing food intake, indicates that this particular nutritional regime affected the biochemical transducing signals around which the body temperature is regulated. The prolonged absence of dietary 18:2(w6) led to the uncoupling of phosphorylation, resulting in decreased combustion of CO_2 and O_2. The drop in both led to altered R.Q., which in turn led to the fall in body temperature (Benedict, 1915), and consequently induced hypothermia in these rabbits. Thus EFA-deficient rabbits were unable to generate a febrile response to intravenously administered HLP, although they responded with fever to intraventricular injections of PGE_2. In spite of the EFA-deficient diet, the ability of the hypothalamus to raise the body temperature was retained to a great extent,

when it was centrally initiated, owing to the unchanged content of brain PC linoleic acid, and PE linolenic and arachidonic acids whereby the brain was capable of retaining its particular unsaturation. Neverthless, it failed to produce prostaglandin in response to endogenous pyrogen (Stitt, 1986). Since the central nervous system is considered to modulate or mediate the host response (Turchik and Bornstein, 1980; Lambert, Harrel and Achtenberg, 1981), defective febrile response to HLP can be attributed to the rise in brain 5,8,11, eicosatrieonic acid level, which is the chemical marker of a EFA-deficiency state (Holman, 1960) and a known inhibitor of 20:4(w6). Inhibition of the 20:4(w6) affected the formation of PGE_2, which is the neural mediator of fever produced by natural pyrogenic stimulus (Stitt, 1986). Furthermore, a lack of both the carboxylic and acyl groups of linoleic and linolenic acid in PC led to defective antibacterial activity of the essential fatty acid (Nieman, 1954), causing the organism to lose its capacity to generate a febrile response to endogenous disease. This led to the recognition of the existence of a relationship between the diseased state and nutritional requirements. In this study this requirement has been identified as essential fatty acids, as impairment of these compounds affects all biological membranes, resulting in failure of the organism to adequately conunteract the stimuli or stresses to which it was subjected, and consequently this led to death.

5.0. PLASMA LIPID INVOLVEMENT IN THE GENESIS OF FEVER

5.1. MATERIALS AND METHODS

5.1.1 Experimental Animals

Five male New Zealand (NZ) rabbits weighing between 2,0 and 2,5 kg, exhibiting a body temperature of 38,88±0,08°C were fed a standard commercial diet of rabbit pellets (Table 1) containing 50 per cent linoleic acid (Table 8). Water and feed was available ad libitum to each rabbit. Animals were housed separately in stainless steel cages and kept on a 12:12/ hours photophase: scotophase regime. To minimise the sudden stress during the experimental period, rabbits were acclimatised to restraining boxes and to thermocouple while a shame test was performed (Kaplan and Timmons, 1962) to eliminate any interference by the carrier solvent (saline) (Fig. 16, Table 9). Each animal was weighed before and after the fever experiment.

5.1.2. Fever Experiment

5.1.2.1. Experimental protocol

Using the same method as described in paragraph 4.1.3.1., the acclimatised rabbits, were first injected with 2,0 4,0 and 6,0 ug/kg purified Salmonella Thyphosa to establish a dose response curve to endotoxin. Rabbits thus exposed to endotoxin were rested for two weeks, before being subjected to daily systemic intravenous (I.V.) injection of 2,0 ug/kg

Table 8 .

FATTY ACID COMPOSITION OF RABBIT STANDARD DIET

Fatty acid	Standard diet
14:0	0,30
14:1	0,10
16:0	16,50
16:1	0,70
18:0	2,0
18:1n9	27,95
18:2n6	50,90
18:3n3	-
20:0	0,65
20:4n6	-
24:0	0,50
22:5n6	0,40

* Values are the mean of two lipid extractions expressed as a percentage of the respective fatty acids.

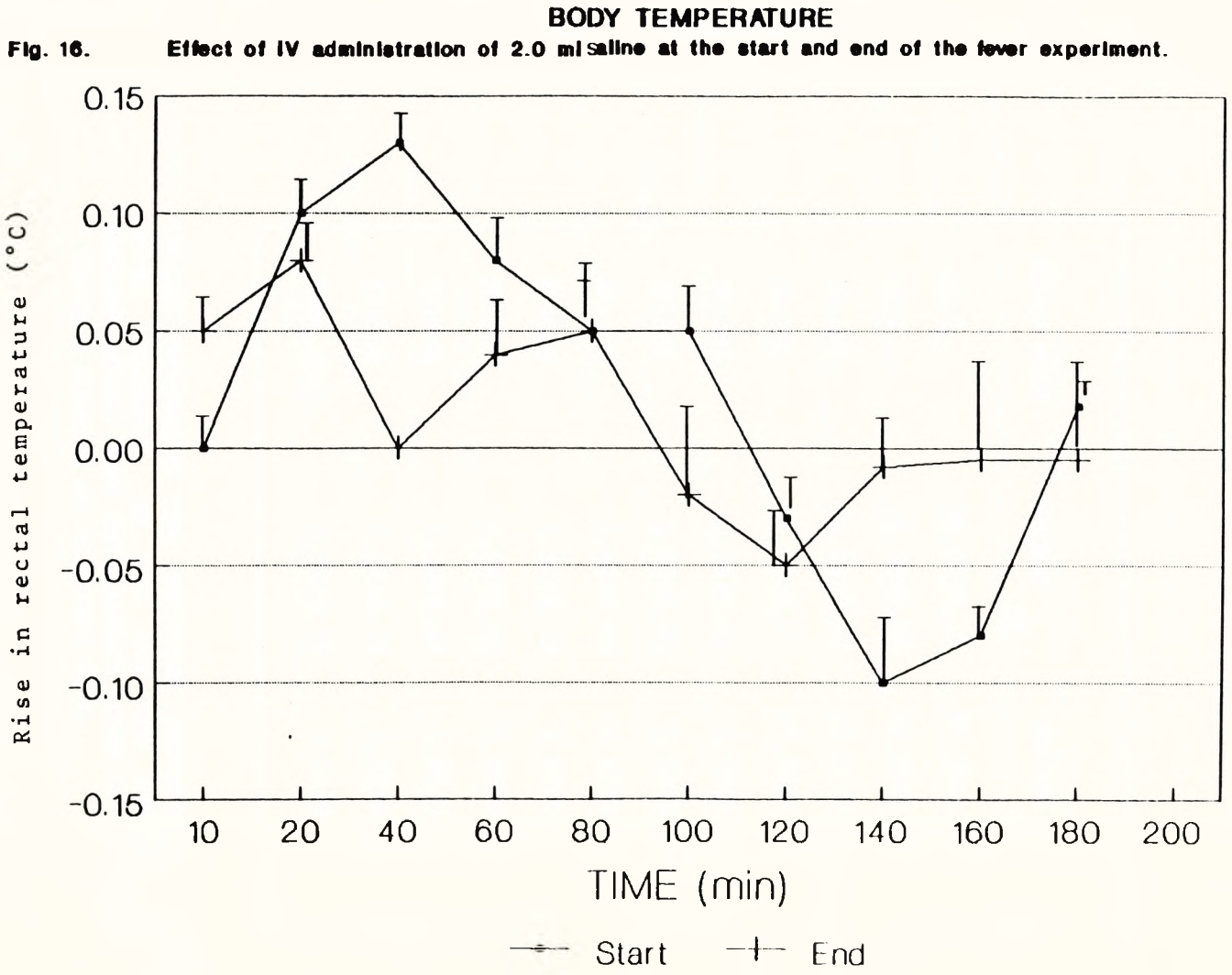


Fig. 16. **Effect of IV administration of 2.0 ml saline at the start and end of the fever experiment.**

Error bars are means ± SE of 4 rabbits. **Saline injected at time 0.**

Table 9.

THE EFFECT OF A SINGLE DOSE OF INTRAVENOUS SALINE INJECTION AT 180 MINUTES.

Fatty acid	Normal SE	Saline SE
16:0	24,07±0,15	26,36±1,00
18:0	15,38±0,58	15,36±0,40
18:1n9	15,99±0,62	16,61±0,63
18:2n6	30,96±0,77	28,35±0,63
18:3n3	0,76±0,04	0,70±0,07
18:3n6	0,40±0,03	0,32±0,04
20:3n6	0,40±0,03	0,32±0,04
20:4n6	3,97±0,19	3,42±0,04
20:5n6	0,64±0,03	0,46±0,17
22:4n6	1,20±0,27	3,85±0,13
22:5n6	3,94±0,27	3,85±0,13
Rest.poly.	2,34±0,28	2,21±0,23

Values represent the relative percentage of fatty acids measured on GLC and the average of five determinatins. Test SE±standard error of the means.
* Group A control on standard diet.

endotoxin (LPS) for 4 days per week for a duration of 3 successive weeks. When the state of minimal fever response was reached, each rabbit received twice the same dose of endotoxin at 60 min interval, until the state of tolerance was reached.

5.1.2.2. Preparation of endotoxin

Purified lipopolysaccharide of Salmonella Thyphosa 0901 (Difco, Michigan), batch N^{o} 3124-25-26, was suspended in pyrogen-free saline (Baxter,Johannesburg) at concentration of 1,0 mg/ml and stored at -15^{o}C. This stock solution, was diluted further on a daily basis to a concentration of 15 ul/ml. Of this, 0,35, 0,70 and 1,05 ml containing 5,0,10,15, ug/ml endotoxin was injected IV into the marginal ear vein of rabbits, giving the required quantity of 2,0, 4,0 and 6,0 ug/kg endotoxin.

5.1.2.3. Temperature measurements

As described in paragraph 4.1.3.4, using a Solomat thermometer model 335K (Ballanvilliers, France) to record the rabbit rectal temperatures.

5.1.2.4. Blood sampling

Blood samples (1,0-2,0 ml) were drawn from the ear artery of each rabbit, with a butterfly needle (G-21) into a heparinised (vacutainer) tube before the start of each experiment (base line), and again at 60 min and 180 min after the dose-dependent fever experiment.

During chronic induction of fever, blood was sampled before each injection, and thereafter at intervals of 180 min. Before the start of the experiment all rabbits were injected with saline alone, their temperatures were measured and serum lecithin fatty acids analysed to eliminate any interference by the carrier solvent. Shortly after collection, the blood samples were centrifuged for 10 minutes at 1800 rpm (Beckman refrigerated centrifuge model TJ6). The resultant serum supernatant was centrifuged a second time to remove any remaining blood cells. The serum was flushed with N_2 and stored at $-20^{o}C$ for fatty acid analysis and $+4^{o}C$ for the analysis of triglyceride and cholesterol.

5.2 CHEMICAL METHODS

5.2.1. Extraction of Total Lipids from Solid Food

2,5 g of pulverised rabbits pellets was macerated in 32,0 ml of 15 per cent KOH in ethanol. The mixture was allowed to saponify overnight in an oven at 60 ^{o}C.
After cooling, 12,0 ml distilled water was added, mixed and the mixture divided into 11,0 ml aliquots. To each aliquot 9,50 ml petroleum-ether (40-60^{o}C) was added, mixed in a vortex mixer for 60 sec, and centrifuged (Beckman TJ 6 refrigerated centrifuge) at 3000 rpm for 5 min. The supernatant layer containing the non saponified portion was then decanted.

This recovery procedure was repeated twice. The remaining residue of rabbit pellets was acidified to ph=2,0 with 3,75 ml of concentrated HCL. The total lipid were extracted by the addition of 9,50 ml petroleum-ether(40-60 ^{o}C), mixed in a vortex mixer for 60 sec and centrifuged as described above for 5 min. The four extracts were combined into one batch. This recovery procedure was repeated twice. The pooled extracts were dried over Na_2SO_4 and the solvent evaporated under nitrogen and methylated in the same manner as serum fatty acids and the resultant fatty acid methyl esters dissolved in 100 ul hexane and analysed on a gas chromatograph.

5.2.2. Thin Layer Separation of Phospholipid Classes

The total lipids extracted from solid food (100 ul) and serum (200 ul) were separated into phospholipid classes as described in paragraph 4.2.3.

5.2.3. Saponification and methylation of lecithin from serum and solid food

The identified serum lecithin fraction was scraped off from the TLC plates into a clean tube, saponified and methylated by the addition of 1,0 ml MeOH in 2,0 ml 0,2M sodium methylate (CH_3NaO) in a 50^{o}C water bath with frequent shaking for 20 minutes. After cooling, 4.0 ml H_2O was added, mixed in a vortex mixer for 60 sec and the methyl esters extracted twice

with 1,0 ml hexane. The hexane layer was transferred to a clean tube and evaporated under N_2 at 60°C, whereupon the methyl esters were dissolved in 100 ul hexane, capped in glass vials and stored at -20°C until analysed.

5.2.4. Gas chromatograph

The separation of methyl esters was performed as described in paragraph 4.2.5.

5.2.5. Serum Triglycerides and Cholesterol Analysis

The method of Bokolo and David (1973) for the quantification of triglycerides and the method of Deeg and Ziegenhorn (1983) for the determination of cholesterol were used in the Department of Chemical Pathology, under the supervision of Professor D. Mendelsohn.

5.2.6. Statistical analysis

All data obtained was subjected to a student t-test and values of P equal to or less than 0,01 were considered a significant difference.

5.3. RESULTS

5.3.1. Introduction

The connection between fever response and essential fatty acids (EFA's) is unknown. Yet it is acknowledged that fatty acids fulfil two primary roles:

"(i) firstly they serve as substrata for oxidative catabolism and production of energy and, (ii) secondly they serve as structural components of cell membranes and subcellular organelles" (Fritz, 1961). A change in the fatty acid content, therefore, reflects altered cellular function, affecting cellular activities. An increase in saturated fatty acids has been recognised to render the membranes less fluid, whereas unsaturated fatty acids have been shown to have the opposite effect (Melchior et al., 1970). Increased membrane fluidity thus becomes dependent on linoleic acid, a major constituent of phospholipids, which also has an established antibacterial activity in vitro. Various studies have shown that temperature is one of the most important factors influencing the unsaturation of phospholipids, thus affecting the growth and metabolism of microorganisms (Crona and Vogelos, 1972; Verma and Küller, 1973; Simiensky, 1974). Changes in the fatty acid composition can occur over a wide range of temperature, resulting in an overall rearrangement of lecithin molecules (Malak et al., 1989), allowing the cells to survive altered conditions (Verma and Küller, 1973). As there is no supporting evidence that C_{18} fatty acids play a key role in the host's defence mechanism against infection, (due to the neutralising effect of protein present in the blood stream) lead to the present investigation into the "in vivo role of essential

fatty acids in the genesis of fever induced by Salmonella Thyphosa.

In this study the following proccedures were preformed:

(a) Number of experiments carried out.

(b) Lecithin fatty acid composition of food

(c) Effect of a single dose of 2,0, 4,0 and 6,0 ug/kg endotoxin on fever pattern and serum lecithin fatty acids composition.

(d) Effect of chronic induction of fever induced by 2,0 ug/kg body of LPS S.Thyphosa.

(e) Effect of chronic induction of fever on serum lipid metabolism

5.3.2. Presentation of data.

Dates are presented as described in paragraph 4.3.2. Due to the small number of rabbits used in the experiment, SD was used to express significance test.

5.3.3. Number of Experiments Carried out

Over the 5 weeks of the experiment a total of 25 injections were administered and 226 blood samples collected from five rabbits. In addition, 22 blood samples were collected at various intervals for triglyceride and cholesterol analysis.

5.3.4. Effect of a Single dose of 2,0, 4,0 and 6,0 ug/kg S.Thyphosa on the Fever Pattern and Serum Lecithin Fatty acid composition

A single dose of endotoxin (2,0 ug/kg) produced a monophasic effect on the pattern of the fever (Fig. 17) and a biphasic effect on the lecithin fatty acid configuration (Table 10 and 11). Within 60 min a reduction in 20:3(w6)->20:4(w6) occurred, while a return to the normal level coincided with the termination of the fever experiment at 180 min (Table 11). The stability of 18:2(w6) under the action of endotoxin 2,0 ug/kg indicates that local synthesis and rapid release of prostaglandin occurred from the periphery via the pathway;

$$20{:}3(w6) \rightarrow PGE_1$$
$$\quad\quad\quad

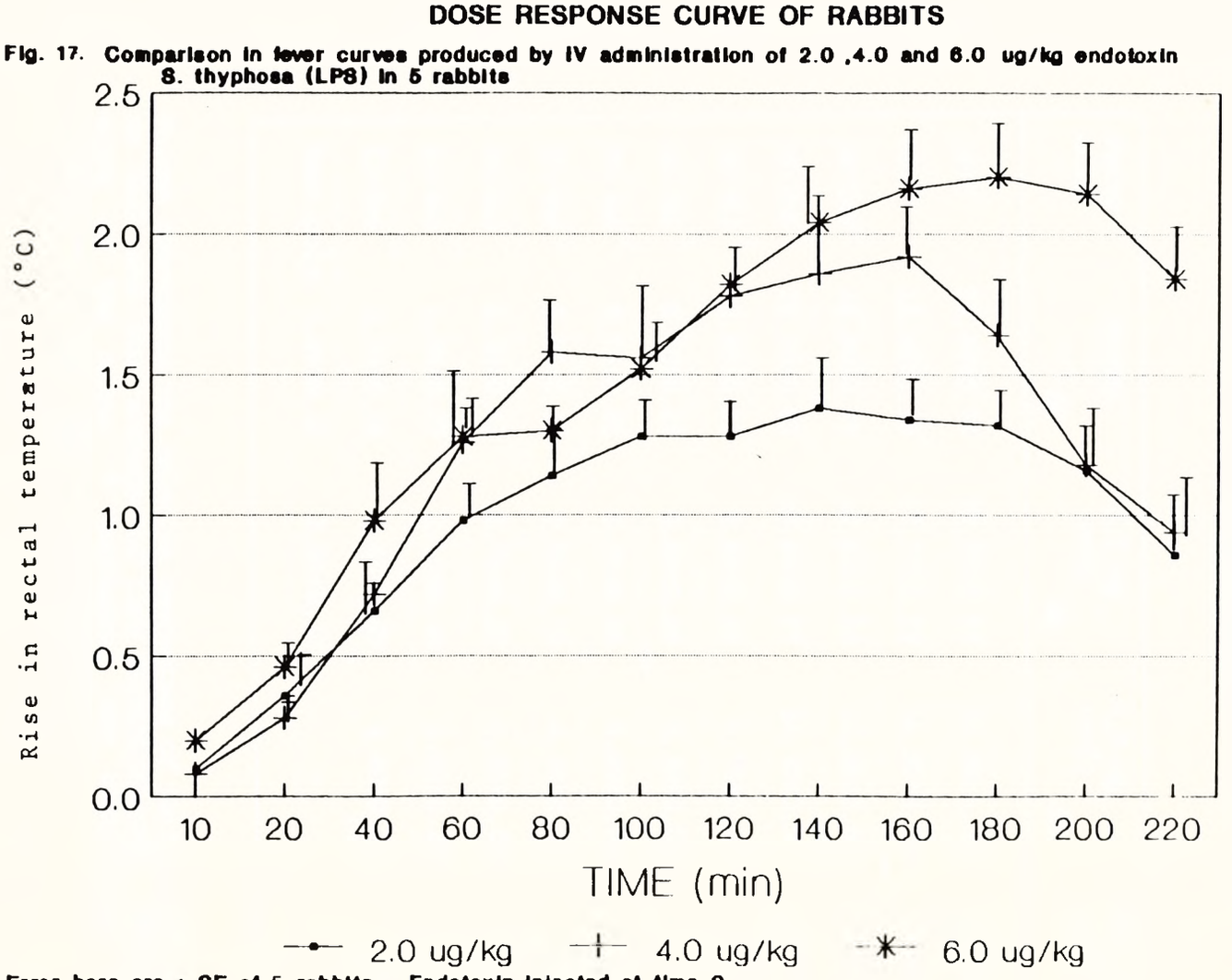


Fig. 17. Comparison in fever curves produced by IV administration of 2.0 ,4.0 and 6.0 ug/kg endotoxin S. thyphosa (LPS) in 5 rabbits

Error bars are ± SE of 5 rabbits. Endotoxin injected at time 0.

Table 10.

DOSE DEPENDENT CHANGES IN THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS TO 2,0 , 4,0 and 6,0 µg/kg ENDOTOXIN (S.THYPHOSA) (60 minutes.)

Fatty acid	Normal SE	2,0 µg (n=5) SE	4,0 µg (n=5) SE	6,0 µg (n=5) SE
16:0	24,40±0,74	25,55±0,65	22,20±1,33	24,75±0,86
18:0	15,40±0,58	17,70±1,25	16,20±0,55 **	15,00±0,68
18:1n9	18,20±0,75	16,83±1,54	13,80±0,66 **	15,20±1215 **
18:2n6	29,10±0,65	28,60±0,97	34,20±0,53	35,90±0,64
18:3n3	0,85±0,08	0,60±0,05	0,75±0,10	0,70±0,07
18:3n6	0,40±0,15	0,40±0,09 **	0,50±0,04	0,30±0,04
20:3n6	0,55±0,05	0,25±0,04 *	0,60±0,08	0,25±0,04
20:4n6	3,85±0,19	2,80±0,19	4,10±0,20	3,00±0,18
20:5n3	0,30±0,03	0,75±0,23	0,65±0,09	0,50±0,09
22:4n6	1,85±0,27	2,10±0,18	2,60±0,25	1,00±0,30
22:5n6	3,70±0,28	4,45±0,65 *	4,40±0,25 **	3,40±0,60 **
Rest.poly.	1,40±0,32	2,00±0,17	-	-

Values represents the relative percentage of fatty acids measured on GLC and the average of five determinations. Test SE±standard error of the means. * represents the statistical significant t-test. * $p < 0,05$ and ** $p < 0,01$-$0,005$.

Table 11 .

DOSE DEPENDENT CHANGES IN THE COMPOSITION OF SERUM LECITHIN FATTY ACID TO 2,0 , 4,0 and 6,0 µg/kg ENDOTOXIN (S.THYPHOSA) (180 minutes.)

Fatty acid	Normal (n=5) SE	2,0 µg (n=5) SE	4,0 µg (n=5) SE	6,0 µg (n=5) SE
16:0	24,40±0,74	26,45±0,64	23,60±1,47	21,75±1,02
18:0	15,40±0,58	15,75±0,93	14,00±2,07	16,40±1,50
18:1n9	18,20±0,75	14,65±1,50	14,75±1,38 **	13,60±1,04 **
18:2n6	29,10±0,65	31,85±1,00	36,75±0,52	37,20±0,67
18:3n3	0,85±0,08	0,75±0,08	0,75±0,11	0,75±0,13
18:3n6	0,40±0,15	0,50±0,05	0,30±0,10	0,40±0,08
20:3n6	0,55±0,02	0,30±0,06	0,45±0,11	0,50±0,08
20:4n6	3,85±0,19	3,40±0,13	4,25±0,66	4,60±0,52
20:5n6	0,30±0,03	0,50±0,10	0,40±0,11	0,50±0,07
22:4n6	1,85±0,27	1,80±0,13	1,35±0,23	1,20±0,27
22:5n6	3,70±0,28	4,05±0,42	3,35±0,48	3,10±0,45
Rest.poly.	1,40±0,32	- **	- **	- **

Values represents the relative percentage of fatty acids measured on GLC and the average of five determinations. Test SE±standard error of the means. * represents the statistical significance t-test. * $p < 0,05$ and ** $p < 0,005$.

produced a dose dependent rise in the linoleic acid 18:2(w6), without any changes in the eicosonoids by 60 and 180 min (Table 10 and 11). The sharp fall in stearic acid (18:0), at 4,0 ug/kg endotoxin was not observed at the higher dose (Table 11). The unchanged content in the precursors of PGE and the dose-dependent increase in the substrata 18:2(w6) reflect a progressive release of linoleic acid via FFA (Frederickson and Gordon, 1958) to meet growing turnover rate of cerebral arachidonic acid for prostaglandin E_2 synthesis. This ensured that the peripheral event became a centrally mediated one, leading to the advent of fever.

5.3.5. Effect of chronic induction of fever induced by 2,0ug/kg body mass of S.Thyphosa (LPS).

Systemic daily injections of 2,0 ug/kg body mass of LPS by the fourth day produced a monophasic fever with great amplitude and a duration of 250 min (Fig.18), becoming biphasic after the eighth injection (Fig. 18). Although the onset of the fever remained the same (20 min), the first peak occurred within 90 min and the second at 180 min, with a duration of 300 min. Extention of the duration of the fever led to a gradual rise in body temperature, which remained high even after carbohydrates were fed (40,01±0,10^{o}C).

A rise in the core temperature thus became dependent on the number of consecutive endotoxin

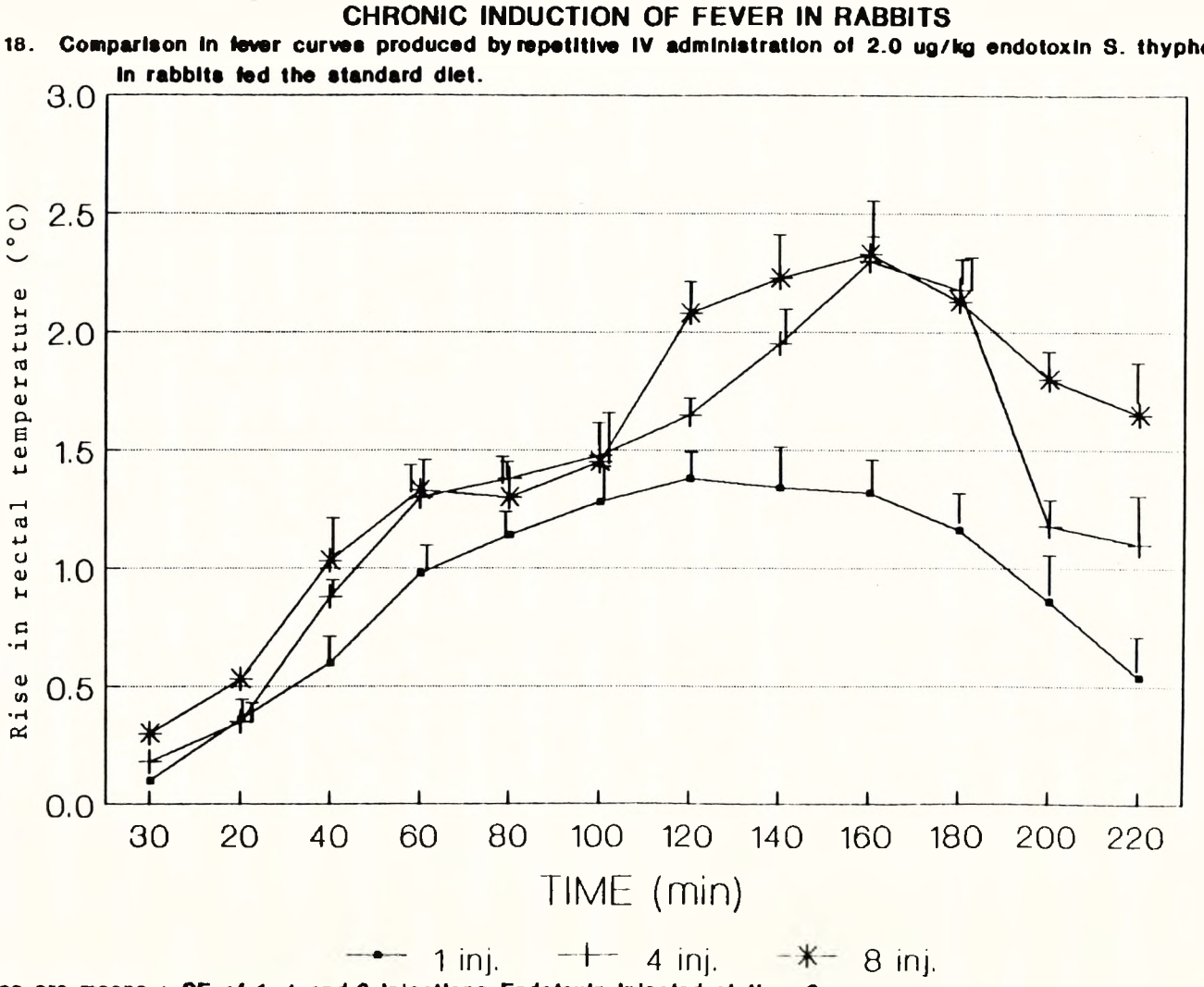


Fig. 18. Comparison in fever curves produced by repetitive IV administration of 2.0 ug/kg endotoxin S. thyphosa in rabbits fed the standard diet.

Values are means ± SE of 1, 4 and 8 injections Endotoxin injected at time 0.

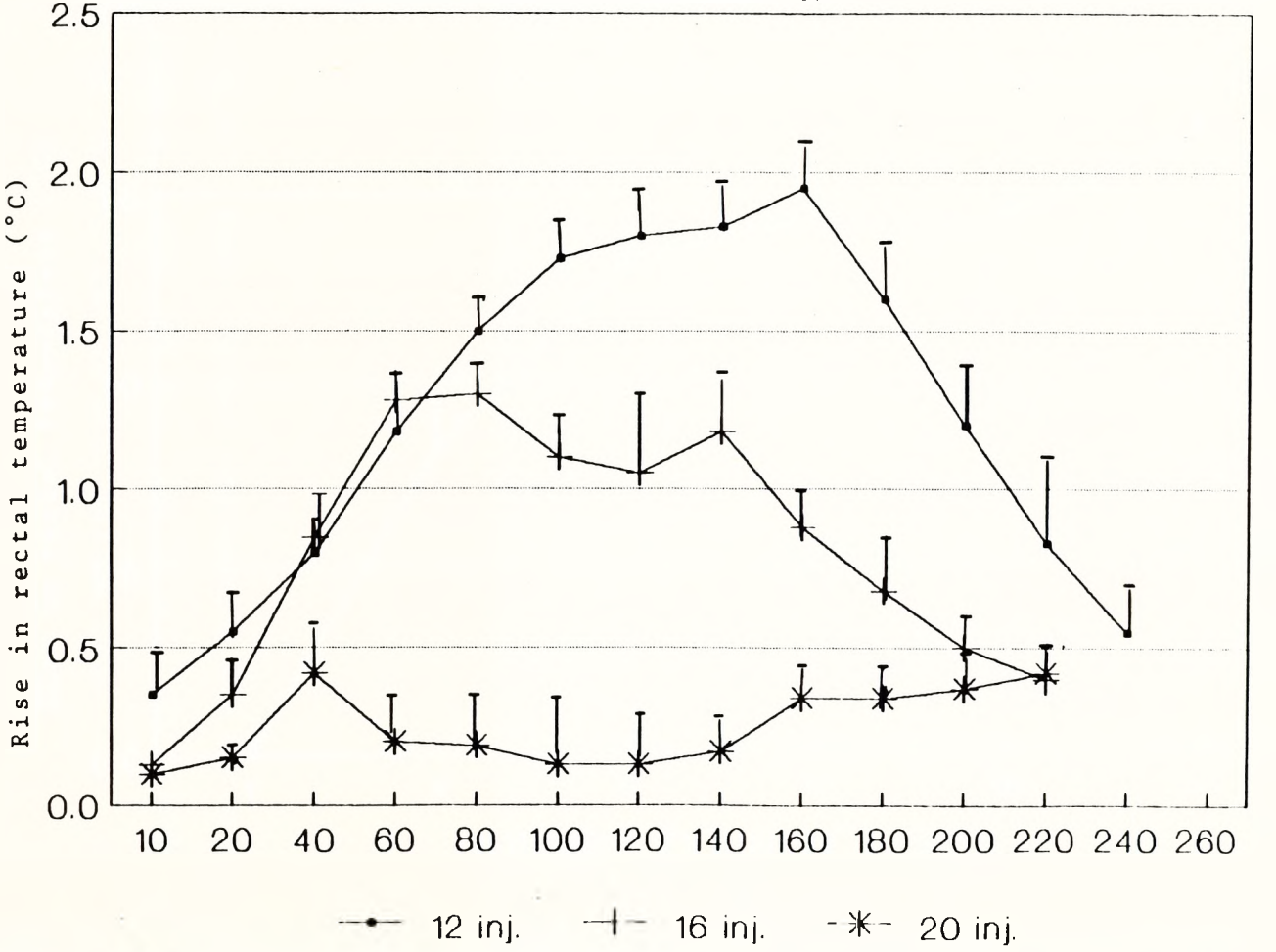
injections (Fig. 19). By the sixteenth injection the body temperature reached 40,7±0,10°C (Fig. 19), entailing only a short monophasic fever (Fig. 20) and by the twentieth injection the rabbit's response to endotoxin was prevented (Fig. 20). These changes led to a disruption of cellular metabolism (Lilly et al.,1984), and inhibition of immune response (Brandt and Banet, 1984). Thus a second administration of endotoxin was unable to rectify the refractory state, causing tolerance to the febrile action of pyrogen to develop (Fig. 20).

5.3.6 Effect of chronic induction of fever on serum lipid metabolism.

Since the formation of new membranes is a prerequisite for phagocytosis induced by the action of endotoxin, this enchanced the need for turnover in phospholipid (PL). Visually, thin layer chromatographic plates (Fig. 21) did in fact show an increased turnover in phospholipids (PL). A single dose of S.Thyphosa led to an increase in the size of various phospholipid class, while reducing the size of lecithin (Fig.21). These changes are inconsistent, as progressive doses of S.Thyphosa (by eight injections) led to reduction in PL lipid classes (Fig. 21), thus securing stability of lecithin. This further supports the contention that turnover and catabolism of lecithin is essential for cell membrane adaptation to environmental changes,

CHRONIC INDUCTION OF FEVER IN RABBITS

Fig. 19. Comparison in fever curves produced by multiple IV administration of endotoxin S. thyphosa (2.0 ug/kg) in rabbits fed the standard diet.



Error bars are ± SE of 4 rabbits. Endotoxin injected at time 0.

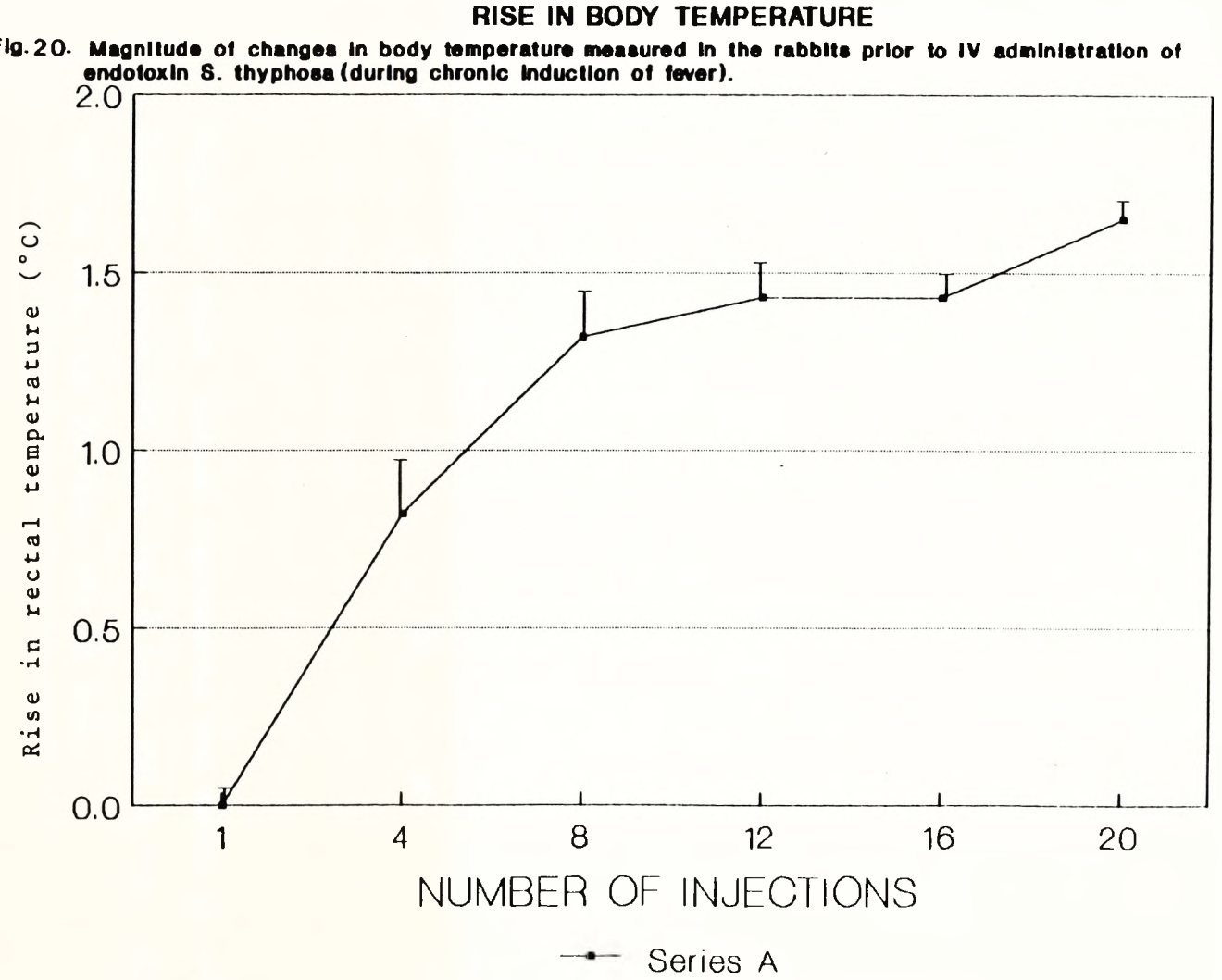


Fig. 20. Magnitude of changes in body temperature measured in the rabbits prior to IV administration of endotoxin S. thyphosa (during chronic induction of fever).

Values are means ± SE of 4 rabbits. Tre, rectal temperature. Body temperature measuredin the morning.

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF SERUM PHOSPHOLIPID DURING CHRONIC INDUCTION OF FEVER.

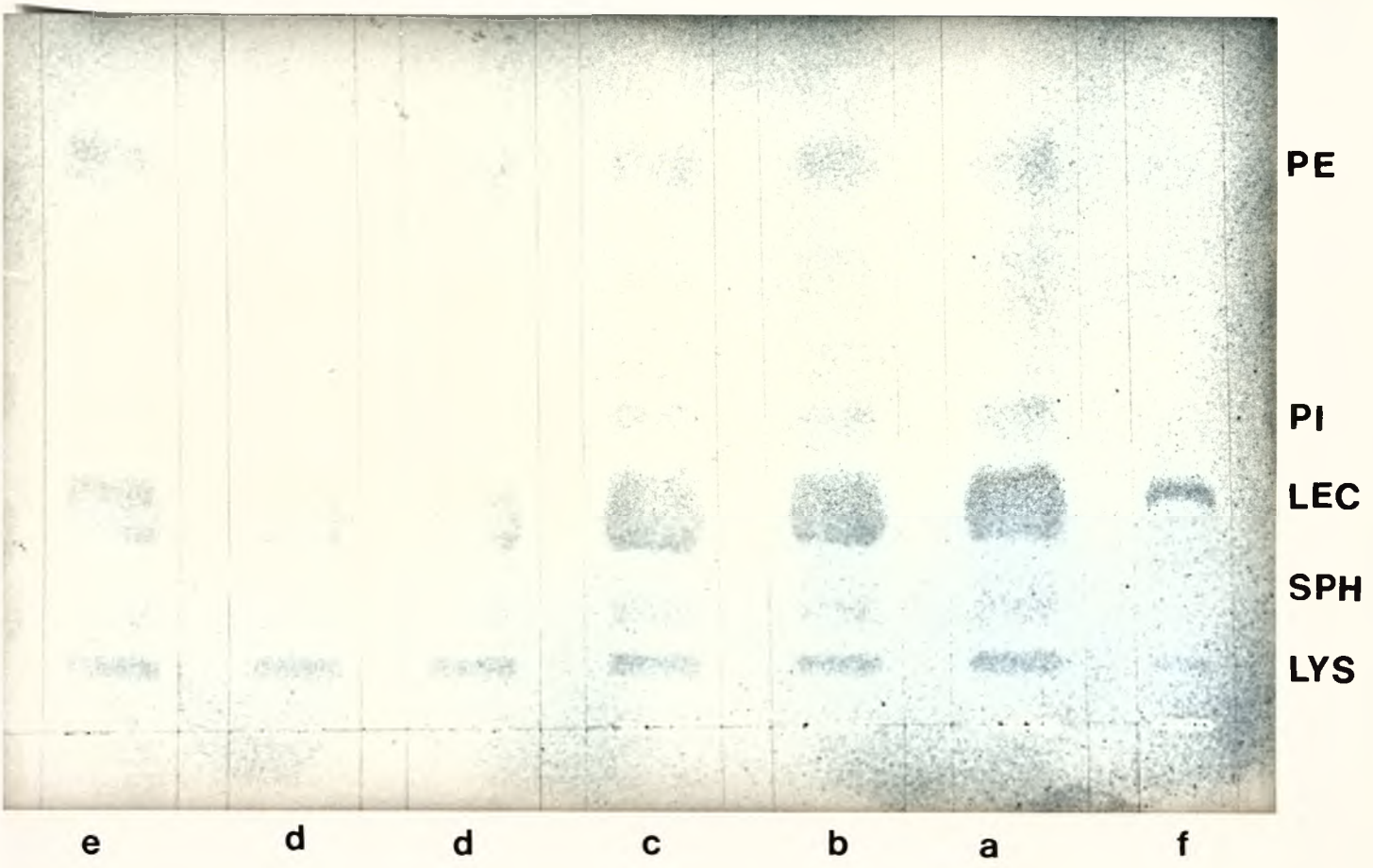


Fig.21. Each column represents the serum of 4 rabbits. Sample taken 180 min. (a) 4 inj. (b) 8 inj. (c) 12 inj. (d) 16 inj. (e) 20 inj. (f) 21 inj.

to enable the cell to surviving altered temperatures. This requires a lipid precursor from the metabolic pool within the host for the replication of the microorganism (Table 1). However, by 16 endotoxin injections this demand on the body cannot be met and defect in the synthesis of PL occurs, leading to inhibition of the formation of lecithin (Fig. 21). These chromatographic changes reflect altered serum lipid metabolism. A single small dose of endotoxin led to insignificant changes within lecithin fatty acid, (Table 10 and 11), while the increasing dose of endotoxin provoked a rise in lecithin linoleic acid, and reduction in polyunsaturates (Tables 11, 12, 13 and 14), together with an increase in triglyceride (TG) and cholesterol (CHO) (Table 15). In the aggregate, this indicates that auxiliary mechanisms have to be activated to meet the caloric demand induced by constant stimulation of S. Thyphosa. The prognosis of fever thus becomes dependent on biochemical processes, i.e. lipolysis and glycogenesis (Fig. 4). Both pathways are exothermic and both are controlled by fluctuation in the content of PGE_1 (Bergström, Carlson and Weeks, 1968) derived from lecithin fatty acids 18:3(w6)->20:3(w6)-> PGE_1. The constant stimulation of this pathway by endotoxin S.Thyphosa led to changes in the biochemical and physiological processes, which became irreversible, and thus pathophysiological (Table 16).

Table 12.

EFFECT OF FOUR REPETITIVE INJECTIONS OF 2,0 ug/kg S.THYPHOSA ON THE COMPOSITION OF LECITHIN FATTY ACIDS (180 minutes).

Fatty acid	Normal (n=4) SE	Four injection Before (n=4) SE	Four injection 180' (n=4) SE
16:0	24,40±0,74	25,05±3,50	24,15±2,02
18:0	15,40±0,58	17,30±1,03 *	12,70±1,87
18:1n9	18,20±0,75	14,55±0,80	15,75±1,12 **
18:2n6	29,10±0,65	31,80±0,84	36,35±0,68
18:3n3	0,85±0,08	0,90±0,07 *	0,80±0,12
18:3n6	0,40±0,15	0,30±0,08 *	0,50±0,17
20:3n6	0,55±0,05	0,35±0,02	0,40±0,16
20:4n6	3,85±0,19	3,10±0,40	4,00±0,57
20:5n6	0,30±0,03	0,65±0,13	0,55±0,14
22:4n6	1,85±0,27	2,25±0,18	1,30±0,36 *
22:5n6	3,70±0,28	3,75±0,47	2,35±0,41
Rest.poly	1,40±0,32	-	-

Values represents the relative percentage of fatty acids mesured on GLC and the average of four determinations. Test SE±standard error of the means of four rabbits. * represents the statistical significance t-test. * $p < 0,025$ and ** $p < 0,005$.

Table 13.

EFFECT OF EIGHT REPETITIVE INJECTIONS OF 2,0 ug/kg ENDOTOXIN S.THYPHOSA ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS IN FED (before injection) AND FASTED (180 minutes)

Fatty acid	Normal (n=5) SE	Eight injections Before (n=4) SE	180' (n=4) SE
16:0	24,40±0,74	27,35±1,19	24,35±0,98
18:0	15,40±0,58	16,65±1,85	13,35±0,72 *
18:1n9	18,20±0,75	17,35±1,81	15,75±0,20 **
18:2n6	29,10±0,65	28,40±1,20	37,40±0,31
18:3n3	0,85±0,08	0,75±0,04 **	0,80±0,13
18:3n6	0,40±0,15	0,20±0,04	0,30±0,13
20:3n6	0,55±0,05	0,20±0,04 *	0,40±0,14
20:4n6	3,85±0,19	2,65±0,36	3,50±0,50
20:5n6	0,30±0,03	1,30±0,70	0,55±0,10
22:4n6	1,85±0,27	1,55±0,15	1,10±0,23 *
22:5n6	3,70±0,28	3,60±0,36	2,50±0,23
Rest,poly.	1,40±0,32	-	-

Values represents the relative percentage of fatty acids measured on GLC and the average of four determinations. Test SE±standard error of the means. * represents the statistical significance t-test. * $p < 0,025$ and ** $p < 0,005$.

Table 14.

EFFECTS OF TWELVE REPETITIVE INJECTIONS OF 2,0 ug/kg ENDOTOXIN ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS IN FED (before injection) and FASTED (180 minutes)

Fatty acid	Normal (n=5) SE	Twelve injection Before (n=4) SE	Twelve injection 180' (n=4) SE
16:0	23,40±0,74	28,15±1,25	24,50±1,24
18:0	15,40±0,58	14,70±2,44	15,40±0,68
18:1n9	18,20±0,75	16,50±2,58	15,45±1,52 **
18:2n6	29,10±0,65	29,75±1,62	37,20±0,84
18:3n3	0,85±0,08	0,75±0,18	0,65±0,10
18:3n6	0,40±0,15	0,30±0,09	0,40±0,03
20:3n6	0,55±0,05	0,30±0,14	0,35±0,06 **
20:4n6	3,85±0,19	3,05±0,44	2,50±0,24
20:5n3	0,30±0,03	1,35±0,70	0,40±0,10 *
22:4n6	1,85±0,27	1,90±0,45	0,75±0,15 *
22:5n6	3,70±0,28	3,25±0,34	2,40±0,49
Rest. poly.	1,40±0,32	-	-

Values represents the relative percentage of fatty acid measured on GLC and the average of four determinations. Test SE±Standard error of the means. * represents the statistical significant t-test. * P < 0,05 and P < 0,005.

Table 15.

EFFECT ON SERUM NEUTRAL LIPIDS (TG,CHO) OF 2,0 ug/kg S.THYPHOSA BEFORE INJECTION AND AFTER 180 MINUTES IN THE COURS OF 5 WEEKS EXPERIMENT (4 INJECTION/DAY)

Number of injection	TG (mm/l) (n=4) Before SE	180' SE	CHO (mm/l) (n=4) Before SE	180' SE
	0,38±0,03 ***	- **	0,39±0,03 **	- **
4	0,86±0,03 **	1,51±0,27 ***	1,24±0,11 **	1,04±0,11 **
8	1,24±0,20 ***	1,69±0,11 **	1,30±0,15 **	1,29±0,07 **
12	1,37±0,33 **	1,65±0,37 ***	1,31±0,29 **	1,50±0,46 ***
16	1,74±0,24	2,05±0,26	1,39±0,23	2,69±0,12
20	1,48±0,11			
1 inj. 60'		0,57±0,02		0,59±0,04
2 inj.180'		0,80±0.13		0,95±0,23

Each values of triglyceride (TG) and cholesterol (CHO) represents the mean of four rabbits. SE±standard error of the mean. * shows the statistical significance of t-test
**P <0,005 , *** P <0,0001.

Table 16.

EFFECT OF SIXTEEN REPETITIVE INJECTIONS OF 2,0 ug/kg ENDOTOXIN S.THYPHOSA ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS IN FED (before injection) AND AFTER INJECTION AT 180 MINUTES.

Fatty acid	Normal (n=5) SE	Sixteen injections Before (n=4) SE	180' (n=4) SE
16:0	24,40±0,74	25,85±1,22	26,65±1,23
18:0	15,40±0,53	19,22±2,38	16,03±1,90
18:1n9	18,20±0,75	17,35±2,96	16,10±1,43 **
18:2n6	29,10±0,65	30,10±1,05	34,50±0,83
18:3n3	0,85±0,08	0,86±0,36	0,65±0,17 ***
18:3n6	0,40±0,15	0,20±0,06 *	- ***
20:3n6	0,55±0,05	0,25±0,10 **	- **
20:4n6	3,85±0,19	2,95±0,20	2,70±0,25
20:5n6	0,30±0,03	0,70±0,19	0,50±0,14 **
22:4n6	1,85±0,27	1,85±0,26 *	0,50±0,18 **
22:5n6	3,70±0,28	2,35±0,45	2,15±0,28
Rest. poly.	1,40±0,32	-	-

Values represents the relative percentage of fatty acids mesured on GLC and the average of four determinations. Test SE±standard error of the means. * represents the statistical significance t-test. * P < 0,025 and ** P < 0,01-0,005.

This ultimately alters the configuration of fatty acids in lecithin, causing a decay in the bioconversion of 18:3(w6)->20:3(w6)->PGE_1 (Table 17) A flaw in the precursors of PGE_1 is recognised to inhibit lipogenesis in favour of glycogenesis (Fig.4) (Bergström, Carlson and Weeks, 1968), in this manner diminishing or inhibiting the breakdown of TG. The mere fact that the concentration of serum TG conforms with the onset, height, and duration of the fever (Fig. 22), implies that inhibition of 18:3(w6)-> 20:3(w6), concurrently with an increase in 18:2(w6) (Table 17) in the febrile state (180 min) prevented desaturation and chain elongation of PUFA, resulting in a reduction or inhibition of 18:3(w6)->20:3(w6) ->20:4(w6)-> 22:4(w6) ->22:5(w6). Defective formation of PUFA's (Table 16 and 17) under constant stimulation by endotoxin indicates inhibition, not only of PGE_1, but also of PGE_2, the direct mediator of endotoxin fever. This demonstrates that chronic induction of fever impairs lipid metabolism, (Fig.4) blocking FFA-uptake from the serum by the tissues and consequently, preventing the breakdown of TG as well as enchancing the recycling of FFA in "linoleic acid" before it is oxidized (Fig.16). A rise in cholesterol (CHO) concentration (Fig. 22) concomitantly with TG is also significant, as it proves to interact with bacteria, at the same time through cholesterylester pathway, provide FFA->PGs (Fig. 3) thus play a useful

Table 17.

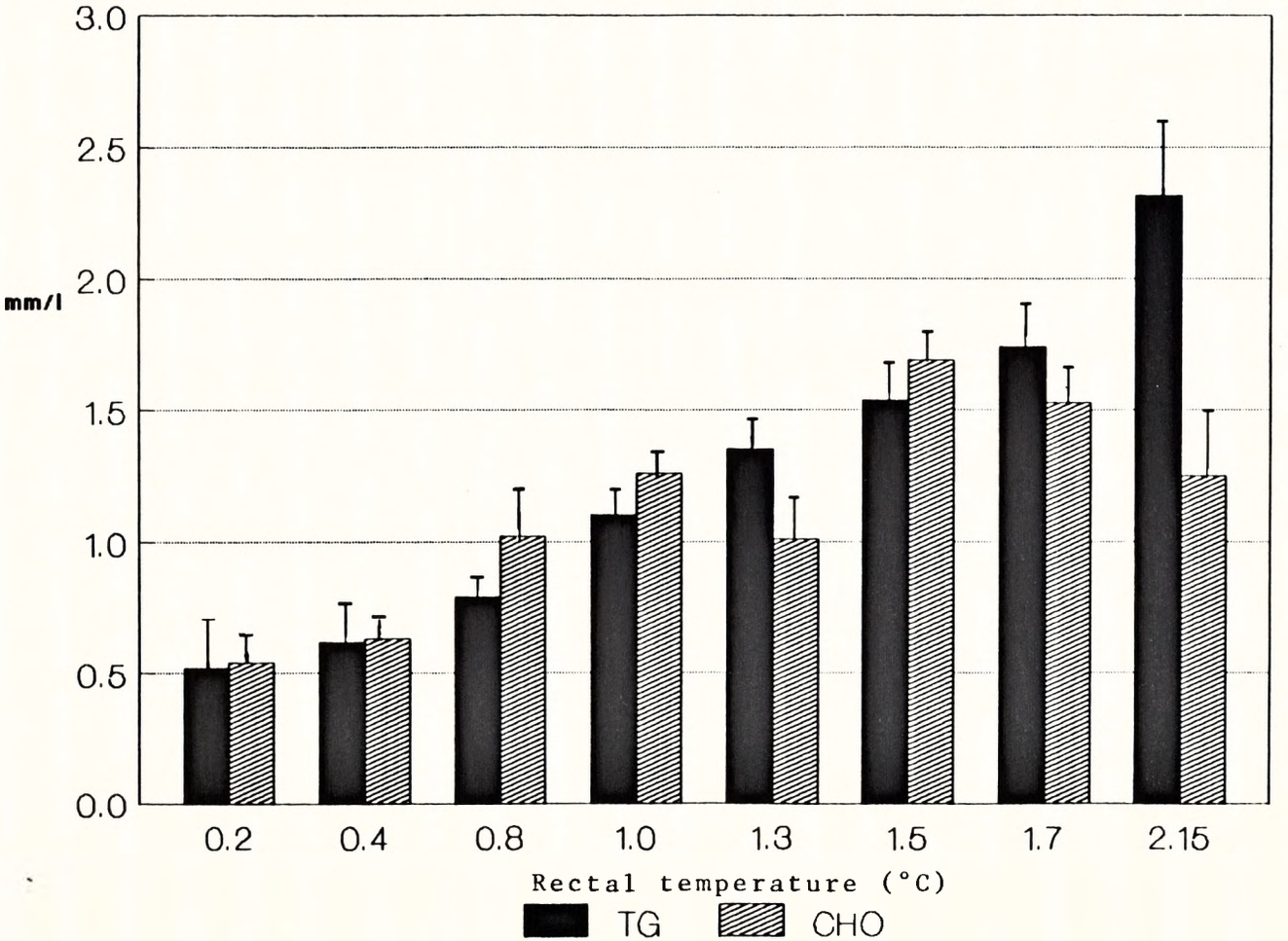
EFFECTS OF 20 AND 21 CONTINOUS INJECTIONS OF 2,0 ug/kg ENDOTOXIN S.THYPHOSA ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS ADMINISTERED ON THE SAME DAY.

Fatty acid	Before injection (n=4) SE	First injection 60' (n=4) SE	Second injection 180' (n=4) SE
16:0	27,25±1,06	25,30±1,32	25,25±1,31
18:0	15,50±0,95	18,75±3,02	13,80±0,86
18:1n9	16,50±1,93	17,60±2,24	16,45±0,96 **
18:2n6	32,00±1,96	29,40±2,82	37,75±1,34
18:3n3	1,32±0,40	0,80±0,31	1,33±0,23
18:3n6	- ***	- ***	- *** **
20:3n6	0,30±0,06 **	0,25±0,12 *	0,20±0,02
20:4n6	2,75±0,11 **	2,84±0,32 *	3,0±0,46
20:5n6	0,86±0,14	0,90±0,30	0,20±0,07
22:4n6	1,65±0,30 *	1,16±0,47 *	- *** **
22:5n6	1,94±0,52	1,70±0,57	2,00±0,40

Values represents the relative percentage of fatty acids measured on GLC and the average of five to four determinations.
Test SE±standard error of the means. * represents the statistical significance t-test. *P < 0,025 , **P <0,005 and
*** means that the compound is not present within the fatty acids.

MOBILISATION OF LIPID DURING FEVER

Fig.22.Fever related changes in serum TG and CHO after 8 injetions of 2.0 ug/kg endotoxin S. thyphosa



Error bars are means ± SE of rabbits. Samples taken in the cours of a day.

role in the defence mechanism of host's against invading microorganisms (Fig. 22, Table 16). Furthermore, the rise in serum TG, CHO, and lecithin linoleic acid indicates active fatty acid transport not only from one fraction to an other but also from the periphery, across the blood-brain barrier (Fig. 23) (Mead and Dopeshwarkar, 1972), thereby "raising" the hypothalamus to a higher degree. The peripheral event, therefore, becomes a centrally mediated one, affecting the respiratory function of mitochondria (Marco et al., 1961). The above resulted in an increase peroxidation (Marco et al., 1961) and in the formation of CO_2 (Fritz, 1961), a known inhibitor of PGE_2 synthesis (Splawinski et al.,1978). The elevated core temperature thus affected heat loss mechanism and also diminished the survival of the rabbits. The hyperthermia however, by the end of the fever experiment led to a block in the uptake of TG, CHO (Table 16) and a fall in lecithin polyunsaturates (Table 17), while the elevated lecithin 18:2(w6) content was maintained. Thus, organism has the ability to burn out the invading microorganism by altering the biological function of lecithin. Although, the antibacterial activity of C_{18}-C_{22} fatty acids "in vitro" has been demonstrated by many researcher, it became evident from the present study it serves the same function "in vivo", providing further support of the vital role played by essential fatty acids in thermoregulation.

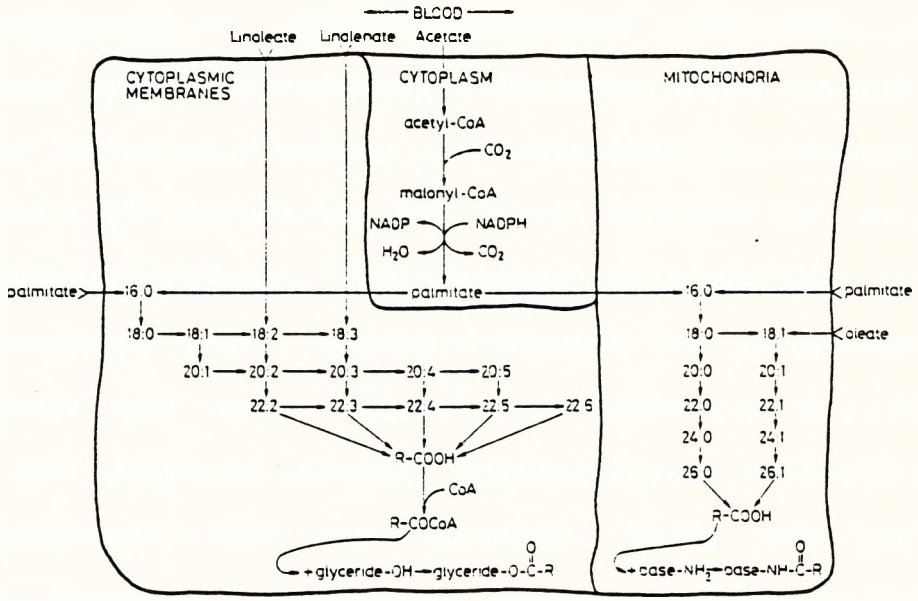


FIG. 2.3 Overall view of fatty acid derivation and metabolism in brain.
Horizontal arrows in the microsomal system do not imply inter-family conversions, which are not known to occur in animals. The figure represents conversions within an individual series, eg. in the *n*-9 series, 18:0 → 18:1 → 18:2; in the *n*-6 series, 18:2 → 18:3, etc.

5.3.5. DISCUSSION

The fact that changes occur in serum lipid metabolism during the febrile state has been acknowledged for over fifty years. However, the cause of these changes have eluded the investigators, as most report have been hampered by the different periods of illness studied, and also by the lack in base-line values at the time that measurements were performed. Furthermore, a single measurement of serum lipid defines only one moment in the continuous movement of lipid moieties, making it difficult to assess the effect of infection on lipid utilisation in the peripheral tissue. The use of triglyceride as the measurement for sepsis induced by Gram-negative bacteria has been suggested by Kaufman et al.(1976), yet to date no clinical application of this has been implemented. In the present study the regular occurrence in the delay between injections and the onset of fever (20 minutes) throughout the experiment suggest that endotoxin does not act directly on the brain (Dascomb and Milton, 1979), but acts through a mechanism which is exothermic and can reach the thermoregulatory centres from the periphery (Dopeswarkar and Mead, 1973) in order to act on appropriate receptors sites in the brain. Brain cells are dependent on glucose as a primary source of fuel (Owen, 1967) and are inflexible in their requirements.

depends on the mobilisation of fat from depot to circumvent nitrogen depletion (Owen, 1967). Fatty acid therefore play an importent role in the genesis of fever induced by Gram-negative bacteria, as they are able to reach the thermoregulatory centres of the brain and can be transferred across the blood-brain barrier (Dopeswarkar and Mead, 1973). Consequently fever is not only a brain induced physiological event, but a peripheral one as well. The recognition of this fact is poorly understood. In the present study, it become apparent that depending on the nutritional state of the rabbits, different fractions of lipids are mobilised. In the fed state, TG, CHO and lecithin 18:3(w6)->20:3(w6)->20:4(w6)->22:4(w6)->22:5(w6) hold the key position in the onset (20 min), fever height (90 min) and the duration (180 min) of the first phase of the biphasic fever. In the fasted state FFA and lecithin linoleic acid not only ensure the prolongation of the fever beyond 180 min., but also raise the fever plateau to a higher level, thus ensuring the second phase of the biphasic fever. The decrease or no fever response to constant stimulation of the pathway with endotoxin further emphasises the drastic changes in lipid metabolism, as it led to unresponsiveness or tolerance in the diseased state.

6.0 THE EFFECT OF DIETARY LINOLENATE ON THE PATHOGENESIS OF FEVER

6.1. Experimental model

Ten white New Zealand (NZ) rabbits weighing 2,20-2,80 kg were divided into two groups. Group A (N=5), designated the control, received the standard commercial diet of rabbit pellets (Table 2), with a fatty acid content which included 50 per cent linoleic acid (Table 18). Group B (N=5) received the standard diet supplemented with hay and containing fatty acids comprising 65 per cent linoleic acid and 48 per cent linolenic acid (Table 18) one week prior to and during the fever experiment. Extraction of total lipid and fatty acid analysis of the diet was performed according to paragraphs 5.2.1., 5.2.2, 5.2.3, and 5.2.4. Water was available to all rabbits ad lib. Both groups were injected once daily intravenously (IV) with 2,0 ug/kg body mass of endotoxin Salmonella Thyphosa (as described in paragraph 5.1.2.2) for 7 days. The animals were housed individually in steel cages and kept on a 12 hr-12 hr photophase-scotophase regimen. To minimise the sudden stress during the experimental period, the rabbits were acclimatised to their restraining boxes and to thermocouples for 1 week before the experiment begun. Each animal was weighed daily before being given its injection and again 180 min thereafter.

Table 18.

FATTY ACID COMPOSITION OF RABBIT STANDARD AND HAY DIET

Fatty acid	standard diet	hay diet
unid.	0,04	3,40
14:0	0,27	2.38
14:1	0,10	1,29
16:0	16,50	15,78
16:1	0,67	1,22
18:0	1,98	2,55
18:1n9	27,94	2,30
18:2n6	50,87	14,70
18:3n3	-	48,30
22:0	0,63	1,97
20:4n6	-	0,04
24:0	0,60	3,40
22:5n6	0.40	2,30

Values are the mean of two lipid extractions expressed as a percentage of respective fatty acids.

The rectal temperature measurements were performed as described in paragraph 5.1.2.3, while blood was sampled as described in paragraph 5.1.2.4., before the start of the experiment (base line), and again at 60 min and 180 min after the first and seventh IV injection of endotoxin. Fatty acid analysis of the serum was performed according to paragraph 5.2.2, 5.2.3. and the methyl esters were separated on a gas chromatograph, as described in paragraph 4.2.5. Results were statistically analysed as described in paragraph 5.3.2. Finally, animals were sacrificed, the brain carefully removed, washed tree time in cold saline and freeze-dried in liquid N_2, before being stored at -70°C. A brain phosphatadylcholine analysis was performed according to paragraphs 4.2.1., 4.2.2., 4.2.3. 4.2.4. and 4.2.5.

6.2. RESULTS

6.2.1. Introduction

A "true" fever, according to Bernheim et al. (1979), is a disorder of thermoregulation by which the body actively seeks to raise its temperature by increasing heat production. Heat is the result of oxidative catabolism in the body, and there is a relation between the amount of O_2 absorbed and the amount of CO_2 eliminated (Benedict, 1907; Fritz, 1961).

Excessive serum unsaturation with C_{18} fatty acids, induced by an increased intake of dietary linoleic, or linolenic acids, results in decreased oxygenation (Stadie, 1945), and increased phosphorylation (Stadie, 1945; Marco et al. 1961). This stimulates CO_2 production via the long chain fatty acids (Fritz, 1961). Heat thus gained affects various cellular metabolic functions, i.e. inhibition of respiratory activity (Sheu and Freese, 1973) or the increase of peroxidation (Marco et al. 1962). Both can cause metabolic disturbances affecting the respiratory quotient (R.Q.), which influences the body's heat loss mechanism. Increased heat production alters the lipophilic and hydrophilic balance within fatty acids, whereby the neutralising effect of serum is inhibited. The increased peroxidation induced by an increase in C_{18} fatty acids, increases the activity of phospholipase A, which alters molecular target cells. All these factors contribute to the cytotoxicity of C_{18} fatty acids, which provokes lysis of the bacterial cell membrane. This illustrates the biochemical importance and physiological role of the essential fatty acids (Burr and Burr, 1930; Friedman, 1979). An excess of the latter appears to influence the body's mechanism for gaining instead of losing heat. An increase in the heat production via essential fatty acids could, therefore, provide the host with fuel to starve out bacterial infection.

However, a rise in both 18:2(w6) and 18:3(w6) leads to competitive inhibition (Pace-Asciak and Wolff, 1968) in the formation of 20:4(w6) -> 22:4(6)->22:5(w6) (Hwang and Carrol, 1980). This combines with chronic induction of fever, contributing to a physio-pathological condition which is diet-induced and not recognised. As lecithin constitutes 70 per cent of human phospholipids (Fredericson and Gordon, 1958) and is rich in both unsaturated and polyunsaturated fatty acids, changes in essential fatty acids cause greater alterations in this class of lipids than in either FFA or TG (Ogburn et al. 1982). Consequently, the present work was undertaken to clarify the effect of an increased intake of dietary linolenate on the specific distribution of serum and brain lecithin fatty acids, and, subsequently, the effect of these changes on thermoregulation and the production of fever induced by Gram-negative bacterial endotoxin. A further objective was to study the pathophysiological consequences of this alteration in brain fatty acids composition.

The data is presented as follows;

(a) A comparison of body weight between the control group (Group A) and rabbits fed the hay supplemented diet (Group B).

(b) The effect of hay on serum lecithin fatty acid composition.

(c) The effect of endotoxin on fever response and on the composition of serum lecithin fatty acids following: 1) single dose of endotoxin.
2) multiple doses of endotoxin.

(d) The effect of hay on brain phosphatadylcholine

6.2.2. Body Weight

No significant difference in body weight was recorded during the course of the fever experiment. In the control (Group A) the average body weight recorded before the experiment was 2,27±0,10 kg and after seven consecutive injections, 2,17±0,06 kg. In Group B the average weight was 2,86±0,5 kg and after the seventh injection, 2,70±0,05 kg. The student t-test indicates that the amount of linolenate in the diet had no significant effect on the body weight of the rabbits as sufficient amounts of linoleic acid was provided by both diet.

6.2.3. The effect of hay on serum lecithin fatty acid composition

Results show (Table 19) that, after 1 week, the increased dietary intake in the linolenate affected the interrelation between the saturated (18:0), unsaturated 18:3(w3) and polyunsaturated 20:4(w6) and 20:5(w3) fatty acids. An increase in the levels of 18:3(w3) and 20:5(w3) occurred with a decrease in

TABLE 19 Effect of a Single Intravenous Injection of 2.0 μg/kg Endotoxin *S. thyphosa* on the Composition of Serum Lecithin Fatty Acid in Rabbits Fed Standard or Hay-Supplemented Diet[a]

	Group A[b]			Group B[c]		
		Post-injection			Post-injection	
Fatty acid	Preinjection	60 min	180 min	Preinjection	60 min	180 min
16:0	24.07 ± 1.15[α]	26.11 ± 2.37[α]	27.01 ± 1.92[α]	22.27 ± 1.44[α]	21.05 ± 1.61[α]	19.06 ± 1.74[α]
18:0	15.38 ± 0.58[α]	12.76 ± 1.36[α]	13.40 ± 0.74[α]	20.91 ± 0.35[β]	21.70 ± 0.50[β]	21.85 ± 0.67[β]
18:1 (ω9)	15.99 ± 0.62[α]	18.97 ± 1.38[α]	14.36 ± 1.15[α]	14.90 ± 1.68[α]	13.82 ± 1.62[α]	13.85 ± 1.02[α]
18:2 (ω6)	30.96 ± 0.77[α]	26.83 ± 0.48[β]	32.17 ± 0.43[α]	32.39 ± 0.91[α]	34.72 ± 0.17[β]	36.40 ± 0.93[β]
18:3 (ω3)	0.76 ± 0.04[α]	0.75 ± 0.04[α]	0.90 ± 0.03[α]	1.52 ± 0.44[β]	0.85 ± 0.12[α]	0.94 ± 0.08[α]
18:3 (ω6)	0.40 ± 0.03[α]	0.27 ± 0.05[α]	0.35 ± 0.05[α]	0.73 ± 0.13[α]	0.63 ± 0.11[α]	0.70 ± 0.22[α]
20:3 (ω6)	0.40 ± 0.03[α]	0.27 ± 0.04[α]	0.38 ± 0.01[α]	0.70 ± 0.17[α]	0.45 ± 0.06[β]	0.55 ± 0.08[α]
20:4 (ω6)	3.97 ± 0.19[α]	2.63 ± 0.35[α]	3.94 ± 0.71[α]	1.82 ± 0.40[β]	2.65 ± 0.38[α]	3.34 ± 0.21[α]
20:5 (ω3)	0.64 ± 0.03[α]	0.46 ± 0.09[α]	0.69 ± 0.18[α]	3.80 ± 0.98[β]	3.22 ± 0.38[β]	2.80 ± 0.30[β]
22:4 (ω6)	1.20 ± 0.11	1.96 ± 0.28	1.64 ± 0.20	—	—	—
22:5 (ω6)	3.94 ± 0.27	3.75 ± 0.31	2.90 ± 0.13	—	—	—
Rest. Poly.	2.34 ± 0.28[α]	2.71 ± 0.31[α]	1.42 ± 0.36[α]	0.62 ± 0.21[β]	0.76 ± 0.11[β]	0.88 ± 0.12[β]

[a]Values represent the relative percentage of fatty acids measured by gas–liquid chromatography (GLC) and the average of four or five determinations. [β] indicates significantly different from control (<0.05) ($p > 0.05$) by Duncan multiple range. Test SE ±SEM.
[b]Standard diet, $N = 4$.
[c]Hay-supplemented diet, $N = 5$.

20:4(w6), combined with an absence of 22:4(w6) and 22:5(w6).

6.2.4. Effect of endotoxin on fever response and on the composition of serum lecithin fatty acids

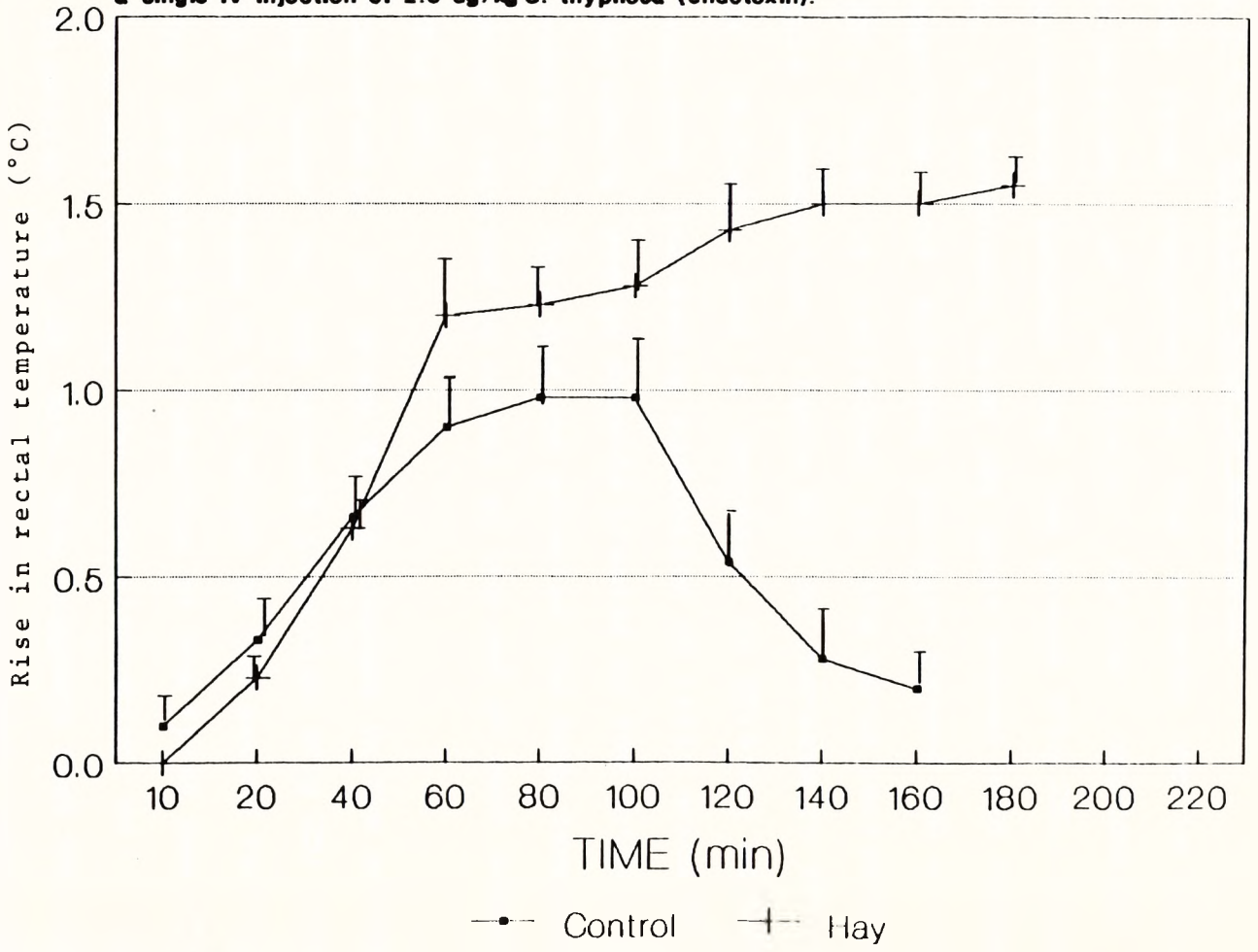
6.2.4.1. Single dose of endotoxin

There was a difference between the two groups in their fever response to a single dose of endotoxin 2,0 ug/kg In group A, the endotoxin produced a short monophasic fever (Fig. 24) with a delay of 20 min before its onset. The fever peaked after 60 min and had a duration of 80 min. In Group B, the endotoxin evoked a magnified biphasic fever with the same delay to its onset as in group A, but with its first peak at 90 min, the second at 140 min and a duration of several hours (Fig 24). Changes in plasma lecithin fatty acid composition appear to coincide with alterations in the fever pattern. In group A, a decrease in the level of all the unsaturates and eicosanoids 20:3(w6), 20:4(w6) and 22:5(w6) occurred within 60 minutes, together with an increase in 20:4(w6) at 180 min. No changes in 20:5(w3) in the plasma level developed (Table 19).

In group B, statistically significant increase in the levels of 18:2(w6) happened within 60 minutes, followed by a decrease in the linolenates 18:3(w3), together with reduced levels of 20:3(w6) and 20:5(w3), while docosatetraeonic acid 22:4(w6) and

INFLUENCE OF HAY ON FEVER IN RABBITS

Fig. 24. Comparison in fever curves of rabbits fed the standard or hay supplemented diet and subjected to a single IV injection of 2.0 ug/kg S. thyphosa (endotoxin).



Error bars indicate ± SE of 4 rabbits. Endotoxin injected at time 0.

docosapentaeonic acid 22:5(w6) remained inhibited (Table. 19). These changes suggest a heightened degree of lipolysis and decreased unesterification of fatty acids within the plasma.

6.2.4.2. Multiple dose of endotoxin

The fever response by the seventh day to daily IV injections of endotoxin further emphasized the difference between the two groups (Fig 25). In group A, a monophasic fever was again evoked with increased magnitude and extended duration beyond 180 min (Fig. 25). This is reflected in the serum lecithin fatty acid composition (Table 20). An increase in the level of 18:2(w6), and a decrease in 22:4(w6) at 180 min indicates increasing combustion of fatty acids to meet increasing energy demands created by the action of the endotoxin. This did not affected the body temperature, which returned to normal at the end of each experiment (Fig 25).

Conversely, in group B, the changes in the pattern of the fever by the seventh injection (Fig 25) resulted in a decrease in the height of the second fever peak. The characteristic retention of the first peak was not reversed, even by the administration of a second injection at 120 min. This suggests that the animals had been made immune to the pyrogenic action of the bacterial pyrogen, not by its continual administration (Fig 25), but more so by the deficiency in the

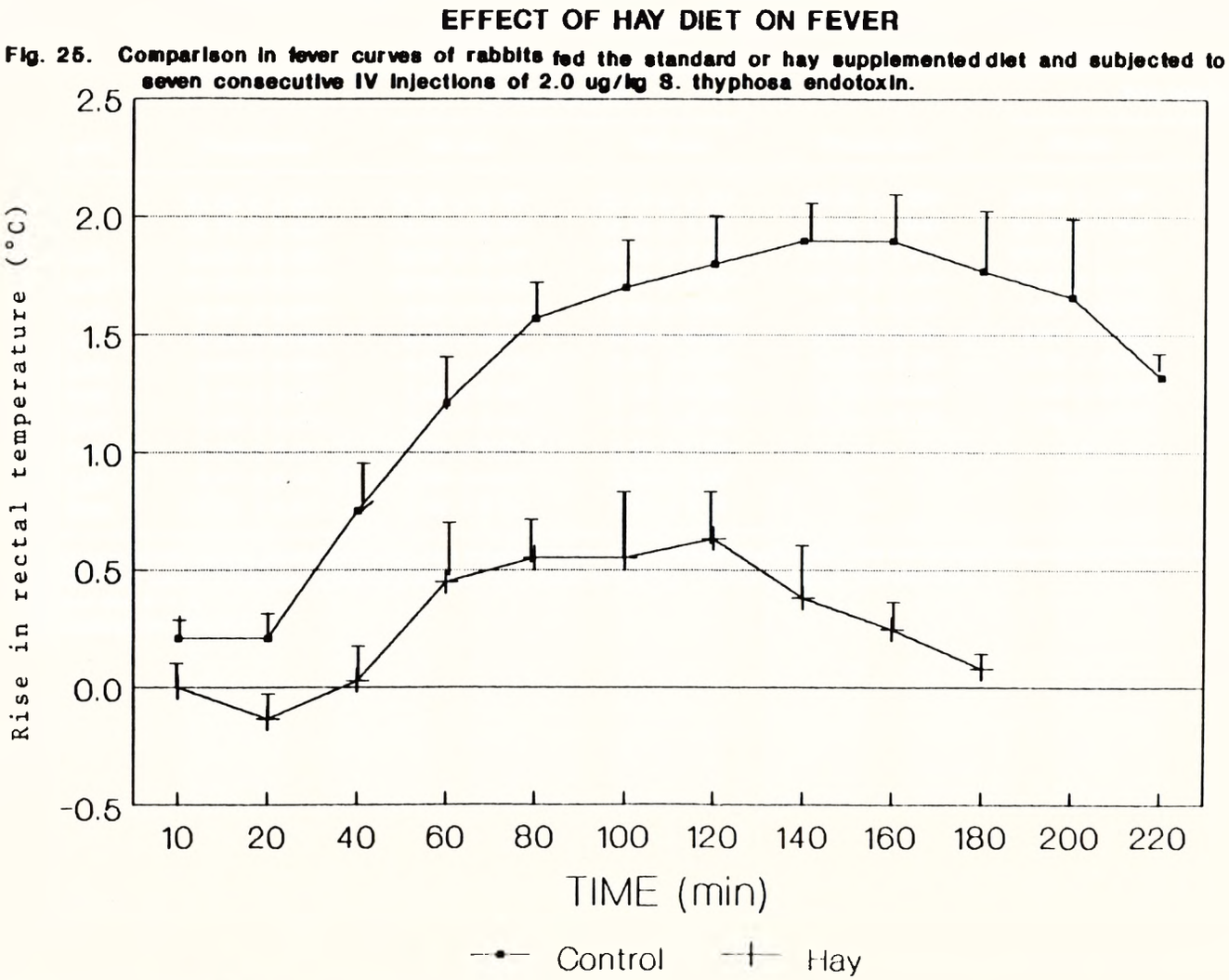


Fig. 25. Comparison in fever curves of rabbits fed the standard or hay supplemented diet and subjected to seven consecutive IV injections of 2.0 ug/kg S. thyphosa endotoxin.

Error bars indicate ± SE of 4 rabbits. Endotoxin injected at time 0.

TABLE 20 Effect of Seven Consecutive Intravenous Injections of 2.0 μg/kg Endotoxin *S. thyphosa* on the Composition of Serum Lecithin Fatty Acid in Rabbits Fed Standard or Hay-Supplemented Diet[a]

	Group A[b]			Group B[c]		
		Post-injection			Post-injection	
Fatty acid	Preinjection	60 min	180 min	Preinjection	60 min	180 min
16:0	22.98 ± 2.64[α]	25.90 ± 0.72[α]	23.17 ± 1.35[α]	20.37 ± 1.35[α]	20.60 ± 1.79[α]	19.91 ± 3.16[α]
18:0	15.73 ± 1.63[α]	13.12 ± 0.72[α]	14.14 ± 1.51	22.02 ± 1.94[α]	21.19 ± 2.98[α]	20.88 ± 1.90[α]
18:1 (ω9)	15.05 ± 0.95[α]	18.91 ± 1.64[α]	16.42 ± 1.92[α]	14.82 ± 1.71[α]	14.82 ± 1.71[α]	12.90 ± 1.99[α]
18:2 (ω6)	31.72 ± 0.77[α]	30.06 ± 0.33[α]	34.57 ± 0.92[α]	34.74 ± 1.65[α]	34.07 ± 0.72[β]	37.27 ± 0.26[β]
18:3 (ω3)	0.94 ± 0.03[α]	0.63 ± 0.02[α]	0.80 ± 0.09[α]	1.50 ± 0.52[β]	1.01 ± 0.18[β]	0.92 ± 0.26[α]
18:3 (ω6)	0.28 ± 0.06[α]	0.33 ± 0.06[α]	0.36 ± 0.06[α]	0.65 ± 0.16[α]	0.66 ± 0.25[α]	0.49 ± 0.31[α]
20:3 (ω6)	0.24 ± 0.05[α]	0.37 ± 0.07[α]	0.31 ± 0.07[α]	0.56 ± 0.18[α]	0.58 ± 0.20[α]	0.42 ± 0.15[α]
20:4 (ω6)	3.15 ± 0.53[α]	2.64 ± 0.69[α]	3.71 ± 0.38[α]	1.79 ± 0.08[β]	1.99 ± 0.59[α]	2.49 ± 0.48[α]
20:5 (ω3)	0.76 ± 0.17[α]	0.42 ± 0.04[α]	0.51 ± 0.10[α]	2.31 ± 0.55[β]	1.96 ± 0.64[β]	3.93 ± 0.40[β]
22:4 (ω6)	2.38 ± 0.45	1.19 ± 0.01	1.62 ± 0.34	—	—	—
22:5 (ω6)	4.47 ± 0.45	2.69 ± 0.01	3.26 ± 0.60	—	—	—
Rest. Poly.	2.03 ± 0.57	2.60 ± 0.45	1.53 ± 0.14	—	—	—

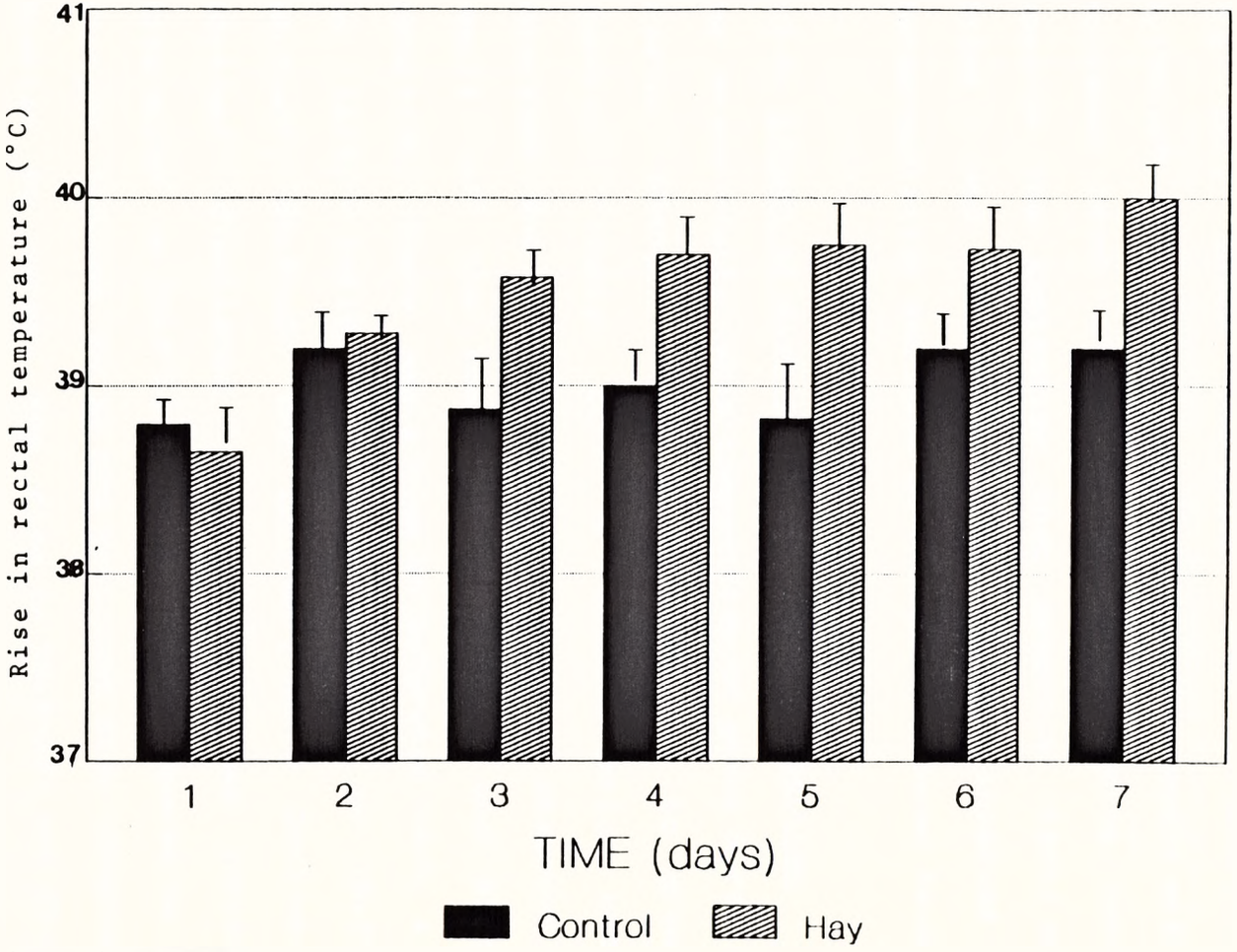
[a] Values represent the relative percentage of fatty acids measured by gas–liquid chromatography (GLC) and the average of four determinations in each group. Test SE ± SEM of four rabbits. [β] indicates significantly different from control (<0.05) ($p > 0.05$) by Duncan multiple range.

[b] Standard diet, $N = 4$.

[c] Hay-supplemented diet, $N = 5$.

BODY TEMPERATURE OF RABBITS

Fig. 28. **Influence of hay on body temperature of rabbits before injection of endotoxin S. thyphosa.**



Error bars indicates ± SE of 4 rabbits.

production of 20:4(w6) induced by the elevated content of the serum 20:5(w3) on the one hand and increased intake of dietary 18:3(w3) on the other hand (Tables 19 and 20). Tolerance to endotoxin in these animals did not prevent the daily rise in body temperature (Fig 26), which increased from 39,28±0,05 to 40,15±0,09 ^{o}C, which occurred simultaneously with an increased level of 18:2(w6). This finding further supports the contention that essential fatty acids 18:2w6 and 18:3(w3) are indeed involved in thermoregulation and PUFA in endotoxin-induced fever.

6.2.5. Effect of diet on brain lipid composition

The brain fatty acid composition of group B reflects drastic changes when it is compared to group A (control) (Table 21). A disequilibrium between saturated and unsaturated fatty acids is observed as a result of the combination of diet and diseased condition. There was a rise in saturated 14:0, a drop in 16:0, followed by an increase in 18:2(w6), 18:3(w3), 20:5(w3) and 22:5(w6), while 20:3(w6) and 20:4(w6) diminished and 16:1 was inhibited. Alteration in 20:3 is striking, when compared to those changes induced by an EFA deficient diet (Chapter 2). Similar to serum, the rise in 20:5(w3) and 22:5(w3) led to the fall in 20:4(w6) and 20:3(w6). This prevented the response of the fever to endotoxin, as both are precursors of PGE's. This further supports

Table 21

FATTY ACIDS COMPOSITION OF BRAIN PHOSPHATIDYLCHOLIN (PC) IN RABBITS FED EITHER THE STANDARD OR HAY SUPPLEMENTED DIET SUBJECTED TO 7 INTRAVENOUS INJECTIONS OF HLP OR S.THYPOSA

Fatty acid	Diet Standard (n=7) SE	Hay suplemented Endotoxin (n=5) SE	Statistical Significance t-test	P
14:0	1,80±0,20	3,75±0,08	6,96	< 0,0001
16:0	41,30±0,89	31,20±0,56	6,97	< 0,0001
16:1	2,05±0,58	-		V.H.S.
18:0	13,85±0,56	12,10±0,48		
18:1n9	31,40±0,99	32,20±0,66		
18:2n6	2,05±0,11	2,90±0,02	5,00	< 0,005
18:3n6	0,95±0,02	1,20±0,14		
18:3n3	-	3,95±0,33		V.H.S.
20:3n6	0,71±0,10	0,10±0,05	4,00	< 0,005
20:4n6	2,53±0,21	0,32±0,04	8,84	< 0,0002
20:5n3	-	0,45±0,00		V.H.S.
22:4n6	0,56±0,16	0,77±0,08		
22:5n6	0,90±0,26	0,70±0,10		
22:5(n3)	-	7,80±0,50		V.H.S.
22:6	1,90±0,30	2,55±0,19		

Values represents the relative percentage of fatty acids measured on GLC and the average of seven to five determinations. Test SE±standard error of the means. * (n=) represents the number of animals used in the experiments. V.H.S. represent the very highly significant test when the compound is not present.

previous findings that the incorporation of peripheral fatty acid occurs within a very short time and is readily incorporated into brain lipids (Fig. 23). This incorporation is necessary, as brain integrity has to be preserved at all costs, primarily to maintain life, and secondarily to regulate changes occurring within the body. The elevated content of the unsaturated 18:2(w6), 18:3(w3) and 20:5(w3) therefore influenced cerebral microsomal elongation and the desaturation system, which is largely concerned with polyunsaturated fatty acids in the ester linkage. This led not only to an alteration in the balance between saturated and unsaturated fatty acids, but also affected the close relation between polyunsaturates. An alteration in this balance due to the action of seven consecutive injections of endotoxin led to hyperthermia instead of fever.

6.4.5. DISCUSSION

Under normal physiological conditions, biochemical changes introduced by the diet can continue unobserved. Under changed physiological conditions that are sensitive to differences in the amounts of arachidonic acid, the absence or an excess of or an imbalance in arachidonate synthesis in the tissues can lead to irreversible pathological changes. As these changes are not recognised, they cannot be diagnosed clinically. In the present study, the existence of a

sensitive balance between 18:2(w6) and 18:3(w3) is clearly defined by an abnormal response of fever to a single small dose of endotoxin on the one hand and the rabbit's tolerance to endotoxin within 7 days on the other hand, together with a gradual rise in the body temperature. Rise in the body temperature in turn is influenced by the equilibrium between 18:2(w6) and 20:4(w6). A change in this balance appears to influence not only the prognosis of fever but also the body temperature as well. A fall in the levels of 18:2(w6) and 20:4(w6) under the action of pyrogen indicates that fever is produced by the metabolites of arachidonic acid. Heat is thus mainly gained by peripheral polyunsaturates provided that R.Q. remains constant. However, if the body heat is increased by either 18:2(w6) or 18:3(w3), or both, and is followed by a decrease in the formation not only of serum 20:4(w6), but of cerebral arachidonate as well, CO_2 production is increased significantly (Fritz, 1961) and, in turn, R.Q. is altered (Splawinski et al. 1978) Since metabolism (R.Q.) and heat production go hand in hand (Benedict, 1907) with increased oxidative catabolism, by way of either 18:2(w6) or 18:3(w6), or both, increased peroxidation (Stadie, 1945, Marco et al., 1961) and alteration in cerebral mitochondrial function occurs in the course of increasing the intake of dietary linolenate (Marco et al., 1962). This affects both cell permeability and the configuration

of fatty acids existing within phospholipids (Melchior et al.,1970). An increase in 18:2(w6) or 18:3(w6) fatty acids promotes membrane fluidity to help the cell to survive raised environmental temperatures. Thus, fluctuations in the level of 18:2(w6) due to endotoxin influence the pattern of the fever, which in turn indicates whether the fever has been centrally or peripherally initiated. It also defines the existence of tolerance to endotoxin in animals rendered resistant to the pyrogenic action of bacterial pyrogen within a short time by the combination of increased amounts of 18:3(w3), 20:5(w3), and regular induction of small doses of endotoxin. Two different mechanisms can be differentiated: (1) initiation by peripheral arachidonic acid metabolites, which induce fever; and (2) hyperthermia induced by peripheral 18:2(w6) and 18:3(w3). These results can therefore be regarded as further evidence that polyunsaturates are actively involved in the induction of fever. Linoleic and linolenic acids appear to be involved in thermoregulation. A change in the thermoregulatory process is manifested by a rise in body temperature and can therefore be considered a change in the life process accompanied by increased heat production (Benedict, 1915). In the present study, an increase in body temperature can therefore be equated with increased heat production induced by the combined action of an increase in 18:3(w3) and 20:5(w3) in the diet. Under the stimulus of S. Thyphosa, the fever had less of an effect on the body temperature.

RABBIT IN DEEP SHOCK



—Illustration showing the appearance of the blood vessels in the ears of a rabbit "in a state of deep shock." The marked vasoconstriction is very plain in the left ear, the vessels of the right ear being dilated because the cervical sympathetic, which carries the constrictor fibers, has been cut. (From Seelig and Joseph.)

7.0 CHRONIC IMBALANCE IN EICOSONOIDS, A COMMON FEATURE OF HYPERTHERMIA IN RABBITS

To provide further support for the postulated role of C_{18} fatty acids in the control of body temperature, and C_{20}-C_{22} in fever, the present study examines several cases encountered over 2 years of experimental work, by which rabbits were found to have pyrexia (39,7-40,40^{o}C), combined with serious signs of distress (lassitude, lethargy, poor appetite, little water consumption, severe panting), together with essential fatty acid deficiency symptoms i.e. loss of body and facial hair and diarrhoea. The cause of the disease in these rabbits was unknown, promted the writer to study the influence of body temperature on febrile response to endotoxin (Case 1), the effect of dietary essential fatty acids on serum lecithin fatty acid composition and on body temperature (Case 2), and finally to asses the fatty acid status of hyperthermic rabbits, prior to death (Case 3). The outcome is discussed in the final chapter of the thesis, in which lecithin fatty acids analysis is used as a diagnostic tool.

7.1. CASE N^{o} 1 (Date, 1985 05.15.)

Ten juvenile white male New Zealand (NZ) rabbits weighing between 1,70 and 1,90 kg and exhibiting a body temperature of 40,0-40,4^{o}C were subjected twice to a single intravenous (IV) injection of 4,0 and 6.0 ug/kg body mass of purified S. Thyphosa

lipopolysaccharide as described in paragraph 5.1.2.2. Each rabbit was weighed daily before injection and at 180 min thereafter. Temperature measurement was recorded according to paragraph 5.1.2.3 and a blood sample was drawn from the ear artery in the same manner as in paragraph 5.1.2.4, 60 min after injection. A serum lecithin fatty acid analysis was done as described in paragraphs 5.2.2., 5.2.3. and 5.2.4.

7. 1.1 Assessment of the physiological condition of the rabbits

Drawing blood from these rabbits was extremely difficult, as the veins and arteries were constricted (see p 129) and when achieved, five out of ten were haemolysed prior to injection. Therefore, only five rabbits were used in this experiment. Rabbits showing haemolysed blood died before being used for the experiment. In the remaining 5 rabbits trauma, such as weight loss, occurred after endotoxin injection.

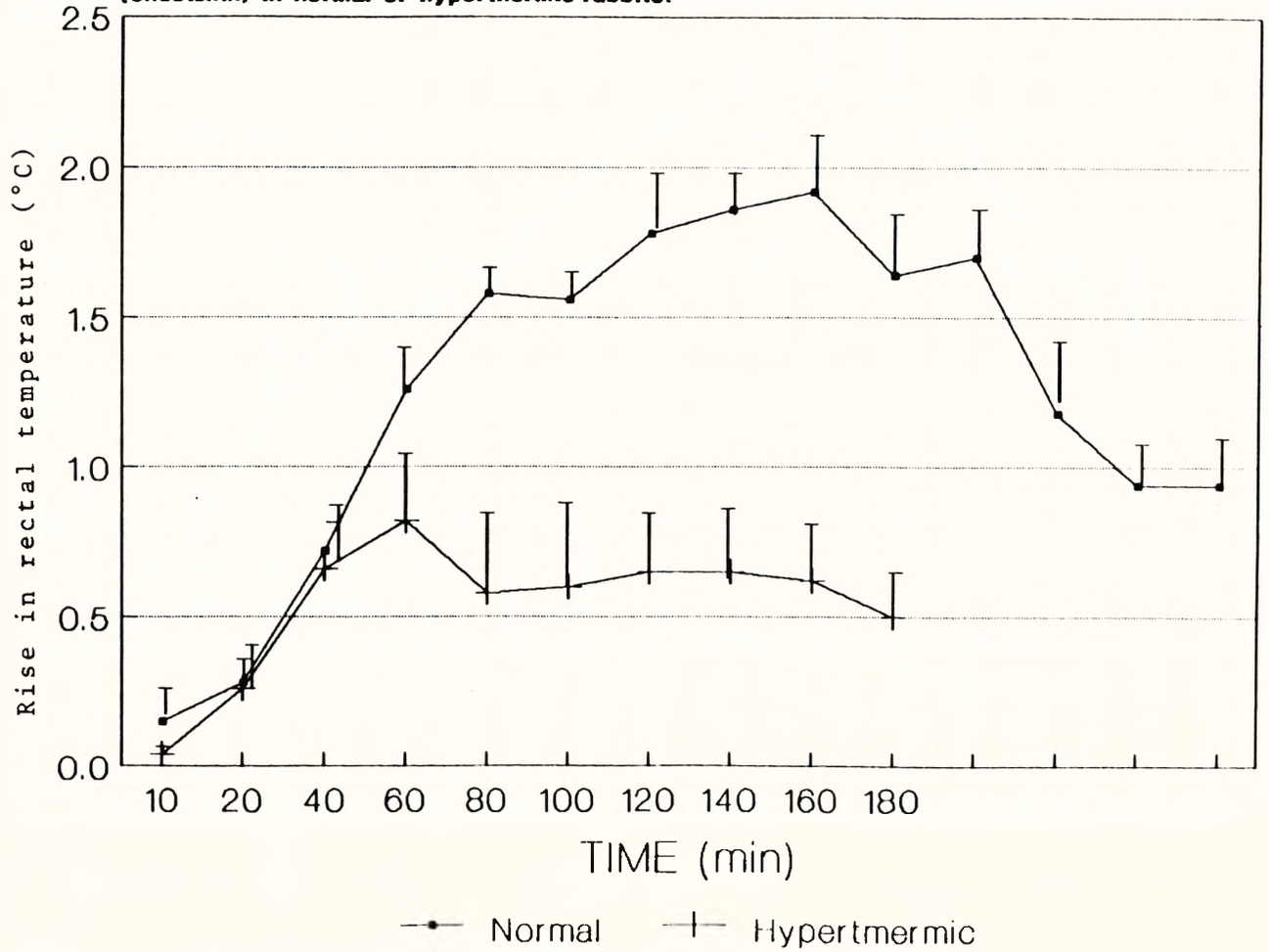
7.1.2 RESULTS

7.1.2.1 Physiological

In the hyperthermic rabbits a single dose of endotoxin 4,0 ug/kg produced a short monophasic fever (Fig. 27) with a latency to its onset of 20 min, and a fever peak at 60 min and a duration of 180 min. The second dose endotoxin (6,0 ug/kg) led to tolerance (Fig. 28)

EFFECT OF BODY TEMPERATURE ON FEVER

Fig. 27. Comparison in fever curves produced by IV administration of a single dose of 4.0 ug/kg S. thyphosa (endotoxin) in normal or hyperthermic rabbits.



Error bars indicates ± SE of 4 rabbits (per group). Endotoxin injected at time 0.

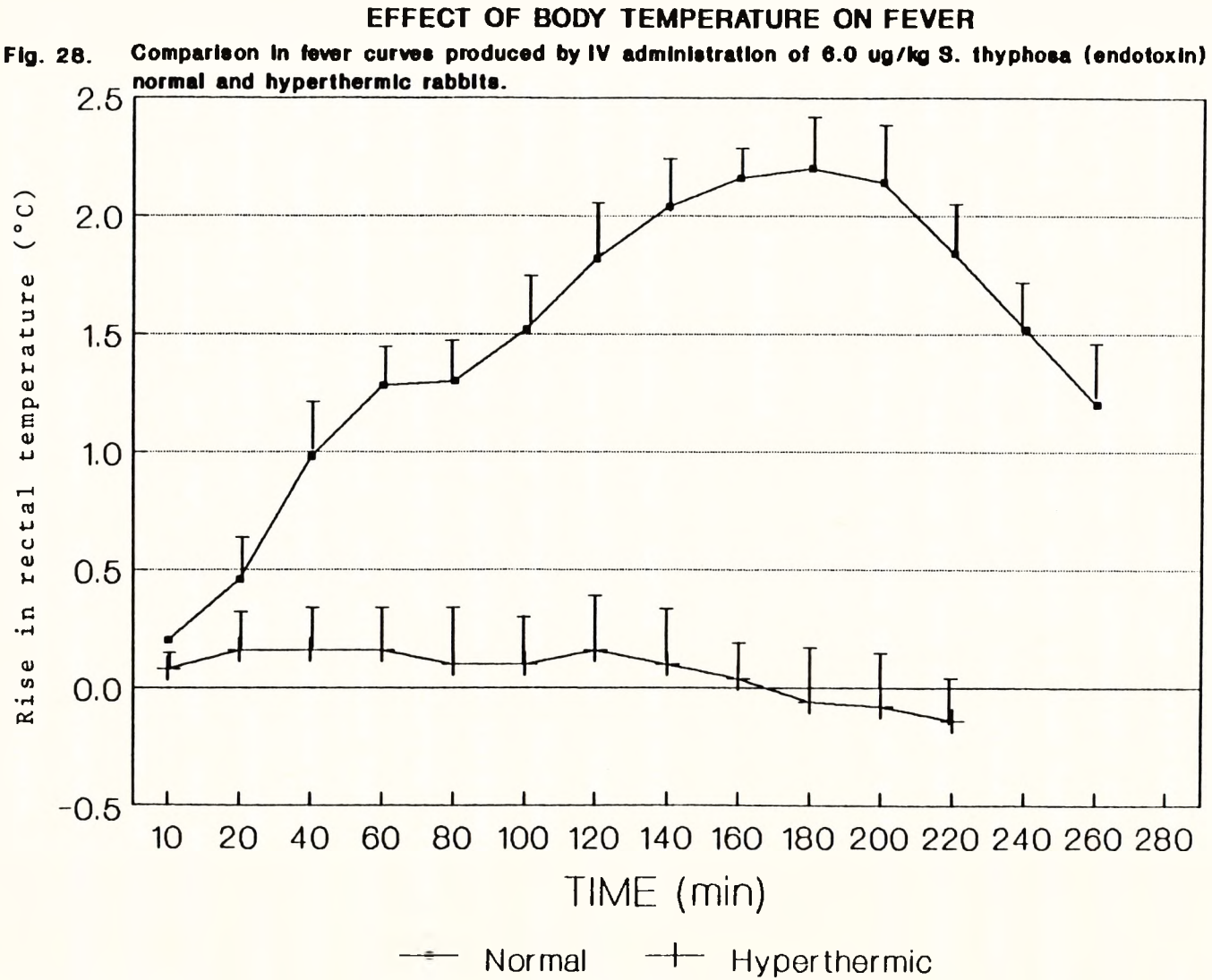


Fig. 28. **Comparison in fever curves produced by IV administration of 6.0 ug/kg S. thyphosa (endotoxin) in normal and hyperthermic rabbits.**

Error bare indicates ± SE of 5 rabbits (per groups). Endotoxin injected at time 0.

and, out of five, two responded with negative feedback This confirms the previous finding by Kenedi and Mendelsohn,(1989) that the body temperature of the host is the major factor influencing susceptibility to bacterial endotoxin. The high mortality rate observed in these rabbits after being subjected only twice to endotoxin injections, could be attributed to the failure of the organism to adequately counteract the stimuli or stresses to which it had been subjected, thus causing disturbances in the alarm mechanism of the body. This resulted in the stage of tolerance to begin with and, finally, to the state of exhaustion.

7.1.2.2 Biochemical

Before injections, the serum lecithin fatty acid pattern of hyperthermic rabbits (fed condition), showed a defect in the formation of 18:3(w6)->20:3(w6) while no changes in the formation of 18:2(w6)-> 20:4(w6)-> 22:4(w6)->22:5(w6) occurred (Table 22). This demonstrates that the elevated body temperature in these rabbits affected the desaturised system and consequently inhibition in PGE_1 occurred. A defect in the release of PGE_1 prevented the breakdown of triglycerides and also the formation of 18:3(w6)-> 20:3(w6) from 18:2(w6). Thus hyperthermia was reached via glycogenesis, instead of via lipolysis.

Table 22

Case 1. INFLUENCE OF 4,0 AND 6,0 ug/kg ENDOTOXIN (S.THYPHOSA) ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS IN HYPERTHERMIC RABBITS (Sample taken after 60 minutes).

Fatty acid	Normal (n=5) SE	Hyperthermic Rabbits Before (n=4) SE	Day 1 4,0 ug (n=5) SE	Day 2 6,0ug (n=4 SE
16:0	24,40±0,74	25,58±1,42	27,10±1,27	20,05±3,13
18:0	15,40±0,58	16,30±0,68	15,50±1,40	15,50±0,14
18:1n9	18,20±0,75	15,60±0,11	13,90±0,30	17,10±0,04
18:2n6	29,10±0,65	29,95±0,88	29,90±0,30	30,40±0,59
18:3n3	0,85±0,08	0,86±0,05	0,83±0,07	0,72±0,13
18:3n6	0,40±0,15	-****	0,04±0,01 **	0,36±0,13 *
20:3n6	0,55±0,05	0,32±0,05	0,75±0,09	0,77±0,16
20:4n6	3,85±0,19	3,52±0,48	3,00±0,27	3,42±0,18
20:5n3	0,30±0,03	0,37±0,08	0,40±0,11	0,30±0,06
22:4n6	1,85±0,27	2,58±0,60	2,88±0,55	2,23±0,73
22:5n6	3,70±0,28	4,25±0,43	2,62±0,33 ***	4,95±0,58 ***
Unid.poly	1,40±0,32	1,10±o.15	4,83±0,63	3,81±0,58

Values represents the relative percentage of fatty acid measured on GLC and the average of four to five determinations. Test SE±Standard error of the means. * represents the statistical significant t- test. *p < 0,05 **p < 0,025 *** p<0,005 **** compund is not present.

Within 60 min of the first dose of endotoxin (4,0 ug/kg), a rise in 20:3(w6) occurred in spite of diminished 18:3(w6) content (Table 22). This consequently affected the release of 20:4(w6), and fever response was mediated via the pathway 20:3(w)->PGE_1, instead of 20:4(w6)->PGE_2. However, the occurrence of high mortality rate in these rabbits after administration of the second dose of 6,0 ug/kg endotoxin, can be attributed to the action of tolerance induced by the combination of elevated body temperature, combined with inhibition of the fatty acid transferred from one position to an other (Table 22). Unchanged fatty acid configuration in the face of endotoxin shows that tolerance in the febrile response occurred peripherally, while the high body temperature was maintained centrally.

7.2 Case_2_(Date 1985. 11. 26.)

The serum fatty acid status (Table 23) was assessed in six hyperpyretic (39,6-40,4 ^{o}C) New Zealand (NZ) rabbits weighing between 3,60 and 4,80 kg and exhibiting essential fatty acid deficiency symptoms. To improve their physical condition, they were put on a diet generously supplemented with hay for 2 weeks. The methodology applied in the analysis of serum fatty acids was the same as described in paragraph 5.2.2, 5.2.3 and 5.2.5.

7.2.1. RESULTS

The thin layer chromatographic separation of phospholipid class indicates an abnormal phospholipid pattern (Fig. 29) prior to the dietary experiment. Rapid incorporation of shingomyeline and serine into lecithin, together with the appearence of increased phosphatadylinositol in 3 rabbits on the one hand and diminished phospholipid classes (including lecithin) in the remaining 3 rabbits exhibiting diarrhoea on the other hand, clearly indicate that the disease is induced by different microorganisms. The increased turnover of phospholipid synthesis (shown on TLC), can be linked with the defence mechanism of the host against the harmful nature of the invading microorganism, since the formation of a new membrane is regarded as a prerequisite for phagocytosis (Day, 1967) during febrile disease. However the diminished phospholipid classes show (on the same TLC plates) a reduction in the formation of lecithin. The decrease in lecithin implies that the metabolic changes induced by the disease are mediated by the activation of TG fatty acids. Fatty acids attached to TG have been shown to possess particular radicals which are able to activate the reticulo-endothelial system (RES), both in vitro and in vivo (Cooper, 1964). Thus different species of microorganisms use different fatty acids for multiplication and for growth, in order to survive the elevated body temperature induced by their presence.

CASE 2. THIN-LAYER CHROMATOGRAPHIC SEPARATION OF SERUM PHOSPHOLIPID IN HYPERTHERMIC RABBITS.

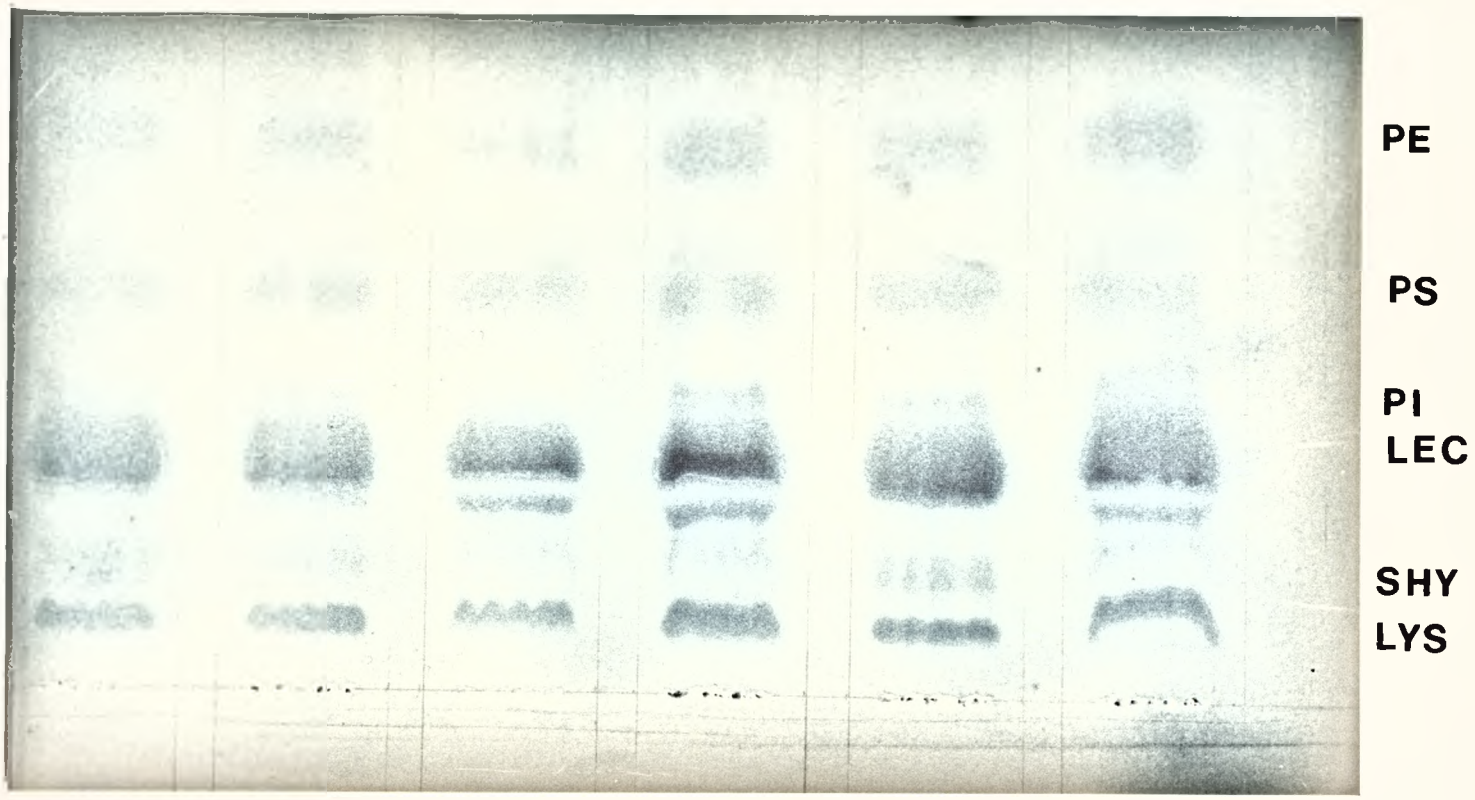


Fig. 29. Each column represents individual rabbit. Blood sample taken at 8 am.

The serum fatty acid analysis shows suppressed 18:3(w6) and 20:3(w6), and an increase in 20:5(w3) fatty acids (Table 23), indicating that the disease is induced by the combination of a diet rich in pentaeonic acid and temperature sensitive bacteria. Both these factors influenced lipogenesis and induced deficiency in PGE_1 and PGE_2. Deficiency in PG's induced the EFA-deficiency symptoms observed in these rabbits (Bergström Carlson and Weeks, 1968; Ziboh and Hsia, 1972). When the diet was supplemented with hay containing 14,85 per cent more 18:2(w6), 48 per cent 18:3(w3) and 2,30 per cent more 22:5(w6) than the normal diet (Table 17) it cured the EFA deficiency symptoms, yet was unable to rectify the amount of 20:3(w3), or restore the suppressed 18:3(w6), nor could it lower the elevated 20:5(w3) fatty acids. As 18:3(w6) remained suppressed, the precursor of PGE_3 became the factor governing the internal temperature of the body (Feldberg and Saxena, 1975).

7.3 Case 3. (Date 1986.12.11.)

The last case study entailed an examination of the serum lipogram of four rabbits in fasted condition (blood taken in late afternoon), prior to death. Blood was collected for serum triglyceride, cholesterol and lecithin fatty acid analysis, according to paragraph 5.1.2.4,5.2.2., 5.2.3.,5.2.4. and 5.2.5.,

Table 23.

Case 2. THE INFLUENCE OF HAY ON THE COMPOSITION OF SERUM LECITHIN FATTY ACIDS IN RABBITS EXHIBITING HIGH BODY TEMPERATURE (Sample taken in the morning)

Fatty acid	Normal Rabbits (n=5) SE	Hyperthermic Rabbits Before (n=5) SE	Hyperthermic Rabbits After hay 1 week (n=5) SE
16:0	24,40±0,74	26,20±1,46	22,75±1,24
18:0	15,40±0,58	13,60±0,37	15,35±0,94
18:1n9	18,20±0,75	16,15±0,43	15,20±0,62
18:2n6	29,10±0,65	31,40±0,75	31,90±1,58
18:3n3	0,85±0,65	0,60±0,05	0,60±0,05
18:3n6	0,40±0,15	- **** ***	-**** ***
20:3n6	0,55±0,05	0,25±0,06	0,30±0,02 **
20:4n6	3,85±0,19	2,50±0,48 *	4,40±0,14 ***
20:5n6	0,30±0,03	1,25±0,33	1,15±0,14
22:4n6	1,85±0,27	1,80±0,36	1,35±0,08
22:5n6	3,70±0,28	3,20±0,80 *	5,00±1,15
Rest.poly	1,40±0,32	3,05±0,31	1,95±0,78

Values represents the relative percentage of fatty acids measuered on GLC and the average of five rabbits. Test SE±standard error of the means. Asteriks represents the statistical significance t-test * P <0,05 ,** P <0,05 , *** P < 0,01 **** the compound is not present.

while body temperature measured as described in paragraph 5.1.2.3. The mean body temperature recorded in these rabbits was 39,65±0,06 ^{o}C.

7.3.1 RESULTS

Abnormal triglycerides (3,38±0,89) and cholesterol (2,30±0,69) and a decay of lecithin fatty acid synthesis (Fig. 30) indicates that the disease process had affected lipid metabolism (Table 24) and body temperature irreversibly. Increased oxidation of 18:1(w9), together with spontaneous synthesis of 18:2(w6), inhibited the formation of C_{18}-C_{22} fatty acids (Table 24). The neutralising capacity of serum was impaired thereby, and linoleic acid thus became toxic. This cytotoxicity of 18:2(w6) in vivo led to hyperthermia, and the occurrence of death in all rabbits.

7.4 DISCUSSION

The cause of chronic mobilisation of C_{18}-C_{22} fatty acids observed in various diseased conditions: i.e. coronary, cancer, chronic inflammation, auto-immune disorder, hypercholesterolaemia has not been defined, nor has it been associated with altered metabolic control affecting thermoregulation, or with tolerance, and therefore the consequences of this phenomenon remained unknown.

CASE 3. THIN-LAYER SEPARATION OF SERUM PHOSPHOLIPID IN HYPERTHERMIC AND CONSTIPATED RABBITS.

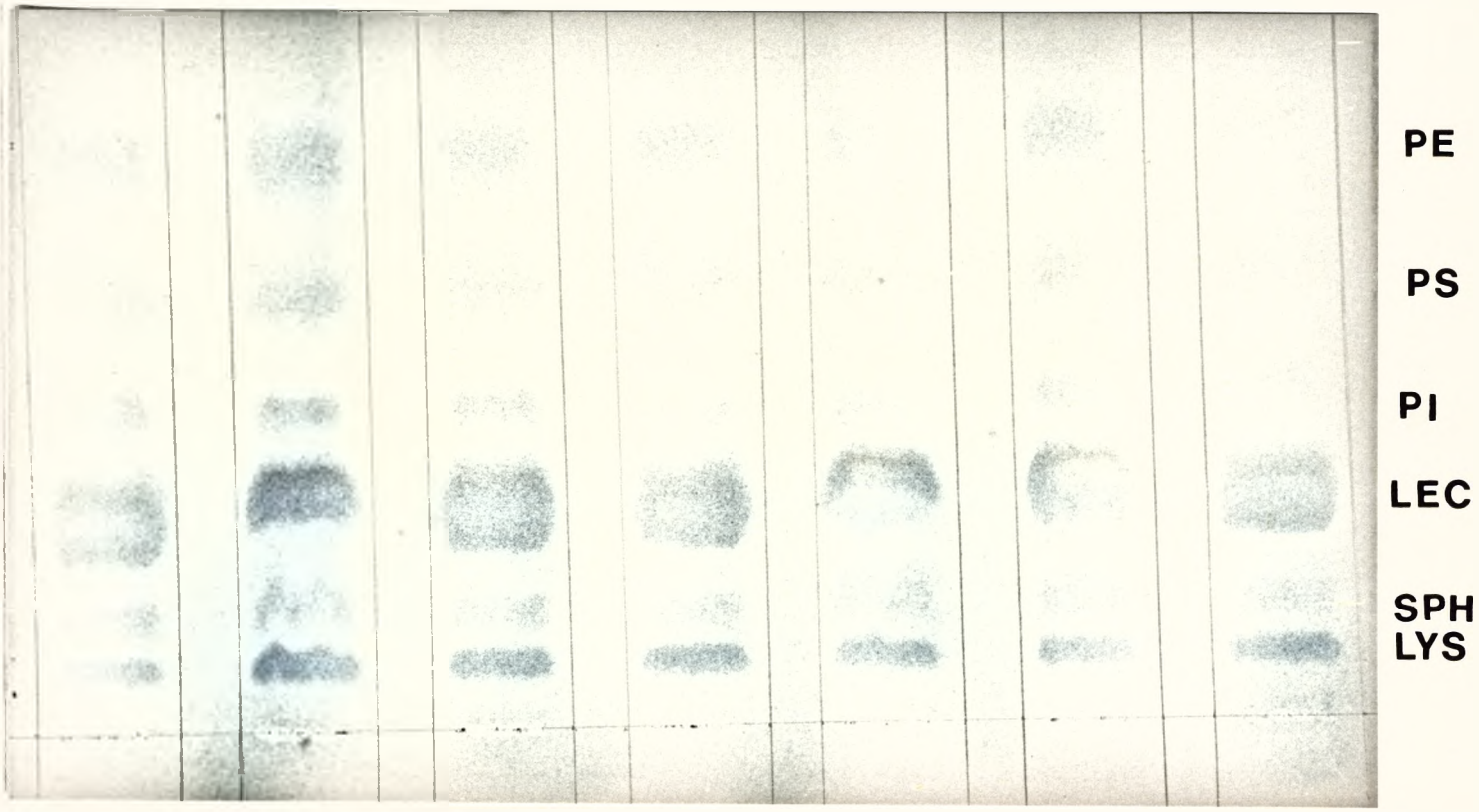


Fig. 30. Each column represents single rabbit serum. Blood sample taken at 5 pm.

Table 24

Case 3. SERUM LECITHIN FATTY ACID PATTERN OF HYPERTHERMIC AND CONSTIPATED RABBITS.

Fatty acid	Normal (n=5)	Hyperthermic (n=4)
16:0	24,40±0,74	26,20±1,46 **
18:0	15,40±0,58	25,26±2,16 **
18:1n3	18,20±0,75	6,20±2,20 ***
18:2n6	29,10±0,65	36,12±0,74 *****
18:3n3	0,85±0,05	- *****
18:3n6	0,40±0,15	- *****
20:3n6	0,55±0,05	- ****
20:4n6	3,85±0,19	0,65±0,07 *****
20:5n3	0,85±0,19	- *****
22:4n6	1,85±0,27	-
22:5n6	3,70±0,28	2,90±0,61
Rest poly.	1,40±0,32	2,67±0,76

Values represents the relative percentage of serum fatty acids measured on GLC and the average of five to four rabbits. Test SE±Standard error of the mean. ** p < 0,005 ***p< 0,0001 **** p < 0,0002 ***** highly significant as the compound is not present.

Nevertheless, in man or in mammalians, apart from the diet (which can affect the nature of the combustion) is a disease process, when the calorie requirements of the body have to be met by an increase in O_2 and CO_2. Thus, depending on the disease process this combustion of C_{20}-C_{22} fatty acids into O_2 or CO_2 is accelerated and, the body temperature is elevated. It follows that, fluctuations in the concentration of blood lipids such as TG, CHO and PL fatty acids reflects the need for energy created by endotoxin stimuli, in order to sustain the increased caloric demand in the diseased state. Under this conditions the biological function of FFA is altered, causing its uptake from the blood by the tissues into the blood to be blocked and, in turn altering endogenous fat metabolism. The resultant rise in the host's body temperature induced spontaneous decomposition of lecithin (Case 1 and 2). The decay of the latter affected the fatty acid transport system between TG-PL-CHO. The resultant hypertriglyceridaemia and hypercholesterolaemia, combined with an increase in lecithin 18:2(w6), inhibited the bioconversion of C_{18}-C_{22} fatty acids (Case 3). These phospholipids cannot provide cellular integrity, nor normal membrane function. The linoleic acid, released from lecithin, induced heat, coma, haemolysis of blood, and the occurrence of death in these rabbits.

8.0 FINAL DISCUSSION

The biochemical mechanisms governing thermoregulation and influencing the production of fever induced by pyrogen are unknown. Adequate understanding (despite intensive investigations) of the physiological mechanism involved in the genesis of fever has not been achieved, as experimental evidence has been based on an isolated cell system (in vitro), of laboratory animals in manipulated circumstances, and not on human patients who are ill. Consequently many unknown factors concerning the genesis of fever have yet to be clarified.

The mechanism responsible for the biphasic fever response induced by endotoxin is not yet understood (Skarnes et al., 1981).

The mechanism whereby endotoxin induces an increase in PGE_2 synthesis in the hypothalamus is unknown (Splawinski et al., 1978).

There is no experimental evidence explaining the differentiation in response to lipid A, endotoxin or leucocyte pyrogen amongst different species of animals (Feldberg and Saxena, 1975; Laburn et al., 1981)

Knowledge is also lacking on how bacterial pyrogen activates the biochemical sequence responsible for the synthesis of prostaglandin (Milton, 1976).

The involvement of periphery in the induction of endotoxin fever has not been acknowledged, as it is

uncertain whether it is produced centrally by PGE_2 (Dascomb and Milton, 1978), by leucocytic pyrogen (IL-1) (Dinarello and Wolff, 1982), or by Thromboxane A_2 (Laburn, Mitchell and Rosendorff, 1978).

According to current hypothesis, leucocyte pyrogen (IL-1) is the key mediator in the biochemical sequence leading to fever (Dinarello and Wolff, 1982), yet many fundamental aspects of its postulated role in the genesis of fever are still obscure and remain to be elucidated.

(1) The initial process whereby leucocyte are activated to produe IL-1,is unknown (Bernheim, Bloch and Atkins,, 1979).

(2) Whilst there is no difficulty in demonstrating that IL-1 circulates in rabbits during fever, there is no proof that IL-1 circulates in humans (Dinarello and Wolff, 1982) during febrile disease.

(3) In vitro stimulation of IL-1 depends on activators such as bacterial endotoxin, hence their ubiquitous presence in the supernatant can interfere with the assays of leucocyte pyrogen (Dinarello and Wolff, 1982).

(4) Regardless of the stimulating agent, the pyrogen formed by blood leucocyte is released over a period of 3-16 hours (Bernheim, Block and Atkins, 1979; Dinarello and Wolff, 1982) an intravenous injection of endotoxin raises the temperature within 20-30 minutes (Grant, 1949; Atkins, 1960).

(5) While fever is blocked by cyclo-oxygenase inhibitors, recent information indicates that neither acute-phase reaction nor proliferation of lymphocytes appears to involve the prostaglandin system (Murakami, 1986).

(6) The mechanism, involving two opposite activities, i.e. tolerance to bacterial pyrogen (Carey, Braude and Zalesky, 1958) and lack of tolerance to IL-1, is not well understood (Bernheim, Block and Atkins, 1979).

(7) Furthermore, in high doses endotoxin is toxic, while highly purified IL-1, even in extremely high doses is non toxic (Dinarello, 1986).

(8) The cause of defective production of IL-1 in elderly patients or malnourished children has not been elucidated (Dinarello, 1984).

(9) The precise mechanism whereby IL-1 is able to trigger the release of PGE_2 from arachidonic acid, thereby altering the thermoregulatory set-point from normothermic to fever level, is presently unknown (Dinarello and Wolff, 1982).

(10) At present it is still unknown whether the acute phase reaction is a direct effect of invading microbes or a secondary release of leucocytic pyrogen (IL-1) from macrophages (Dinarello and Wolff, 1982).

(11) Furthermore, there is no recognition of tolerance induced by hyperthermia or hypothermia, as both events are considered to be exogenously induced, rather than endogenously (Stitt, 1979; Mouiton, 1979).

(12) Hypothermia in adults is not a recognised disease, and is only considered as such in neonates (Mestyàn et al., 1962, Olivier,; 1965).

In addition, little attention has been paid to the time sequence of different events occurring during generalised infection, therefore the consequence of reduced food intake during fever has not been taken into account. Physiologists do not concede to the effect of the nutritional state of the host (fed or fasted state) on febrile response, therefore, clinicians cannot understand complex fever charts, when temperature readings are taken of patients every 3 hours. There is also no rational explanation of the mechanism of different events occurring during febrile disease. Therefore, by recognising, (i) the role of w6 and w3 fatty acids in the maintenance of stable body temperature, (ii) the effect of the nutritional state on the production of fever induced by endotoxin or endogenous pyrogen, and (iii) that a rise in TG and CHO occurs to spare lipid oxidation (thus extending life during starvation), the present thesis was able to shed light on the complex interaction between host/fuel/ agent in the genesis of fever. The implication being that activation of peripheral C_{18} fatty acids in vivo direct the centrally mediated event, leading to the advent of fever. A shift in lecithin configuration therefore, becomes a requirement for the production of fever induced by an endogenous or exogenous pyrogen.

In both events there is a dependence on linoleic acid, the substrata for the precursors of PGE's, which are the mediators of febrile response. Neither endotoxin nor endogenous pyrogen (EP) is able to pass through the blood-brain barrier (Dascomb and Milton, 1978; Dinarello, 1984), while phospholipid (lecithin) fatty acids are incorporated readily into cerebroside (within seconds). The speed of this incorporation has also been found to be greater than for acetate (Mead, and Dopeswharkar, 1972). Although both kinds of fever (endogenous and exogenous) depend on lecithin fatty acids, the difference between the two lies in their caloric requirement of energy for the induction of fever. In endogenous pyrogen induced fever linoleic acid within lecithin molecules is activated without any requirement for an auxiliary mechanism. Conversely, bacterial fever can only be induced not only by the activation of lecithin fatty acids, but also by the release of an auxiliary mechanism i.e. TG, CHO or both, to meet and sustain the energy demand during an infectious disease. All these foregoing points lead to the need for the recognition of the biochemical mechanisms involved in thermoregulation and fever which to date has not been taken into consideration. It should be noted that according to Benedict (1916) the maintenance of body temperature is an energy requiring process, which is regulated by periodic feed. The ingested food provides the source of fuel which is

converted by a metabolic process into energy. If this demand can not be met, the body's reserve has to be drown upon to such an extent as to make the process essentially catabolic (Benedict, 1916). Thus, in a fasting condition, the body mass lives on its own substance and is markedly influenced by the composition of fat in the diet (Wertheimer and Shapiro, 1948). The nature of fat deposited in the adipose tissue is, in turn influenced by the balance between fat in the diet and fat synthesised in the body (Wertheimer and Shapiro,1948). Adipose tissue has the ability to activate desaturation as well shortening the carbohydrate chain (Wertheimer and Shapiro, 1948; Jeanreanud, 1967), and therefore it can play an active role in fat synthesis, as the newly synthesised fat contains mainly C_{16}-C_{18} fatty acids (Fritz, 1961). The assumption that desaturation is carried out within adipose tissue (Shöenheimer and Rittenberg, 1936; Longenecker, 1939; Shapiro and Wertheimer, 1948) arose from the fact that fat deposited in various part of the body is not identical in iodine value (Wertheimer and Shapiro, 1948), the deeper layers being more saturated than the outer part. A correlation between temperature at the site of storage and the degree of saturation implies dependence of body temperature on saturated and unsaturated fatty acids in the fat body (Wertheimer and Shapiro, 1948). It has also been acknowledged

that adipose tissue is able to synthesise long-chain fatty acids from acetate (Favergàe and Gerlach, 1955; Steinberg, Vaughan and Margolis, 1960) and has the ability to synthesise and release PL (lecithin), which is not the precursor of tissue triglyceride (Kennedy, 1957), but is formed independently (Jeanreanud, 1961) in response to external stimuli (Raetz, 1978). Under pathological conditions e.g. febrile disease (induced by bacteria), fatty acids existing within lecithin molecules secure the formation of a new membrane, which is a prerequisite for phagocytosis and also provides the energy to meet caloric demand in a stressful and fasting state. Fatty acids (which are an essential component of complex lipids such as triglycerides, cholesterol and phospholipid), thus plays an important role, not only in the maintenance of stable temperature, but also in the induction of fever. Consequently fever becomes dependent on diet, and on the nutritional state of the host. This realisation led to the investigation of the relation between host/fuel/agent in the genesis of fever.

In chapter 1 of the thesis a diet rich in saturated and poor in unsaturated fatty acids affected the host/fuel relation in a way that in the first instance increased the susceptibility to infection by endogenous pyrogen, and, in the second instance,

played a decisive role in the resistance to the same pyrogen. Both events can be attributed to lack in dietary 18:2(w6). An increased in the saturated and decrease in the unsaturated fatty acids in the diet affected the fat deposits, and consequently, decay in the desaturation system occurred. This prevented not only the formation of eicosonoids, but also immune response to blood-born pyrogen. The decreased caloric intake led to reduced metabolic rate, and this in turn reduced the core temperature. The lowering of body temperature (hypothermia) induced by increased 18:1(w9) combined with 20:3(w9), led to unresponsiveness to endogenous pyrogen. This consequently altered the immune response to blood born pyrogen and provoked the state of exhaustion in these rabbits, which, finally leding to death. Thus connection between diet/body temperature/disease/death can be established.

The following investigation illustrates (Chapter 2) that, under normal dietary conditions, plasma lipids are not only involved in the genesis of fever,induced by the endotoxin S.Thyphosa but have the effect of changing the fever pattern from monophasic to biphasic. This alteration in the pattern of the fever depends on two factors, i.e. the amount of circulating endotoxin within the blood, and the the nutritional state of the moment. In the fed state release of

lecithin eicosonoids, together with triglycerides and cholesterol released from the liver, secure the first phase of the biphasic fever. In the fasted state (afternoon) release of linoleic acid from lecithin combined with TG and CHO secures the second phase of the biphasic fever. The return to normal level in all the fatty acids in lecithin coincides with the termination of the fever. In chronic induction of fever, the metabolic cost is very high, and can induce chronic mobilisation of lipids to sustain caloric demand in a stressful and fasting state. This means that body reserves have to be drawn upon, which makes the process essentially catabolic (Benedict, 1916). This uncontrolled rise in serum lipid elevates the fever plateau beyond 180 minutes, thereby securing the rising phase of the fever. This involves release of linoleic acid (from lecithin) independently of TG, primarily to activate immune response and secondly to alleviate the effect of stress on the body, induced by the rising temperature. Although fever is not the cause of an actual disturbance of balance between heat production and heat loss, neither of these processess proceeds at its normal rate. In continuous fever (induced by endotoxin), there is a relative inefficiency in the mechanism of heat dissipation, which reflects an increase in the metabolic heat production during the rising phase of the fever (after 180 minutes). It follows that the core temperature is

elevated (40,0-40,7 ^{o}C) and once this level is reached, it affects the formation of eicosonoids. This cannot be reversed by either carbohydrate or protein feed, and the physiological event becomes a pathophysiological one, as the rabbits lost the power to eliminate heat, due to increased unsaturation of serum by linoleic acid. Heat thus gained disrupted cellular function (Lilly et al.,1984) and consequently, inhibited the immune response to endotoxin (Brandt and Banet, 1984). Inhibition of the immune response led to tolerance instead of fever reaction to the action of exogenous pyrogen. However, the increase in body temperature and linoleic acid content occurred to a point beyond which there were no further increases and the metabolic cost of hyperthermia was stabilised and mainly occurred to burn up microorganisms. In this case, the rise in body temperature did not lead to the death of the rabbits, thus further supporting the contention that hyperpyrexia induced by the combination of host/fuel/ agent is the consequence of antibactericidal effects of C_{18} fatty acid. Furthermore, it has to be pointed out that both the monophasic and biphasic events of the fever are biochemical processes, controlled by the prostaglandin system (Skarnes et al., 1981). A rise in TG and fall in 18:3(6)->20:3(w6) diminishes the formation of PGE_1, thus securing free arachidonic acid for the formation of PGE_2. The synthesis of

PGE_2 is the criterion for endotoxin fever (Splawinski et al., 1978). The second phase of the fever (180 min) is independent of the prostaglandin system (Skarnes et al., 1981) and appears to be governed by the same mechanism that controls the temperature of the body.

Chapter 3 illustrates that a diet rich in 18:2(w6), 18:3(w3) and 20:5(w3), led to increased serum unsaturation and, in turn, affected brain fatty acid composition. It diminished the synthesis of 20:3(w6) and 20:4(w6) acids, while inhibiting the formation of 22:4(w6) and 22:5(w6) in the serum. Combined with seven consecutive IV injections of 2,0ug/kg of S. Thyphosa, prevented the the rabbits to mount a febrile response (to endotoxin), this led to hyperthermia and to tolerance. The brain fatty acids composition showed greater changes than the serum. A rise in 20:5(w3) and 22:5(w3) led to a fall in 20:3(w6) and 20:4(w6), while an increase in the unsaturated 18:2(w6) and 18;3(w3), led to an increase in 14:0, reduced 16:0, and inhibition of 16:1. These changes in the balance between saturated and unsaturated fatty acids in phosphotadylcholine decreased the survival rate of the rabbits, which is attributable to the adverse effect of increased w3 fatty acids in the diet. Combined with the action of temperature sensitive microorganisms impaired immune reaction and thereby affected the

defence mechanism of the host. This points to the fact that both defective intake (chapter 1) or uncontrolled intake of the unsaturated fatty acids in the diet affects brain fatty acid composition and impairs immune reaction to infection. Both events lead to unresponsiveness to a disease condition when it occurs.

Chapter 4 demonstrates that phospholipid turnover and distribution are species-dependent and crucial to the counteracting of bacterial diseases. Differences in PL distribution reflects changes in serum fatty acid composition. A defect in the formation of 18:3(w6) and 20:3(w6) in Case 1 and 2, combined with an infectious disease, induced essential fatty acid deficiency symptoms (Burr and Burr 1930), which are unrelated to diet, and are induced by a defect in the saturation system, affecting the formation of PG's in a diseased condition. Although a hay supplemented diet was able to rectify the clinical symptoms of EFA-deficiency, it was unable to rectify suppressed 18:3(w6) and 20:3(w6) content, which remained suppressed. Thus the disease process in these rabbits was mediated by glycogenesis, which also altered the body temperature in response to temperature-sensitive bacterial mutants. In case 4, the elevated linoleic acid induced chronic mobilisation of all the eicosonoids, resulting in a metabolic cost which was too high and led to the death of all the rabbits tested.

To conclude: (1) consistency of body temperature depends on the stability of lecithin fatty acid configuration, as a shift in their balance in the serum has been found to affect the thermoregulatory mechanism, and (2) increased combustion of polyunsaturates becomes the biochemical transducing signal by which the hypothalamus readjust itself to higher or lower metabolic rate (R./Q.) and, consequently, diminishes the body's response to endotoxin.

9.0 CONCLUSION

There is a widespread tendency to consider disease due to malnutrition to be attributable solely to dietary deficiencies induced by vitamins, protein or essential fatty acids. However, such disturbances are not necessarily due to deficient diet, as not only a deficiency, but also an excess, of one or more nutrients may be responsible. It is a fact that either under-feeding or over-feeding could result in metabolic disturbances. Throughout of the present work the modifying effect of the diet on febrile diseased state is manifested by changes in body temperature (i.e. drop or rise). Upon the induction of tolerance this became clearly evident. The conclusion reach by the author is that essential fatty acids (namely linoleic acid) have the most pronounced effect on the

body's temperature, while febrile response to bacterial disease is dependent on the polyunsaturated fatty acids (PUFA). The normal healthy rabbits fed a balanced diet and subjected to a prolonged febrile state by chronic induction of S. Thyphosa, had the ability to cope with the effect of thermal stress induced by endotoxin, thus elevating the body temperature above the normal resting range. This was bought about by an increase in serum lecithin linoleic acid content. The rise in the latter occurred in spite of the fact that the balance between saturated and polyunsaturated fatty acids being maintained. The stability of both, combined with an increased content of linoleic acid, activated the immune response of the body. Tolerance to the febrile action of endotoxin occurred only after a long period time.

Conversely, animals made deficient in essential fatty acid (in the presence of sufficient amounts of protein, vitamins and carbohydrates), failed to counteract the stimuli or stress to which they were subjected. This provoked a disturbance in the response to blood-born pyrogen, while no impairment to the febrile action of PGE_2 occurred. This demonstrates that brain integrity as a whole was maintained, by reducing caloric requirements, thereby lowering the body temperature and, consequently, inactivating the immune response to pyrogen. Thus using the word of Platt (1958) " the sum of the forces to resist death" was impaired. In contrast, an excess of w3 elements in

the rabbit's diet resulted in a drastic changes in the formation of the w6 PUFA, in both serum and brain phosphotadylcholine. These changes led to a disequilibrium between unsaturated and saturated fatty acids. Combined with a diseased condition, this led to failure of the organism to react to endotoxin stimuli, and tolerance of febrile action of pyrogen occurred, due to over-feeding. An excess in dietary intake of fat became similarly deleterious to the organism, causing deficiency in PUFA and consequently impairment to the host resistance to infection. Unresponsiveness to endotoxin led to death in these rabbits.

Death is non-specific response to many different types of endotoxin, while the development of fever is specific physiological response (Wood, W.B, 1958; Cooper, K.E., Cranston, W.I., Snell, E.S. 1964) which is greatly dependent on "the host defence mechanism". Throughout of this work the occurrence of death was associated with a regime lacking in w6, or with an excess of w3 dietary fatty acids. This had a direct bearing on the lecithin metabolism of infected animals. A shift in fatty acid configuration of lecithin either depressed or stimulated the infection-enchancing effect of the disease in the rabbits tested. This led the author to recognise that essential fatty acids possess a "dual characters" i.e. physiological and biochemical. This dual role enables the EFA's to activate the body's mechanism against the

invading microorganisms, thereby substantiating the existence of the antibactericidal property of $C_{18:2(w6)}$ "in vivo". These discoveries brought the author to a realisation of the true essentiality of the essential fatty acids.

By recognising this changes in serum lecithin fatty acid configuration, the present thesis provides the fundamental theory of the "Biochemistry of Thermoregulation and Fever," it also establish a new diagnostic tool which is delicate enough to follow every step of the process of changes in the organism. Thus, equipping the clinicians with a diagnostic tool which can: (1) dissociate fever from hyperthermia, (2) assess the cause of hypothermia, (3) establish the state of tolerance induced by hyperthermia or hypothermia, (4) determine the origin of fever, i.e. whether endogenous or exogenous and (5) last but, not least the involvement of the periphery in the centrally mediated event.

These conclusions introduce into the biochemistry of thermoregulation and fever, with the recognition of the fact that all the changes involved depend on the sum total of organic changes within the body.

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APPENDIX

PUBLISHED WORKS

Pyrogens fail to produce fever in a cordylid lizard

H. P. LABURN, D. MITCHELL, E. KENEDI, AND G. N. LOUW
Department of Physiology, University of the Witwatersrand Medical School, Johannesburg 2000, and Department of Zoology, University of Cape Town, Cape Town 7700, South Africa

LABURN, H. P., D. MITCHELL, E. KENEDI, AND G. N. LOUW. *Pyrogens fail to produce fever in a cordylid lizard.* Am. J. Physiol. 241 (Regulatory Integrative Comp. Physiol. 10): R198-R202, 1981.—We investigated the effects on body temperature of the lizard *Cordylus cataphractus* of intracardiac injections of leucocyte pyrogen (LP) synthesized from rabbit blood and of killed *Aeromonas hydrophila*, a gram-negative bacterium reputed to be pathogenic in lizards. Lizards were placed in a photothermal gradient that allowed them to select a preferred body temperature following the injections. Neither injection of 0.5 ml rabbit LP nor of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline caused body temperature of lizards to differ from that of control lizards injected with sterile saline. Following injection of these solutions in the lizards placed in a thermal gradient where ambient temperature ranged from 20–88°C, body temperature was maintained between 32 and 34°C. Pyrogens failed to elevate body temperature even when body temperature was elevated artificially to 36°C before injection. We conclude that *C. cataphractus* does not respond with fever to either rabbit LP or *A. hydrophila*. Fever may not be ubiquitous even among lizards.

leucocyte pyrogen; body temperature; *Aeromonas hydrophila*; *Cordylus cataphractus*

FEVER IN ECTOTHERMS was first observed in lizards (20) and has since been reported in amphibia, fish, and even some invertebrates (5–7, 12, 17–19). The existence of an ability to develop fever in such phylogenetically disparate animals has led some authors to suggest fever has an ancient evolutionary origin. Moreover, in at least some of the ectotherms, fever has survival value (8, 13), an observation of clinical importance if a similar advantage prevails in mammals.

The literature is remarkably devoid of reports of animals which fail to develop fever in response to pyrogen injection. Is fever really ubiquitous? We noticed that all work on fever in lizards appears to have been carried out using *Dipsosaurus dorsalis* and *Iguana iguana*, both iguanid lizards (2, 11). We, therefore, decided to investigate whether lizards of other families also had the ability to develop fever.

We describe here experiments in which we tested the responses of a cordylid lizard to pyrogens. *Cordylus cataphractus* inhabits arid regions of southern Africa and is well known for its sun-basking thermoregulatory behavior. The pyrogens selected for testing were those previously used by Kluger and his colleagues (2, 11) in studying iguanid lizards, namely the killed gram-negative bacterium *Aeromonas hydrophila* and rabbit leucocyte pyrogen.

Some of the results have been reported briefly at the Pecs Satellite Symposium on Thermal Physiology (15).

METHODS

Animals. Lizards (*C. cataphractus*) were trapped in the wild and maintained in the laboratory at an ambient temperature of 20–25°C. For several months before experimentation lizards were maintained on natural daylight cycles; light and heat were supplied by tungsten lamps that allowed the lizards to select their preferred body temperature. The lizards weighed between 33 and 60 g (mean 45 g; $n = 10$). New Zealand White rabbits of both sexes weighing between 2.5 and 3.0 kg were used.

Experimental chamber. During experiments, lizards were exposed in a photothermal gradient chamber with a sand base. Heat was supplied by tungsten lamps at one end of the chamber. The temperature at the cool end of the gradient was measured by exposed copper-constantan thermocouples and at the hot end by a copper-constantan thermocouple in a blackened copper tube of approximately the same dimensions as the lizards. Two gradient chambers were used. The first was an asbestos cement chamber with floor dimensions approximately 1.8 x 0.3 m. The temperature of the cool and warm ends of the chamber was 20–25°C and 80–88°C, respectively. The second chamber was a metal chamber of smaller dimensions, approximately 0.5 x 0.3 m, designed to fit inside an air-conditioned container. Here the cooler and warmer ends of the chamber were maintained at 35–37°C and 55–57°C, respectively.

Body temperature measurements. Lizard body temperature was measured by inserting a fine copper-constantan thermocouple (36 gauge) via the cloaca to a depth of approximately 30 mm. The thermocouple was secured to the lizard's tail with adhesive tape. Restriction of the lizard's general mobility within the chamber was small.

Rabbit body temperature was measured by inserting a copper-constantan thermocouple mounted in polyethylene tubing via the rectum to a depth of about 100 mm.

All thermocouple outputs were detected on a Bailey BAT4 thermometer at 15-min intervals. The thermocouples were calibrated in water against a standard mercury thermometer; precision of the calibrated thermocouples was better than 0.2°C.

Pyrogen injections. All injections in lizards were intracardiac and, except for the rabbit leucocyte pyrogen, were 0.2 ml. That the injectate indeed entered the heart was checked in a few lizards by injecting the same volume

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of dye, killing the animals, and locating the dye at post-mortem. Intravenous 3-ml injections into rabbits were via an ear marginal vein.

Rabbit leucocyte pyrogen was prepared by incubating whole rabbit blood with purified endotoxin of *Salmonella typhosa* in a dose of 30 μg/100 ml of blood. Details of the technique have been described previously (4).

A. hydrophila was grown on blood-agar plates for 24 h before being killed by suspension in 70% ethyl alcohol. The organisms were then washed twice with sterile 0.9% sodium chloride, centrifuged, and resuspended. Concentrations of the killed bacteria in sterile 0.9% sodium chloride were adjusted using Burroughs-Wellcome turbidity tubes and checked by direct counting under the microscope. Injections into lizards consisted of 4×10^9 organisms in 0.2 ml of sterile saline. Injections into rabbits consisted of 4×10^9 organisms in 3 ml of sterile saline.

Control injections consisted of 0.2 ml of sterile saline in the case of the lizards and of 3 ml of sterile saline in the case of rabbits.

Experimental procedure. All experimental injections of the lizards were carried out at the same time of day, 1000 h. Lizards were kept 2 h in the experimental chambers before receiving any injections. Thereafter injections were made of either a pyrogen suspension or of sterile saline, and body temperature measurements were monitored for a further 7 h. In certain experiments temperature measurements were also made on the day following that of injection. Experiments on rabbits were carried out on conscious animals restrained in conventional stocks.

Statistical analysis of data. Data were subject to Student's *t* test and values of *P* less than or equal to 0.05 were considered to be significant.

RESULTS

Responses of lizards to injections of rabbit leucocyte pyrogen. Figure 1 shows the body temperature of lizards after injection of 0.5 ml of rabbit leucocyte pyrogen or of 0.5 ml sterile saline. Experiments were carried out in the chamber with the temperature range of 20–88°C; there were up to five lizards in the chamber at any one time. Each lizard received both leucocyte pyrogen and saline on separate days; the order of injection alternated from lizard to lizard.

Body temperatures of lizards receiving leucocyte pyrogen did not differ at any time from those of lizards receiving saline. Following both treatments, body temperatures stabilized on average between 32 and 34°C.

Responses of lizards to injections of A. hydrophila. Figure 2 shows the body temperature of lizards after injection of suspension of killed *A. hydrophila*. Conditions of the experiment were the same for injections of rabbit leucocyte pyrogen; control experiments consisted of the injection of 0.2 ml of sterile saline.

Body temperatures of all lizards rose slowly throughout the period of exposure, but at no time were temperatures of lizards receiving the pyrogen significantly different ($P > 0.05$, *t* test) from those of lizards receiving saline. Final body temperature following both treatments was about 32°C.

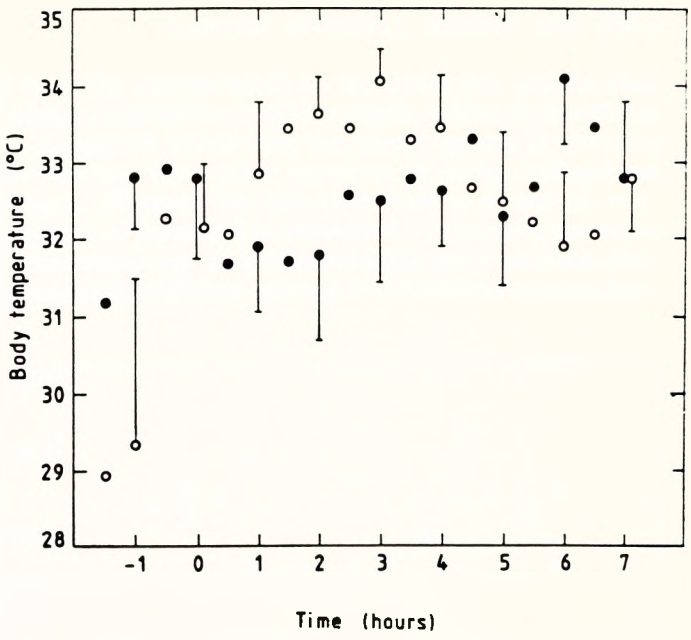


FIG. 1. Changes in body temperature of lizards following injection of 0.5 ml rabbit leucocyte pyrogen (LP) or of 0.5 ml sterile saline. Temperature of gradient was 20–88°C. Mean responses are shown for injections of rabbit LP (●) $n = 5$ or for injections of sterile saline (○) $n = 5$. One SEM is shown at hourly points. *Ordinate*: body temperature of lizards in °C. *Abscissa*: time (in hours) after injection (at zero).

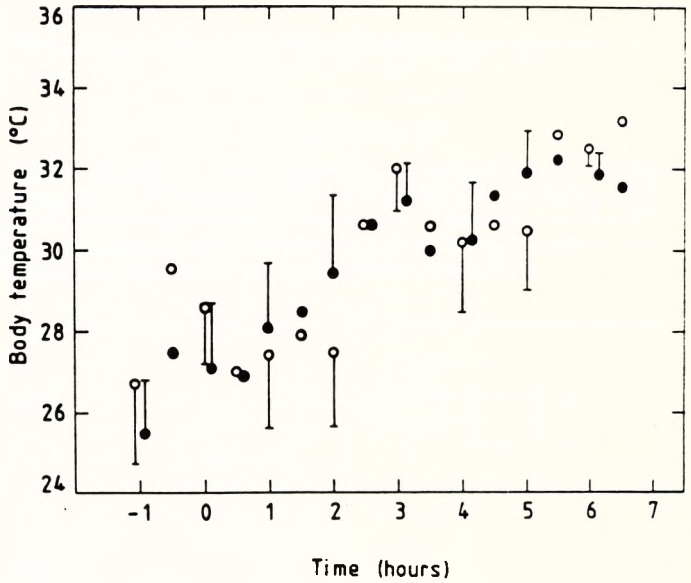


FIG. 2. Changes in body temperature of lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 20–88°C. Mean responses are shown for injections of *A. hydrophila* (●) $n = 5$, or for injection of saline (○) $n = 5$. Additional details as in Fig. 1.

The results shown in Fig. 3 derived from experiments in which lizards were placed in the gradient chamber one at a time, rather than in groups. Body temperatures then stabilized at a somewhat lower level, on average 29–32°C. Once again there was no evidence that body temperature was different in the lizards following injection of the pyrogen.

In the next series of experiments, lizards were placed individually in the gradient chamber in which tempera-

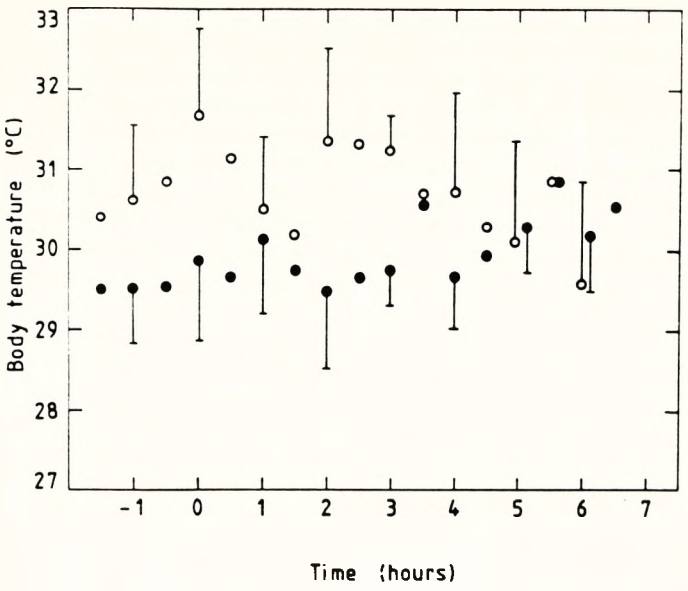


FIG. 3. Changes in body temperature of lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 20–88°C and lizards were tested in gradient one at a time. Mean responses are shown for injections of *A. hydrophila* (●) $n = 6$ or for injection of saline (○) $n = 5$. Additional details as in Fig. 1.

ture ranged from 35 to 57°C. Injections were made of *A. hydrophila* or of sterile saline. Figure 4 shows the body temperature of the lizards. At the time of injection temperatures were approximately 36°C and stabilized between 35 and 37.5°C throughout the experiment.

Figure 5 shows body temperature responses of the same lizards in the same chamber on the day following injection. There was considerably less variation in temperature on the 2nd day, but body temperatures nevertheless were maintained within the same temperature ranges as on the 1st day. On neither the 1st nor the 2nd day were the temperatures of the lizards receiving the pyrogen significantly different from that of lizards receiving sterile saline ($P > 0.05$, t test).

Responses of rabbits to injections of A. hydrophila. The pyrogenicity of the suspension of killed *A. hydrophila* was verified by injecting 4×10^9 organisms in 3 ml of sterile saline into the marginal ear vein of four rabbits. Control injections consisted of intravenous sterile saline. Responses of the rabbits are shown in Fig. 6. Changes in rectal temperatures of rabbits receiving *A. hydrophila* were significantly different ($P < 0.05$, t test) from rabbits receiving saline from 30 to 120 min after injection.

DISCUSSION

Our results show that *C. cataphractus* did not develop fever in response to injections of rabbit leucocyte pyrogen or of endotoxin of *A. hydrophila*, an organism reported to be pathogenic in lizards (13). In all our experiments, body temperature changes in the lizards were the same following pyrogen injections as they were following saline injections.

That the lizards failed to develop fever in response to injections of rabbit leucocyte pyrogen was not due to a lack of pyrogenicity of the leucocyte pyrogen (4). It could be that the lizards failed to recognize the protein that constitutes rabbit leucocyte pyrogen, although Bernheim and Kluger (3) found that the desert iguana *D. dorsalis* does develop fever after injection of rabbit endogenous pyrogen. Whereas the idea that fever is of ancient evolutionary origin would be enhanced by the demonstration of cross-reactivity of endogenous pyrogens between phylogenetically different species, considerable species spec-

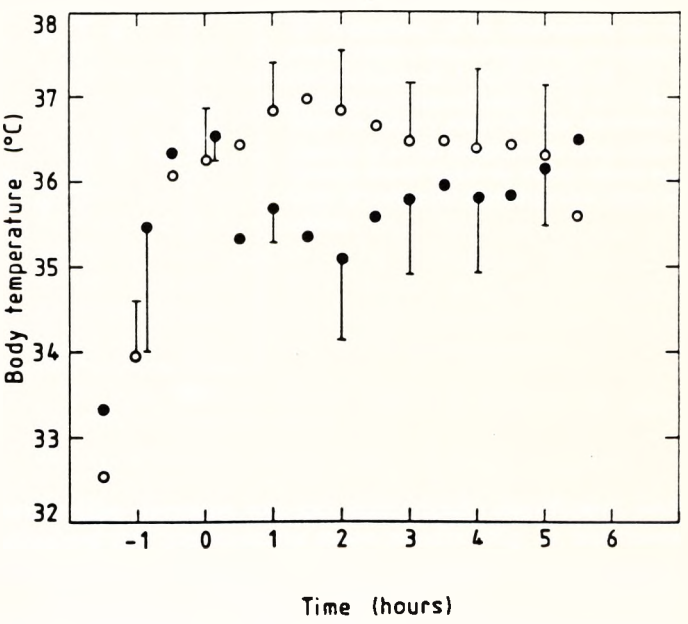


FIG. 4. Changes in body temperature of solitary lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 35–57°C. Mean responses are shown for injections of *A. hydrophila* (●) $n = 5$ or for injections of saline (○) $n = 5$. Additional details in Fig. 1.

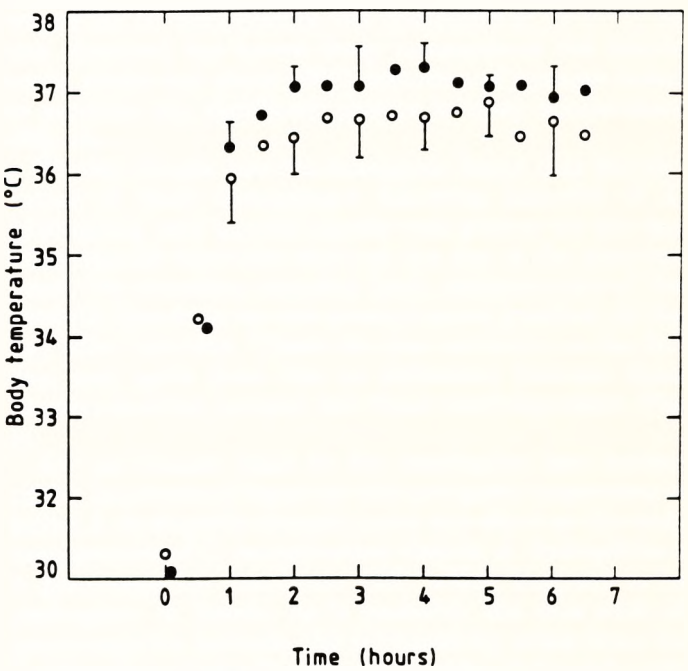


FIG. 5. Changes in body temperature of lizards on day following injection of either *A. hydrophila* (●) $n = 5$ or sterile saline (○) $n = 5$. Temperature of gradient was 35–57°C. All other details as in Fig. 4.

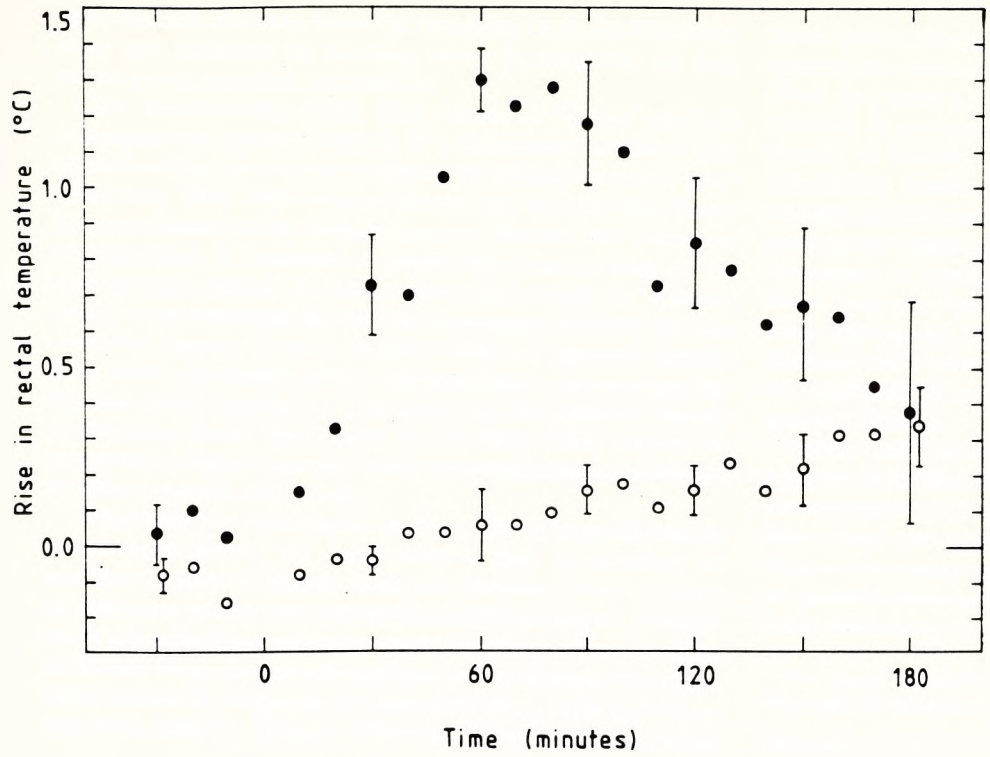


FIG. 6. Changes in rectal temperature of rabbits following injection of 4×10^9 organisms of *A. hydrophila* or of sterile saline. Mean responses are shown for injections of *A. hydrophila* (●) $n = 4$ or for injections of saline (○) $n = 5$. One SEM is shown every 30 min. *Ordinate*: change in rectal temperature from preinjection levels (zero on scale). *Abscissa*: time (in minutes) after injection (zero on scale).

ificity appears to exist even among species of mammals (4).

Kluger (13) and Bernheim and Kluger (2) found that two species of iguanid lizards, *D. dorsalis* and *I. iguana*, responded to the injection of *A. hydrophila* with a rise in body temperature brought about by selecting warmer environments. We prepared a suspension of killed *A. hydrophila* in a fashion identical to that of Kluger and his co-workers and injected it intracardically into *C. cataphractus* lizards free to select their thermal environment. The lizards did not select a warmer environment than they did when injected with saline (Fig. 2).

We attempted to discover why our lizards did not react to the injection of pyrogen, while the lizards studied by Kluger and his colleagues routinely did so. First, we established that the suspension of killed bacteria was indeed pyrogenic by injecting it intravenously into conscious rabbits. Injection of approximately the same absolute dose into rabbits, or about 1/16 of the dose per unit of body mass, caused a significant fever of rapid onset (Fig. 6). Thus the bacterial suspension was undoubtedly pyrogenic.

The second possibility that may have accounted for the difference between our results and those of Kluger lay in the nature of the behavior of the lizards. We observed *C. cataphractus* to display marked social facilitation (16); the lizards tended to cluster on top of and next to one another in all environments. It may have been that this social behavior masked any possible subtle differences in thermoregulatory behavior resulting from pyrogen injections. No mention was made of social facilitation in the case of the iguanid lizards. We therefore repeated the experiments exposing the lizards one at a time in the thermal gradient chamber. When exposed alone, the lizards selected somewhat lower body temperature (the decrease was not statistically significant) and attained this temperature more rapidly (Fig. 3), but there was still no evidence of a pyrexic response to the pyrogen.

The body temperature selected by *C. cataphractus* was in the region of 32°C (Figs. 1–3). This temperature is lower than the 36–38°C preferred by the iguanid lizards (2). We investigated the possibility that the lizards only react to pyrogen when their body temperature is already elevated to 36°C by exposing them, one at a time, to a photothermal gradient inside an air-conditioned chamber where the air temperature was controlled at 35–57°C. In this chamber, the lowest body temperature the lizards could attain was about 36°C. Injection of pyrogen failed to produce any significant change in body temperature (Fig. 4).

Finally, Kluger and his colleagues found that the elevation of body temperature following injection of *A. hydrophila* into iguanid lizards was greater on the day following the injections than on the day of the injection itself. We therefore recorded body temperatures of the cordylid lizards in the hot photothermal gradient on the day after injection. There was less variation on the 2nd day, and again there was no difference between those lizards receiving pyrogen and those receiving saline (Fig. 5).

All our results point to the conclusion that neither rabbit leucocyte pyrogen nor the pyrogenic suspension of killed *A. hydrophila* bacteria have any effect on the body temperature of *C. cataphractus* lizards. *A. hydrophila* is a gram-negative bacterium (10), which happens to be pathogenic in lizards. As is probably the case with all gram-negative bacteria, the pyrogenicity arises from the lipid moiety of the lipopolysaccharide of the cell walls (9). The lipid moiety, lipid A, is common to most, if not all, pyrogenic lipopolysaccharides. Thus, because the liz-

ards did not develop fever in response to *A. hydrophila*, they are unlikely to develop fever in response to any other gram-negative bacterium.

Our observations differ from those of Kluger and his colleagues on iguanid lizards. We attempted to follow the experimental procedure adopted by Kluger in every possible way. Why the cordylid lizards did not develop fever in circumstances in which iguanid lizards did remains unexplained. It remains a possibility that a dose of *A. hydrophila* higher or lower than that used by Kluger could have resulted in the development of fever in the cordylid lizards. It is a characteristic of iguanid lizards that their preferred body temperatures are among the highest of all lizards (1). The ability of the iguanid lizards to develop fever may have to do with their ability to maintain a higher normal body temperature than occurs in other species of lizards.

Whatever the mechanism is that allows iguanid lizards to develop fever, we have shown that a member of another family of lizards does not develop fever, at least in response to the two pyrogenic solutions tested. Preliminary observations we have made on three other families of lizards have demonstrated that none of these develops fever either in response to injection of *A. hydrophila* at the dose used by Kluger. Whether the ability to develop fever never existed, or has been lost, in these species is a matter for speculation. In any event, it may well be that fever is a rare phenomenon among lizards, and perhaps reptiles in general, and that the discovery of fever in one family of lizards was fortuitous. Such a possibility has important implications; fever may not have a common, ancient evolutionary origin in all species. In reptiles, and perhaps in other ectotherms, fever may have arisen spontaneously in isolated species, which in turn implies that fever may not have been of survival value during evolution. Moreover, the demonstration that fever enhances survival after infection by pathogenic organisms may also be confined to certain species of ectotherms, including iguanid lizards.

Fever may be of survival value in mammals, but we suggest that this possibility should not be derived from extrapolation of results obtained from ectotherms, where fever does not appear to be ubiquitous. Moreover, the chance discovery of fever in one family of lizards has led to generalizations and speculation about the nature of fever than may turn out to be unwarranted.

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ON THE PYROGENIC ACTION OF INTRAVENOUS LIPID A IN RABBITS

BY EVA KENEDI, HELEN LABURN, D. MITCHELL AND F. P. ROSS
From the Departments of Physiology and Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg 2001, South Africa

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SUMMARY

1. Previous evidence purporting to show that lipid A is the pyrogenic moiety of endotoxin is demonstrably inconclusive.

2. We have extracted lipid A from endotoxin of *Salmonella typhosa* and tested the pyrogenic action of the lipid A, the residual polysaccharide and the parent endotoxin, by intravenous injection in conscious rabbits.

3. Lipid A dissolved in an aqueous solution of rabbit serum albumin induced a significant pyrexia of short latency, while neither rabbit serum albumin alone, nor the polysaccharide from *S. typhosa*, affected body temperature. The physical presence in the injectate of the polysaccharide from *S. typhosa* did not enhance the pyrogenicity of the lipid.

4. Dose–response curves for lipid A and the parent endotoxin, over the dose range 10 ng–20 μg, showed that lipid A incorporated in endotoxin was much more pyrogenic than pure lipid A in solution. When separated from the polysaccharide component of endotoxin, lipid A lost more than 99·9 % of its pyrogenic activity, at threshold doses.

INTRODUCTION

Current dogma holds that the toxic and pyrogenic properties of bacterial endotoxin derive from lipid A, the lipid component of the lipopolysaccharide molecule (Bradley, 1979; Dey, Feldberg, Gupta & Wendlandt, 1975; Galanos, Freudenberg, Luderitz, Rietschel & Westphal, 1979). Lipid A certainly exhibits many of the biological actions of the parent lipopolysaccharide: it gives a positive response in the *Limulus* lysate assay for endotoxin (Yin, Galanos, Kinsky, Bradshaw, Wessler, Luderitz & Sarmiento, 1972), activates complement (Galanos, Rietschel, Luderitz & Westphal, 1971), and induces the synthesis of antibodies effective against endotoxin (Rietschel & Galanos, 1977).

The claim that the pyrogenicity of endotoxin derives from lipid A is usually supported by citation of a single paper (Dey *et al.* 1975), although that conclusion was actually reached in a paper published two years earlier (Rietschel, Kim, Watson, Galanos, Luderitz & Westphal, 1973). Lipid A injected intravenously into cats and rabbits resulted in a rise in body temperature. Lipid A is not soluble in water and, in the studies mentioned, the lipid was either solubilized by admixture to bovine

serum albumin or other albumin, or dissolved in an aqueous solution of the organic solvent triethylamine. We have reported recently that intravenous bovine serum albumin itself is pyrogenic in rabbits (Hattingh, Laburn & Mitchell, 1979). Is it possible that the putative pyrogenicity of lipid A is an artifact resulting from the use of pyrogenic vehicles? Dey and his colleagues do not appear to have tested their vehicles for pyrogenicity. Rietschel and his colleagues stated that bovine serum albumin was not pyrogenic in their rabbits. However, their figures show pyrexias of more than 0·5 °C following injections of bovine serum albumin; they performed no statistical analyses of their results.

The published evidence supporting the hypothesis that lipid A is the only pyrogenic fraction of endotoxin therefore seems equivocal. We have re-investigated the putative pyrogenicity of intravenous lipid A. We have also compared the pyrogenicity of lipid A derived from *Salmonella typhosa* with that of the parent endotoxin.

Some of the results have been reported to the Physiological Society of Southern Africa (Kenedi, Laburn, Mitchell & Ross, 1980).

RESULTS

Animals. All measurements were made on New Zealand White Rabbits weighing between 2·5 and 3·5 kg. The rabbits were conscious and restrained in conventional rabbit stocks.

Temperature measurements. Rectal temperature was measured using copper-constantan thermocouples inserted via the rectum to a depth of about 100 mm. The output from the thermocouples was recorded every 10 min on a data logger. All thermocouples, connected to their appropriate channels on the data logger, were calibrated by water immersion against a certified mercury thermometer. The precision of the temperature measurements was better than 0·2 °C. Temperature responses were expressed as the rise in rectal temperature compared to that prevailing at the time of the injection. For some experiments, a 3 h thermal response index was calculated as described by Clark & Cumby (1975). The thermal response index is the time integral (°C . h) of the rise in rectal temperature following injection.

Procedure. Rectal temperature was monitored for at least 1 h prior to injection. Test or control samples were then injected into an ear marginal vein. All injections were made in a volume of 2 ml. Rectal temperatures were measured for 3 h following injection.

All experiments took place at an ambient temperature between 18 and 22 °C. No animal was febrile at the time of the injection.

Lipid A preparation. Lipid A was prepared from the purified lipopolysaccharide of *Salmonella typhosa* 0901 (Difco 3124-25-6) using the method of Galanos *et al.* (1971). Batches of 100 mg of lipopolysaccharide were hydrolysed in 10 ml of 1% acetic acid at 60 °C for 16 h. The cooled hydrolysate was centrifuged for 30 min at 4 °C and 10^5 g. The precipitate was washed, resuspended in sterile distilled water, and recentrifuged three times. The final precipitate constituted the lipid A, and the pooled supernatants contained the polysaccharide fraction of the endotoxin.

The lipid A and polysaccharide fractions were lyophilized and weighed. A ratio of 1 : 13·5 of lipid A : polysaccharide by mass was obtained, with an over-all recovery of about 96%. Both lipid A and the polysaccharide were kept in the lyophilized form at 4 °C until used.

Samples of the lipid A were analysed for 2-keto-3-deoxyoctonic acid, which covalently links the lipid A to the polysaccharide in endotoxin (Waravdekar & Saslaw, 1959). None of this acid could be detected, indicating that the lipid A preparations were free of all bound saccharides.

Solubilization of lipid A. For injection, lipid A was solubilized in one of three ways, namely by making aqueous solutions of lipid A and bovine serum albumin (Armour) in a one-to-one ratio by mass, or by dissolving lipid A in triethylamine (Merck) in a concentration of 400 g/l. In addition lipid A was suspended in aqueous solutions of sodium chloride (9 g/l) containing the polysaccharide residue remaining after lipid A extraction, in the mass ratio of 13·5 units of polysaccharide to one unit of lipid A, or sodium chloride alone. The fine suspensions so formed were thoroughly vortexed immediately before injection.

Prior to injection, aliquots of the above solutions or suspensions were diluted to a volume of 2 ml using sterile pyrogen-free saline. Control injections in each case consisted of the vehicle alone, made up to a volume of 2 ml with the sterile pyrogen-free saline.

RESULTS

Fig. 1 shows the effects on rectal temperature of intravenous injection of lipid A (10 μg) in aqueous solutions of bovine serum albumin (10 μg, *A*), rabbit serum albumin (10 μg, *B*), or triethylamine (25 nl, *C*). Injection of an aqueous solution of bovine serum albumin alone, even in a dose as low as 10 μg, resulted in a rise in rectal temperature significant after 90 min ($P < 0{\cdot}05$, Student's t test). The rise in temperature following injection of 10 μg lipid A dissolved with the same amount of bovine serum albumin was never significantly different from that which followed injection of bovine serum albumin alone.

Triethylamine (Fig. 1*C*), injected alone in aqueous solution, also caused rectal temperatures of the rabbits to rise, to a level which was significant after 60 min ($P < 0{\cdot}05$, Student's t test). Addition of lipid A (10 μg) to the triethylamine solution resulted in a somewhat greater rise in rectal temperature, but the rise was significantly different from that caused by triethylamine alone only in the last half hour of the experiment ($P < 0{\cdot}05$, Student's t test).

Injection of 10 μg homologous rabbit serum albumin (Fig. 1*B*) had no effect on rectal temperature apart from a trivial rise at the end of the experiment. However, injection of lipid A (10 μg) solubilized with the same amount of rabbit serum albumin resulted in a pyrexia of rapid onset. The rise in rectal temperature was significantly different from that which followed injection of rabbit serum albumin alone by 20 min after injection, and remained significantly different thereafter ($P < 0{\cdot}05$, t test).

The other major component of endotoxin, namely the polysaccharide, had no pyrogenic action. Fig. 2 shows that injection of an aqueous solution of 135 μg polysaccharide, the amount apparently bound to 10 μg of lipid A in *S. typhosa* endotoxin, had no significant effect on rectal temperature.

Fig. 2 also shows the consequences on rectal temperature of injecting 10 μg lipid A together with 135 μg polysaccharide, and, for comparison, the consequences of injecting 10 μg endotoxin. The physical presence of the polysaccharide from *S. typhosa* did not enhance the pyrogenicity of lipid A: the pyrexia following injection of lipid A plus polysaccharide had a time course and magnitude indistinguishable statistically from that following injection of lipid A plus rabbit serum albumin (Fig. 1*B*). However, 10 μg endotoxin, in which were incorporated less than 1 μg lipid A and about 9 μg polysaccharide, induced a pyrexia significantly larger in the last hour of the experiment ($P < 0{\cdot}05$, t test) than a physical mixture of 10 μg lipid A and 135 μg polysaccharide.

The results displayed in Fig. 2 also lead us to suggest that lipid A does not need to be in solution in order to manifest its pyrogenic action. The mixture of lipid A with polysaccharide, in an aqueous vehicle, did not form a solution but a cloudy suspension of fine particles. Injection of this suspension caused a pyrexia indistinguishable from that caused by the clear solution of the same amount of lipid A in rabbit serum albumin. Our contentions that lipid A does not need to be in solution, and that

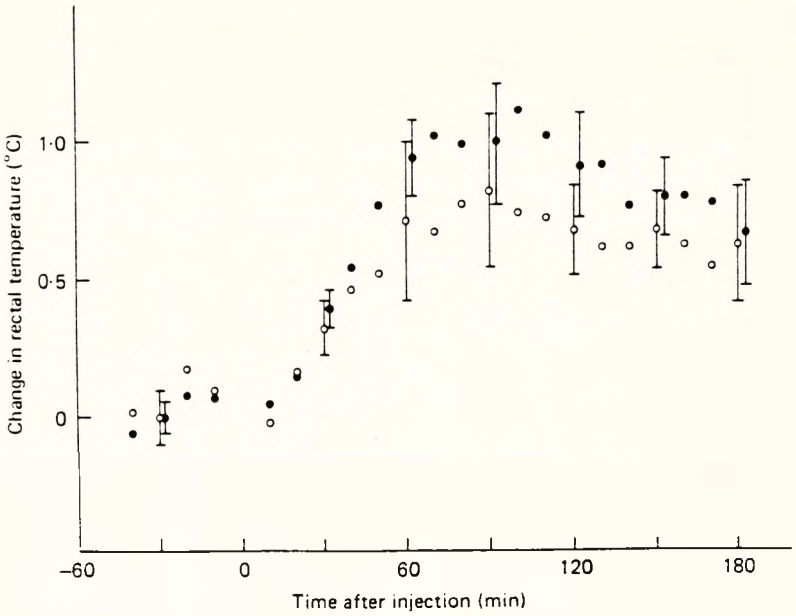


Fig. 1*A*

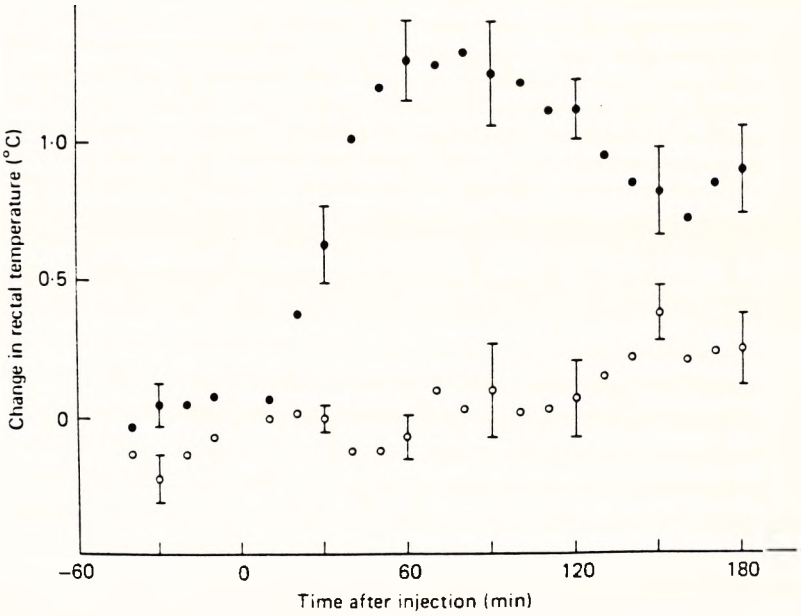


Fig. 1*B*

lipid A pyrogenicity is not enhanced by the physical presence of its related polysaccharide, were confirmed by injecting 10 μg of lipid A in suspension in 2 ml of sterile, non-pyrogenic saline. A pyrexia ensued which was statistically indistinguishable ($P < 0{\cdot}05$, t test, $n = 6$) from that which followed injection of suspensions of 10 μg lipid A together with 135 μg of the related polysaccharide, and indeed from that which

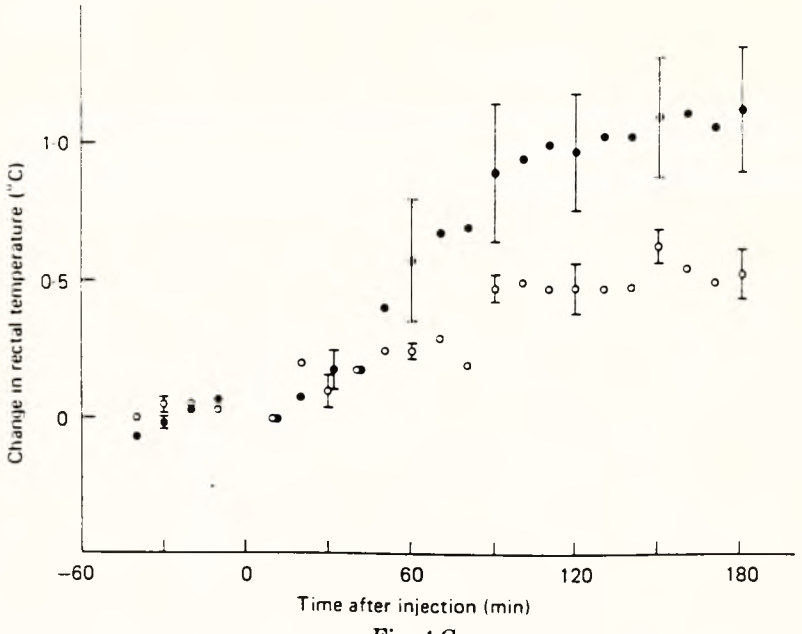


Fig. 1*C*

Fig. 1. Rise in rectal temperature in conscious rabbits following intravenous injection of 10 μg lipid A, in various vehicles, at time 0. Each point represents a mean for the group of rabbits, and error bars indicate ± 1 S.E. of mean. *A*: filled circles, lipid A plus 10 μg bovine serum albumin, in 2 ml sterile saline, $n = 6$; open circles, 10 μg bovine serum albumin alone, in 2 ml sterile saline, $n = 6$. *B*: filled circles, lipid A plus 10 μg rabbit serum albumin in 2 ml sterile saline, $n = 6$; open circles, 10 μg rabbit serum albumin alone, in 2 ml sterile saline, $n = 6$. *C*: filled circles, lipid A in 25 nl triethylamine and 2 ml sterile saline, $n = 6$; open circles, 25 nl triethylamine and 2 ml sterile saline alone, $n = 4$.

followed injection of aqueous solutions of 10 μg lipid A with 10 μg rabbit serum albumin.

Although the polysaccharide component of endotoxin had no pyrogenic action of its own, the covalent coupling of polysaccharide to lipid A in endotoxin significantly augmented the pyrogenicity of the endotoxin. This conclusion, illustrated by the one example in Fig. 2, is confirmed by the results in Fig. 3, which shows dose–response curves, expressed in two ways, for lipid A extracted from the endotoxin of *S. typhosa*, and for the parent endotoxin itself. Fig. 3*A* shows the responses to the pyrogenic solutions expressed in terms of the thermal response index over the three hour period following injection: Fig. 3*B* shows the responses expressed in terms of the rise in rectal temperature prevailing 90 min after the injection, when the pyrexia was close to its peak (Fig. 1*B*).

Fig. 3 shows that 10 μg doses of endotoxin and lipid A produced the same rise in rectal temperature after 90 min, namely about 1·5 °C. However, for lower doses, endotoxin produced a significantly higher rise in rectal temperature at 90 min. Moreover, at all doses, the thermal response index following endotoxin injection was significantly greater ($P < 0{\cdot}01$, t test) than that following lipid A injection. Finally, the threshold dose of lipid A, whether measured in terms of thermal response index

or temperature rise at 90 min. was two orders of magnitude higher than that of endotoxin.

DISCUSSION

Endotoxin, the toxic and pyrogenic agent in gram-negative bacteria, is a lipopolysaccharide. Our results provide conclusive evidence—we believe for the first time—that the lipid component of the lipopolysaccharide, lipid A, is pyrogenic, at

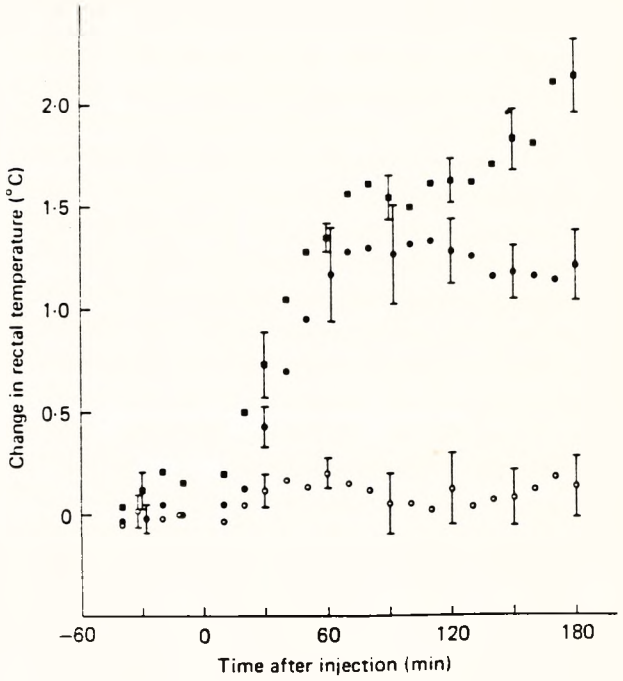


Fig. 2. Rise in rectal temperature following intravenous injection at time 0, in 2 ml of sterile saline, of 135 μg polysaccharide from *Salmonella typhosa* (open circles, $n = 6$), 10 μg lipid A and 135 μg polysaccharide (filled circles, $n = 6$), and 10 μg *S. typhosa* endotoxin (squares, $n = 6$).

least in rabbits, while the polysaccharide component is not. We employed the endotoxin of *Salmonella typhosa* in our experiments, but since lipid A is chemically similar (though not identical) in all endotoxins derived from enterobacteria (Hase & Rietschel, 1976), our conclusion that lipid A is the only pyrogenic fraction is likely to be valid for all such endotoxins.

Lipid A may well be the only pyrogenic fraction of endotoxin, but the pyrogenicity of endotoxin depends on the integrity of the whole lipopolysaccharide molecule. Lipid A does not exhibit quantitatively the pyrogenic potency of endotoxin. The polysaccharide component is more than a 'solubilizing carrier' for lipid A (Bradley, 1979). Lipid A taken into solution using rabbit serum albumin as a solubilizer (Fig. 1*B*) was no more pyrogenic than lipid A in particulate suspension in saline, and the physical presence of the related polysaccharide, in the same proportion that occurs in the parent endotoxin, did not affect the pyrogenicity of lipid A. What role the polysaccharide component plays has yet to be determined.

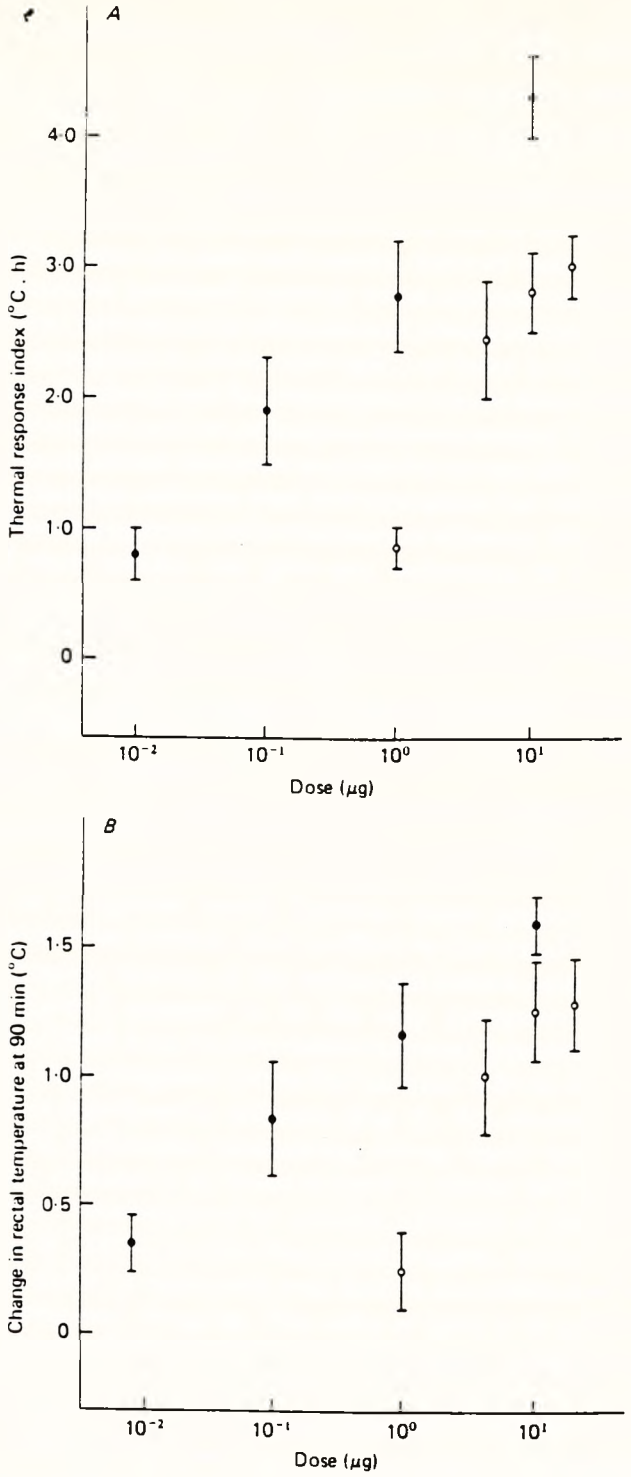


Fig. 3. Dose–response curves for lipid A with an equal mass of rabbit serum albumin in 2 ml sterile saline (open circles) and the parent *S. typhosa* endotoxin in 2 ml sterile saline (filled circles). Each point represents the mean ± S.E. for six rabbits. *A*, response expressed as thermal response index, the time integral of the rise in rectal temperature for the three hours following the injection. *B*, response expressed as the rise in rectal temperature prevailing 90 min after injection.

Previous papers attributing the pyrogenic action of endotoxin to lipid A (see, for example, Culbertson & Osburn, 1980) cite as evidence the work of Dey *et al.* (1975), although prior credit ought to be given to Rietschel *et al.* (1973). Dey and his colleagues injected lipid A, dissolved in an aqueous solution of bovine serum albumin or triethylamine, into cats, and observed a rise in rectal temperature. Feldberg & Saxena (1975), from the same laboratory, showed that an aqueous solution of lipid A in triethylamine produced pyrexia when injected intrathecally in rats. We have now shown that bovine serum albumin and triethylamine, in the doses used to convey lipid A, are pyrogenic in their own right, at least in rabbits. Neither Dey and his colleagues nor Feldberg and Saxena appear to have tested whether their vehicles were pyrogenic. Rietschel and his colleagues, experimenting in rabbits, used bovine serum albumin, human serum albumin, and rabbit serum albumin as vehicles for lipid A. They claim that the vehicles were not pyrogenic but their results show appreciable pyrexias following injection of bovine serum albumin. Since they report no statistical analyses of their results, nor give any indication of the variability of responses between animals, it is difficult to draw quantitative conclusions from their paper. Moreover, neither Dey nor Feldberg nor Rietschel appear to have injected the polysaccharide moiety of the endotoxin, to test whether or not it was pyrogenic. We submit, therefore, that the supposed evidence offered by Dey *et al.* (1975), Feldberg & Saxena (1975), and Rietschel *et al.* (1973) that lipid A is the only pyrogenic fraction of endotoxin is inconclusive.

We have now shown (Fig. 1) that the lipid A from *S. typhosa*, dissolved in an aqueous solution of rabbit serum albumin, is pyrogenic in rabbits, whereas the same quantity of the vehicle alone is not pyrogenic. Moreover, a mass of the polysaccharide component of *S. typhosa* nearly fourteen times greater than the mass of lipid A producing maximum pyrexia, had no effect on the rectal temperatures of the rabbits. Thus the pyrogenic moeity of the endotoxin indeed appears to be the lipid moeity.

Although lipid A appears to be the only pyrogenic fraction of endotoxin, the pyrogenicity of lipid A on its own cannot account quantitatively for the pyrogenicity of endotoxin. Dose–response curves for the lipid A from *S. typhosa* and for the parent endotoxin were significantly different (Fig. 3). We obtained significant pyrexia with 10 ng doses of endotoxin (containing 0·3 ng lipid A/kg body weight) injected intravenously, whereas 1 μg doses of lipid A (about 300 ng/kg), dissolved in rabbit serum albumin, were required to produce pyrexias of the same magnitude.

Rietschel *et al.* (1973), on the other hand, suggest, without statistical support, that the threshold doses of endotoxin and lipid A in rabbits are about the same, and lie between 1 ng/kg and 10 ng/kg. The ratio of lipid A to polysaccharide in endotoxin varies with the number of repeating oligosaccharide units in the polysaccharide part of the molecule, which in turn varies with the species of bacterium from which the endotoxin is derived. Our extraction procedure yielded a mass ratio of about 1 part of lipid A to 13·5 parts of polysaccharide, though in some species of endotoxin the ratio might be as high as 1 part to 2 parts of polysaccharide (Morrison & Leive, 1975). Nevertheless, lipid A always constitutes a minor part of endotoxin molecule. Therefore, whether one accepts Rietschel's values for the threshold doses or ours, the pyrogenicity of the endotoxin, per unit mass of lipid A, is greater than the pyrogenicity of lipid A alone. Our results suggest that the threshold dose of lipid A

combined in endotoxin is more than 1000 times lower than that for lipid A dissolved in rabbit serum albumin. In other words, when separated from the polysaccharide component of endotoxin, lipid A lost more than 99·9 % of its pyrogenic activity.

It is characteristic of fever, as distinct from other hyperthermias, that there is a limit to the extent to which body temperature rises (Bligh, 1973). According to the results shown in Fig. 3, high doses of endotoxin produced fevers approximately equal in magnitude to those which followed similar doses of lipid A. The responses to the two pyrogens appeared to saturate at a thermal response index of 3–4 °C . h, or a 90 min temperature of 1·0–1·5 °C. One explanation of this saturation would be that there is a limited number of active sites, perhaps receptors in phagocytic cells, at which both lipid A and endotoxin act. The intact endotoxin molecule interacts more potently at these sites than does isolated lipid A. The process saturates when all sites are occupied. Another possibility is that the effector mechanisms responsible for the genesis of fever saturate; for example the synthesis of leucocyte pyrogen, or the activity of leucocyte pyrogen, may be rate-limiting.

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Heat Stroke and Endotoxaemia in Rabbits

N. Butkow, D. Mitchell, Helen Laburn, and Eva Kenedi

Department of Physiology, University of the Witwatersrand Medical School, Johannesburg 2193, South Africa

Endotoxin has been detected in the plasma of patients and experimental animals with heatstroke (6, 8), and many of the pathological features of heatstroke are similar to those of endotoxaemia (6). Fine (5) suggested that the failing liver in heatstroke was unable to clear the blood of endotoxin originating from intestinal bacteria. Sterilizing of gut of dogs before exposing them to heat reduced their mortality from heatstroke (2), which implies that endotoxaemia of intestinal origin was sufficiently severe to contribute to the fatal outcome. We now have evidence that endotoxaemia occurs early in rabbits exposed to lethal heat stress, and contributes to their body temperature rise during the heat exposure.

METHODS

Adult New Zealand White rabbits (2.3-3.5 kg) of either sex were used. One group of rabbits received chloramphenicol (25 mm/day) and dihydroxystreptomycin (25 mm/day) orally for three days prior to experimentation. The other group was treated identically but received no antibiotics.

Conscious rabbits were exposed in conventional stocks to a dry still environment at 40°C dry-bulb temperature. Their rectal temperatures were monitored with indwelling thermocouples. The rabbits were under constant observation in case of unacceptable distress. Five control rabbits were allowed to reach a rectal temperature of 43.5°C; all were conscious and mobile when removed from the hot environment, but died within 24 hours with pathology (including disseminated intravascular coagulation) typical of heatstroke. When exposures were terminated at rectal temperatures of 42.5°C, the rabbits recovered uneventfully. Rabbits reaching 43.5°C in subsequent exposures were killed by overdose of barbiturate, at the end of the exposure.

We took blood from an ear artery, using sterile procedures, before heat exposure, and at 1°C steps of rectal temperature, beginning at 40.5°C. The blood was analysed for endotoxin using the *Limulus* amoebocyte lysate assay (10), modified for use with plasma samples (7).

RESULTS

Figure 1 shows the rates of rise in rectal temperature of two groups of rabbits, one pretreated with oral antibiotics, and the other a control group. There was no significant difference in the mean temperatures of the groups before heat exposure. Thereafter the group pretreated with antibiotics showed a slower rate of rise of rectal temperature.

Figure 2 shows the proportion of animals with plasma positive for endotoxin, at various rectal temperatures. No rabbit had a detectable concentration of endotoxin in its plasma before the heat exposure. At a rectal temperature of 42.5°C, all control rabbits had endotoxin in their plasma, and some had concentrations of 500 ng/ml. Only one of six rabbits pretreated with antibiotics had detectable endotoxaemia. According to a chi-square test, mortality in pretreated rabbits was reduced significantly.

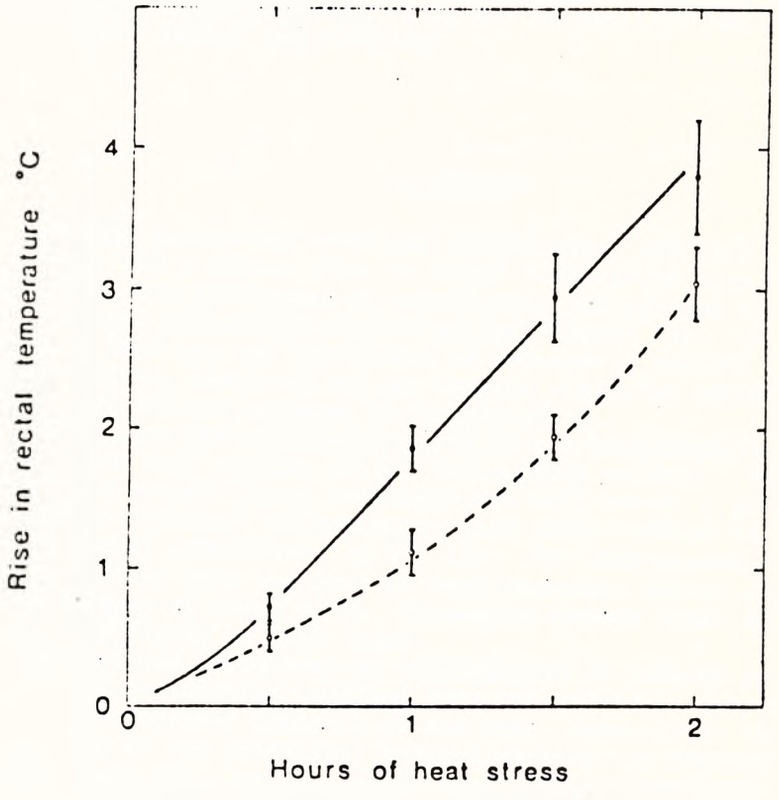


FIG. 1. Rise of rectal temperature (mean ± SE) in control rabbits (solid line, n = 9) and rabbits pretreated with oral antibiotics (dashed line, n = 6).

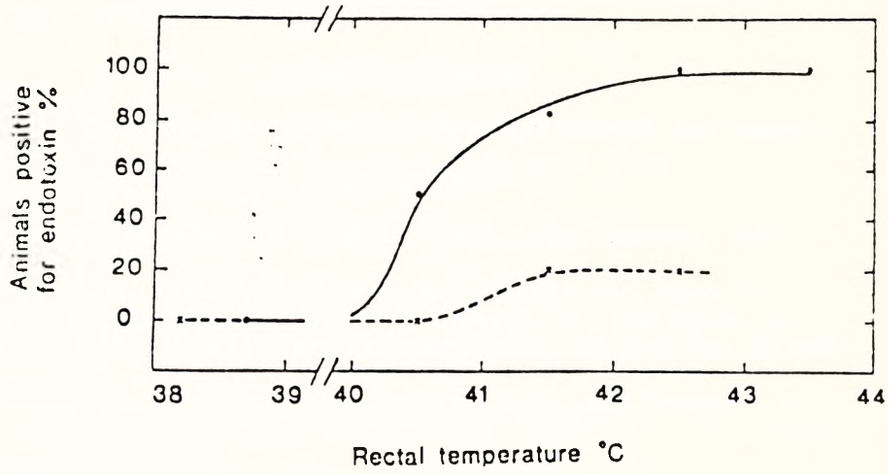


FIG. 2, Proportion of control rabbits (solid line, n = 12) and pretreated rabbits (dashed line, n = 6) positive for endotoxin, at various rectal temperatures.

DISCUSSION

Rabbits pretreated with oral antibiotics, and then exposed to heat, had a slower rise in rectal temperature than did control rabbits. The difference in temperature had nothing to do with the central antipyretic action of antibiotics (3); the antibiotics we used are not inhibitors of eukaryotic protein synthesis. Bynum, Brown, DuBose *et al* (2) noticed a similar phenomenon in dogs, but did not attempt to explain it.

We believe the explanation lies in the endotoxaemia evident, during the heat stress, in all the control animals. The pyrogenic properties of endotoxin are well known; 1 μg of endotoxin injected intravenously into adult rabbits is enough to raise rectal temperature by 1°C (9). The endotoxaemia became evident after only one hour of heat exposure, and at rectal temperatures of about 41°C. Thermally-induced liver damage would have been likely at that stage.

The reduced prevalence of endotoxaemia in rabbits pretreated with oral antibiotics confirms that the endotoxin originated from gram-negative bacteria of the gut. Presumably, the integrity of the intestinal mucosal barrier was destroyed by high temperature (6). Selective heating of the abdominal contents causes fatal circulatory collapse in dogs (1). Whether intestinal endotoxin leaks into the circulation, or whether the bacteria themselves invade, and subsequently release endotoxin, is not yet known. One would predict that endotoxin-tolerant animals

would be resistant to lethal heat stress; this prediction has been confirmed recently in rats (4).

ACKNOWLEDGMENT

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Effect of Dietary Linolenate on the Pathogenesis of Fever

E. KENEDI and D. MENDELSOHN

1. INTRODUCTION

The effects of a reduction in the level of arachidonic acid 20 : 4(ω6) by increases in linoleic acid 18 : 2(ω6) and/or linolenic acid 18 : 3(ω3) in a febrile condition, induced by gram-negative bacterial pyrogens, are unknown. Yet it is recognized that endotoxin fever in humans and rabbits is arachidonate dependent (Spawinski *et al.*, 1978). It is also recognized that increasing amounts of linolenate, 18 : 3(ω3), reduce the metabolites of linoleic acid, 18 : 2(ω6) (Machlin, 1962; Mohrhauer and Holman, 1963; Rahm and Holman, 1964; Anding and Hwang, 1986) by acting as a competitor for the same cyclo-oxygenase (Pace-Asciak and Wolfe, 1968), thereby not only influencing the formation of arachidonic acid but suppressing its metabolites as well. The metabolites of arachidonic acid have been implicated in the biochemical sequences leading to fever induced by bacterial endotoxin (Feldberg and Saxena, 1975; Milton, 1976; Skarnes *et al.*, 1981). Therefore, deficiency in arachidonic acid diminishes the fever response (Kenedi *et al.*, 1984), which, in the course of constant stimulation by endotoxin, leads to tolerance of the fever (Atkins, 1960; Kanoh *et al*, 1977). The physiological consequences of tolerance in the body, induced by increased intake in linolenate, together with chronic induction of fever, have not been associated with a diseased condition, as the biochemical mechanism leading to tolerance had been unknown until the present study. According to Bernheim *et al.* (1979), a "true" fever is a disorder of thermoregulation in which the body actively seeks to raise its temperature by increasing heat production (Bernheim *et al.*, 1979). Heat is the result of oxidative catabolism in the body, and there is a relationship between the amount of O_2 absorbed and the amount of CO_2 eliminated (Benedict, 1907; Fritz, 1961).

Excessive unsaturation of plasma by 18 : 2(ω6) and 18 : 3(ω3) induced by increased dietary linolenate results in decreased oxygenation (Stadie, 1945), which, coupled with increased phosphorylization (Stadie, 1945; Marco *et al.*, 1961), stimulates CO_2 production

E. KENEDI and D. MENDELSOHN • Department of Chemical Pathology, School of Pathology, Medical School, University of the Witwatersrand; and The South African Institute for Medical Research, Johannesburg 2000, Republic of South Africa.

via the long-chain fatty acids (Fritz, 1961). Heat is thus gained mainly by a change in the respiratory quotient (R.Q.), which in turn increases the body temperature (Harper, 1967). This illustrates both the biochemical importance and the physiological role of the essential fatty acids (Burr and Burr, 1930; Friedman, 1979). Excess or lack of the latter could impair R.Q. in such a way as to influence the body's mechanism for gaining or losing heat. Thus, body temperature depends on the equilibrium between unsaturated and polyunsaturated fatty acids. Fluctuation in the concentration of 18 : 2(ω6) can thus alter the equilibrium between thermolysis and thermogenesis. Body temperature, which is the index of the resultant two factors, can be affected by either (Benedict, 1915). An increase in body temperature can therefore be equated with increased R.Q. induced by excessive formation of 18 : 2(ω6) and the development of fever with rapid oxidation of polyunsaturates (eicosanoids) (Fritz, 1961), provided R.Q. remains constant.

Therefore, a deficiency in 20 : 4(ω6) and its metabolites (Hwang and Carrol, 1980) in spite of an abundance in essential fatty acids, combined with chronic induction of fever, could contribute to a physiopathological condition that is diet-induced and not recognized. As lecithin constitutes 70% of human phospholipids (Frederickson and Gordon, 1958) and is rich in both unsaturated and polyunsaturated fatty acids, changes in essential fatty acids causes greater alterations in this class of lipids than in either free fatty acids or triglycerides (Ogburn *et al.*, 1982).

The present work has been undertaken to clarify the effects of an increased intake in dietary linolenate on the specific distribution of fatty acids and consequently on the production of fever induced by gram-negative bacterial endotoxin and the pathological consequences of this alteration in thermoregulation.

2. MATERIALS AND METHODS

2.1. Experimental Model

Ten white New Zealand (NZ) rabbits weighing 2.20–2.80 kg were divided into two groups. Group A (N = 5), designated the control, received the standard commercial diet of rabbit pellets (Table I). Its fatty acid content included 50% linoleic acid. Group B (N = 5) received the standard diet supplemented with hay and contained fatty acids comprising 65% linoleic acid and 48% linolenic acid (Table II). Water was available to all rabbits *ad lib.* Both groups were injected once daily intravenously (IV) with 2.0 μg/kg body mass of the endotoxin Salmonella thyphosa for 7 days.

The animals were housed individually in stainless steel cages and kept on a 12-hr–12-hr photophase–scotophase regimen. To minimize the effect of sudden stress during the experimental period, the animals were acclimatized to their restraining boxes and to thermocouples for 1 week before the start of the experiment. Each animal was weighed daily before being given its injection and again 180 min thereafter.

2.2. Endotoxin

Purified lipopolysaccharide of *S. thyphosa* 0901 (Difco 3124-25-6) was suspended in pyrogen-free saline at 1.0 mg/ml and stored at −15°C. This stock solution was further diluted daily to a concentration of 15 μg/ml. Of this, 0.35 ml containing 5 μg endotoxin was injected daily into the marginal ear vein of each rabbit, giving the required quantity of 2.0 μg/kg endotoxin.

TABLE I. Composition of Standard Commercial Diet

Composition	Normal maintenance diet
Fat	5% corn oil
Protein	20% milk powder (vitamin free)
Fiber	4% cellulose
Carbohydrate	64% sucrose
Salt	4% salt mix[a]
Vitamin mix	2%[b] (ICN Pharmaceuticals)

[a]As recommended by Jones and Foster (1942).
[b]ICN Pharmaceuticals, Inc. (Cleveland, Ohio) g/kg, vitamin A 90.2000 IU, vitamin D 100.000 IU, α-tocopherol 1.5 g, ascorbic acid 45.0 g, inositol 1.0 g, choline chloride 75.0 g, *p*-aminobenzoic acid 5.0 g, niacin 4.5 g, pyrodoxin hydrochloride 1.0 g, thiamine hydrochloride 1.0 g, calcium pentotenate 3.0 g, vitamin B_{12} 1.4 g, biotin 20.0 mg, folic acid 90.0 mg.

2.3. Temperature Measurements

The rectal temperature was measured using copper constant thermocouples covered with polyethylene tubing and inserted into the rectum to a depth of ≈100 mm. All thermocouples were calibrated by immersion in water against a certified mercury thermometer. Temperature measurement was accurate to within 0.2°C. The output from each thermocouple was recorded at 10-min intervals for 1 hr before and 3 hr after every injection, using a Solomat thermometer, model 335K (Ballainvillers, France). The temperature was compared with that

TABLE II. Fatty Acid Composition of Rabbit Standard Diet and Hay Diet

Fatty acid	Standard diet[a]	Hay diet[a]
Unidentified	0.04	3.40
14 : 0	0.27	2.38
14 : 1	0.10	1.29
16 : 0	16.50	15.78
16 : 1	0.67	1.22
18 : 0	1.98	2.55
18 : 1	27.94	2.30
18 : 2 (ω6)	50.87	14.70
18 : 3 (ω3)	—	48.30
22 : 0	0.63	1.97
20 : 4 (ω6)	—	0.04
24 : 0	0.60	3.40
22 : 5 (ω6)	0.40	2.30

[a]Values are the mean of two lipid extractions expressed as a percentage of the respective fatty acids.

prevailing before the injections. All experiments were carried out at an ambient room temperature of 21° ± 1°C.

2.4. Blood Sampling

Blood samples of 1.0–2.0 ml were drawn from the ear artery of each rabbit with a butterfly needle (21-gauge) into heparinized (vacutainer) tubes before the start of the experiment (baseline) and again at 60 min and 180 min after the first and seventh injections, respectively. Shortly after collection, the blood samples were centrifuged for 10 min at 1800 rpm. The resultant serum supernatant was centrifuged for a second time to remove any remaining blood cells. The serum was flushed with N_2 and stored at −20°C for fatty acid analysis. Three control rabbits (group A) were injected with saline alone. Their temperatures were measured and serum lecithin fatty acids analyzed to eliminate any interference from the carrier solvent (Fig. 1, Table III). Two rabbits died during the course of the experiment; measurements for these two rabbits are not included in the results.

2.5. Extraction of Fatty Acids from Solid Food

Pulverized rabbit pellets of 2.50 g or hay were macerated in 32.0 ml 15% KOH in ethanol; the mixture was allowed to saponify overnight at 60°C. After cooling, 12.0 ml distilled water was added, mixed, and divided into four 11.0-ml aliquots. To each aliquot, 9.50 ml petroleum ether (40–60°C) was added, mixed on a vortex mixer for 60 sec, and the

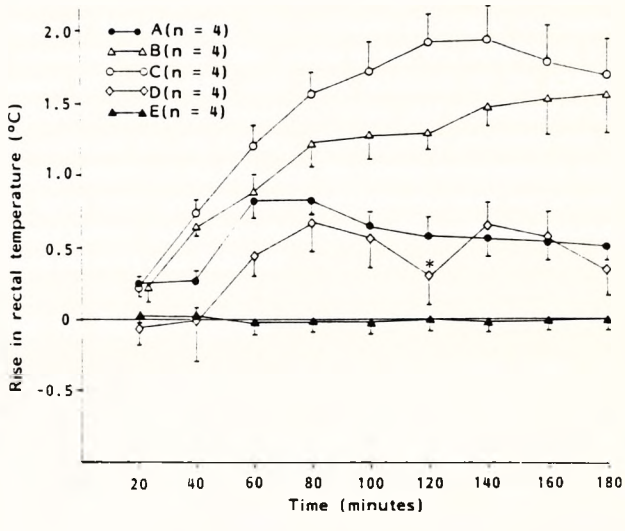


FIGURE 1. Rise in the rectal temperature in conscious rabbits following a single or seven consecutive intravenous (IV) injections of 2.0 μg/kg *S. thyphosa* (endotoxin). Each point represents a mean of four rabbits; error bars indicate SE ±SEM. (●) Single injection in rabbits on standard diet (N = 4). (△) Single injection in rabbits on hay-supplemented diet (N = 4). (○) Seven consecutive IV injections in standard diet group (N = 4). (◇) Seven consecutive IV injections in hay-supplemented group (N = 4). (▲) Pyrogen-free saline 0.35 ml alone (N = 4).

TABLE III. Effect of a Single Dose of Intravenous Saline Injection at 180 min[a]

	Group A[a]	
Fatty acid	Control	Saline
16:0	24.07 ± 0.15	26.36 ± 1.00
18:0	15.38 ± 0.58	15.36 ± 0.40
18:1 (ω9)	15.99 ± 0.62	16.61 ± 0.63
18:2 (ω6)	30.96 ± 0.77	28.35 ± 0.63
18:3 (ω3)	0.76 ± 0.04	0.70 ± 0.70
18:3 (ω6)	0.40 ± 0.03	0.36 ± 0.05
20:3 (ω6)	0.40 ± 0.03	0.32 ± 0.04
20:4 (ω6)	3.97 ± 0.19	3.42 ± 0.04
20:5 (ω6)	0.64 ± 0.03	0.46 ± 0.17
22:4 (ω6)	1.20 ± 0.11	1.70 ± 0.29
22:5 (ω6)	3.94 ± 0.27	3.85 ± 0.13
Unidentified polyunsaturate	2.34 ± 0.28	2.21 ± 0.23

[a]Values represent the relative percentage of fatty acids measured on gas–liquid chromatography (GLC) and the average of three determinations. Test SE ± SEM of three rabbits.
[b]Group A control on standard diet.

resulting supernatant centrifuged (model TG 6 refrigerated centrifuge, Beckman Instruments, Palo Alto, California) at 3000 rpm for 5 min. The supernatant layer containing the non-saponifiable portion was then decanted. This extraction procedure was repeated twice.

The residue remaining from the rabbit pellets or hay was acidified to a pH of 2.0 with 3.75 ml concentrated HCl. The fatty acids were recovered by the addition of 9.50 ml petroleum ether (40–60°C) mixed on a vortex for 30 sec, and centrifuged as above for 5 min. The extracts were combined into one fraction. This recovery procedure was repeated twice. The pooled extract was dried over Na_2SO_4, and the solvent evaporated to dryness under N_2. The extracted total lipids were saponified and methylated the same way as serum fatty acids, the resultant fatty acid methyl esters were dissolved in 100 μl hexane and analyzed on a gas chromatograph (Packard 427) (see Table II).

2.6. Extraction of Serum Lipids

Serum of 0.20 ml was extracted with 1.0-ml AR grade MeOH–$CHCl_3$ (1 : 1, v/v) for 10 min by gentle stirring. After standing for 5 min at room temperature, the mixture was centrifuged for 10 min at 3000 rpm and the supernatant decanted into a clean test tube. The residue was further extracted with MeOH–$CHCl_3$ (1 : 1) following the same procedure. After separation of the two phases with 0.1 M NaCl, the $CHCl_3$ phase subnatant was filtered through solvent-washed filter paper (Whatman No. 1), dried under N_2 at 50°C, and immediately dissolved in 100 μl $CHCl_3$.

2.7. Thin-Layer Chromatographic Separation of Phospholipids

The 100 μl extracted lipid was separated on precoated silica gel G-plastic baked plates 20 × 20 cm, 0.25-mm layer (Machery-Nagel) prewashed in MeOH–$CHCl_3$ (1 : 1) and

activated for 1 hr at 100°C. The solvent system $CHCl_3$–MeOH–CH_3COOH (glacial)–H_2O (50 : 25 : 7 : 1), (v/v) was used for the separation of phospholipid classes. After drying and exposure to iodine vapor, the lecithin fraction was outlined and the thin-layer chromatography (TLC) plate redried at 100°C for 15 min to remove the iodine.

2.8. Saponification and Methylation of Lecithin

The lecithin fraction was scraped off into a clean test tube, saponified, and methylated by the addition of 1.0 ml MeOH in 2.0 ml 0.2 M sodium methylate (CH_3NaO) in MeOH for 20 min in a 50°C water bath with frequent shaking. After cooling with 4.0 ml H_2O, the methyl esters were extracted twice with 1.0 ml hexane. The separated hexane layer was transferred to a clean test tube and evaporated under N_2 at 60°C. The plasma lecithin fatty acid methyl esters were dissolved in 100 μl hexane, capped in glass vials, and stored at −20°C until analyzed.

2.9. Gas Chromatography

A Packard 427 gas chromatograph fitted with a flame ionization detector and a glass column measuring 1.8 m × 4 mm (internal diameter) packed with 10% SP 2330 on 100/100 chromasorb WAW was used for the separation of the fatty acid methyl esters. The samples were run at 200°C with a carrier gas (N_2) flow rate of 25 ml/min. Both the detector heater and injector were set at 220°C. The fatty acid methyl esters were identified using retention volumes of known GLC standard Pufa-2, NHIF, RM3, 68A (animal source, Supelco, Inc., Bellefonte, Pennsylvania). Peak areas were measured using an SP 4100 (Spectrophysics) integrator.

3. RESULTS

3.1. Influence of Hay on Plasma Lecithin Fatty Acid Composition

The results shown in Table IV indicate that within 1 week, increased dietary linolenate affected the interrelationship between the unsaturated and polyunsaturated fatty acids. Prior to the fever experiment, an increase in levels of 18 : 2(ω6), 18 : 3(ω3), and 20 : 5(ω3) followed by a decrease in 20 : 4(ω6) was observed, together with an absence of 22 : 4(ω6) and 22 : 5(ω6).

3.2. Effect of Endotoxin on Fever Response and on the Composition of Serum Lecithin Fatty Acids

3.2.1. Single Dose of Endotoxin

A difference between the two groups in their response of fever to a single dose of endotoxin (2.0 μg/kg body mass) became apparent. In group A, the endotoxin produced a short monophasic fever with a delay of 20 min before its onset. Fever peaked after 60 min and had a duration of 80 min. In group B, the endotoxin evoked a magnified biphasic fever with the same delay to its onset as group A, but with its first peak at 80 min and a second at 140

TABLE IV. Effect of a Single Intravenous Injection of 2.0 μg/kg Endotoxin *S. thyphosa* on the Composition of Serum Lecithin Fatty Acid in Rabbits Fed Standard or Hay-Supplemented Diet[a]

	Group A[b]			Group B[c]		
		Postinjection			Postinjection	
Fatty acid	Preinjection	60 min	180 min	Preinjection	60 min	180 min
16:0	24.07 ± 1.15[α]	26.11 ± 2.37[α]	27.01 ± 1.92[α]	22.27 ± 1.44[α]	21.05 ± 1.61[α]	19.06 ± 1.74[α]
18:0	15.38 ± 0.58[α]	12.76 ± 1.36[α]	13.40 ± 0.74[α]	20.91 ± 0.35[β]	21.70 ± 0.50[β]	21.85 ± 0.67[β]
18:1 (ω9)	15.99 ± 0.62[α]	18.97 ± 1.38[α]	14.36 ± 1.15[α]	14.90 ± 1.68[α]	13.82 ± 1.62[α]	13.85 ± 1.02[α]
18:2 (ω6)	30.96 ± 0.77[α]	26.83 ± 0.48[β]	32.17 ± 0.43[α]	32.39 ± 0.91[α]	34.72 ± 0.17[β]	36.40 ± 0.93[β]
18:3 (ω3)	0.76 ± 0.04[α]	0.75 ± 0.04[α]	0.90 ± 0.03[α]	1.52 ± 0.44[β]	0.85 ± 0.12[α]	0.94 ± 0.08[α]
18:3 (ω6)	0.40 ± 0.03[α]	0.27 ± 0.05[α]	0.35 ± 0.05[α]	0.73 ± 0.13[α]	0.63 ± 0.11[α]	0.70 ± 0.22[α]
20:3 (ω6)	0.40 ± 0.03[α]	0.27 ± 0.04[α]	0.38 ± 0.01[α]	0.70 ± 0.17[α]	0.45 ± 0.06[β]	0.55 ± 0.08[α]
20:4 (ω6)	3.97 ± 0.19[α]	2.63 ± 0.35[α]	3.94 ± 0.71[α]	1.82 ± 0.40[β]	2.65 ± 0.38[α]	3.34 ± 0.21[α]
20:5 (ω3)	0.64 ± 0.03[α]	0.46 ± 0.09[α]	0.69 ± 0.18[α]	3.80 ± 0.98[β]	3.22 ± 0.38[β]	2.80 ± 0.30[β]
22:4 (ω6)	1.20 ± 0.11	1.96 ± 0.28	1.64 ± 0.20	—	—	—
22:5 (ω6)	3.94 ± 0.27	3.75 ± 0.31	2.90 ± 0.13	—	—	—
Rest. Poly.	2.34 ± 0.28[α]	2.71 ± 0.31[α]	1.42 ± 0.36[α]	0.62 ± 0.21[β]	0.76 ± 0.11[β]	0.88 ± 0.12[β]

[a] Values represent the relative percentage of fatty acids measured by gas liquid chromatography (GLC) and the average of four or five determinations. β indicates significantly different from control (<0.05) ($p > 0.05$) by Duncan multiple range. Test SE ±SEM.

[b] Standard diet, $N = 4$.

[c] Hay-supplemented diet, $N = 5$.

min, and a duration of several hours (see Fig. 1). Changes in the serum lecithin fatty acid composition appear to coincide with alteration in the fever pattern.

In group A, decrease in the level of all the unsaturates and of the polyunsaturates 20 : 3(ω6), 20 : 4(ω6), 22 : 5(ω6) occurred within minutes, together with an increase in 22 : 4(ω6). No change in 20 : 5(ω3) in the plasma levels occurred (Table IV). The return to normal in most of the fatty acids at 180 min coincided with the termination of the fever (Table IV).

In group B, an increase in levels of 18 : 2(ω6) and 20 : 4(ω6) occurred within minutes, followed by a decrease of the linolenate 18 : 3(ω3) 18 : 3(ω6), together with reduced levels of 20 : 3(ω6) and 20 : 5(ω3) while both docosatetraeonic acid 22 : 4(ω6) and docosapentaeonic acid 22 : 5(ω6) remained inhibited. These changes suggest heightened degree of lipolysis and decreased unesterification of fatty acids within the plasma (Table IV).

3.2.2. Multiple Doses of Endotoxin

The fever response by the seventh day to daily IV injections of endotoxin further emphasized the difference between the two groups. In group A, a monophasic fever was again evoked with increased magnitude and extended duration beyond 180 min (Fig. 1). This is reflected in the serum lecithin fatty acid composition (Table V). An increase in levels of 18 : 2(ω6) and 20 : 4(ω6), and a decrease in 22 : 4(ω6) at 180 min indicates increasing combustion of fatty acids to meet increasing energy demands under the action of endotoxin (Table V). This did not affect the body temperature, which returned to normal at the end of each experiment (Fig. 2).

Conversely, in group B, the change in the pattern of fever by the seventh injection (see Fig. 1) resulted in a decrease in the size of the second fever peak. The characteristic retention of the first peak was not reversed even by the administration of a second injection at 120 min. This suggests that the animals had been made immune to the action of bacterial pyrogen not only by its continual administration (Fig. 1), but more so by the deficiency in the production of 20 : 4(ω6) induced by increased intake in dietary 18 : 3(ω3) (Tables IV and V). Tolerance to endotoxin in these animals did not prevent the rise in body temperature (Fig. 2), which

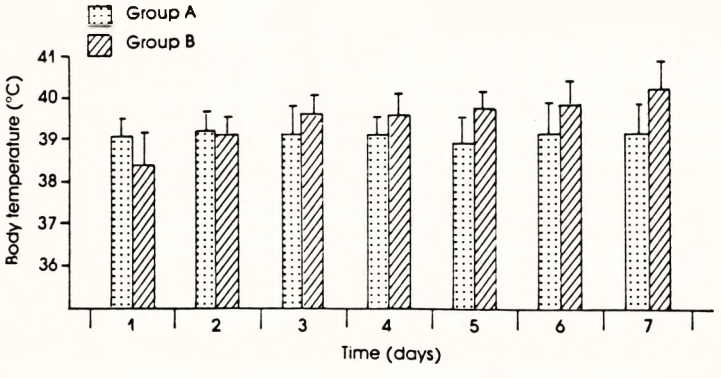


FIGURE 2. Body temperature of rabbits before injection of endotoxin *S. thyphosa*. Group A fed on standard diet ($N = 4$). Group B fed on hay-supplemented diet ($N = 4$). Error bars indicate SE ±SEM.

TABLE V. Effect of Seven Consecutive Intravenous Injections of 2.0 μg/kg Endotoxin *S. typhosa* on the Composition of Serum Lecithin Fatty Acid in Rabbits Fed Standard or Hay-Supplemented Diet[a]

	Group A[b]			Group B[c]		
		Postinjection			Postinjection	
Fatty acid	Preinjection	60 min	180 min	Preinjection	60 min	180 min
16:0	22.98 ± 2.64[α]	25.90 ± 0.72[α]	23.17 ± 1.35[α]	20.37 ± 1.35[α]	20.60 ± 1.79[α]	19.91 ± 3.16[α]
18:0	15.73 ± 1.63[α]	13.12 ± 0.72[α]	14.14 ± 1.51	22.02 ± 1.94[α]	21.19 ± 2.98[α]	20.88 ± 1.90[α]
18:1 (ω9)	15.05 ± 0.95[α]	18.91 ± 1.64[α]	16.42 ± 1.92[α]	14.82 ± 1.71[α]	14.82 ± 1.71[α]	12.90 ± 1.99[α]
18:2 (ω6)	31.72 ± 0.77[α]	30.06 ± 0.33[α]	34.57 ± 0.92[α]	34.74 ± 1.65[α]	34.07 ± 0.72[β]	37.27 ± 0.26[β]
18:3 (ω3)	0.94 ± 0.03[α]	0.63 ± 0.02[α]	0.80 ± 0.09[α]	1.50 ± 0.52[β]	1.01 ± 0.18[β]	0.92 ± 0.26[α]
18:3 (ω6)	0.28 ± 0.06[α]	0.33 ± 0.06[α]	0.36 ± 0.06[α]	0.65 ± 0.16[α]	0.66 ± 0.25[α]	0.49 ± 0.31[α]
20:3 (ω6)	0.24 ± 0.05[α]	0.37 ± 0.07[α]	0.31 ± 0.07[α]	0.56 ± 0.18[α]	0.58 ± 0.20[α]	0.42 ± 0.15[α]
20:4 (ω6)	3.15 ± 0.53[α]	2.64 ± 0.69[α]	3.71 ± 0.38[α]	1.79 ± 0.08[β]	1.99 ± 0.59[α]	2.49 ± 0.48[α]
20:5 (ω3)	0.76 ± 0.17[α]	0.42 ± 0.04[α]	0.51 ± 0.10[α]	2.31 ± 0.55[β]	1.96 ± 0.64[β]	3.93 ± 0.40[β]
22:4 (ω6)	2.38 ± 0.45	1.19 ± 0.01	1.62 ± 0.34	—	—	—
22:5 (ω6)	4.47 ± 0.45	2.69 ± 0.01	3.26 ± 0.60	—	—	—
Rest. Poly.	2.03 ± 0.57	2.60 ± 0.45	1.53 ± 0.14	—	—	—

[a]Values represent the relative percentage of fatty acids measured by gas–liquid chromatography (GLC) and the average of four determinations in each group. Test SE ±SEM of four rabbits. [β] indicates significantly different from control (<0.05) ($p > 0.05$) by Duncan multiple range.

[b]Standard diet, $N = 4$.

[c]Hay-supplemented diet, $N = 5$.

increased daily from 39.2 ± 0.05°C to 40.25 ± 0.09°C, and occurred simultaneously with an increase in levels of 18 : 2(ω6). This finding further supports the contention that essential fatty acids are involved in thermoregulation and polyunsaturates in endotoxin-induced fever.

4. DISCUSSION

Under normal physiological conditions, biochemical changes introduced by the diet and their effect on serum lipid composition can go unobserved. Under changed physiological conditions that are sensitive to differences in the amounts of arachidonic acid, absence of, excessive, or imbalanced arachidonic synthesis in the tissue can lead to irreversible pathological changes. As these changes are not recognized, they cannot be diagnosed clinically. In the present study, the existence of a sensitive balance between 18 : 2(ω6) and 18 : 3(ω3) is clearly defined by an abnormal response of fever to a single dose of endotoxin on the one hand, and the rabbit's tolerance to endotoxin within 7 days on the other, together with a gradual rise in the body temperature. This in turn governs the equilibrium between 18 : (ω6) and 20 : 4(ω6). A change in this balance appears to influence not only the prognosis of fever but also the body temperature. A fall in levels of 18 : 2(ω6) and 20 : 4(ω6) under the stimulus of endotoxin indicates that fever is produced by the metabolites of arachidonic acid. Heat is thus mainly gained by peripheral polyunsaturates provided that R.Q. remains constant. However, if body heat is increased by either 18 : 2(ω6) or 18 : 3(ω3), or both, it is followed by a decrease in the formation of not only serum 20 : 4(ω6) but of cerebral arachidonite as well (Kenedi and Mendelsohn, unpublished results), thereby significantly increasing CO_2 production and in turn altering R.Q. (Splawinski *et al.*, 1978).

Since metabolism (R.Q.) and heat production go hand in hand (Benedict, 1907) in increasing oxidative catabolism by way of either 18 : 2(ω6) or 18 : 3(ω3), or both, increased peroxidation (Stadie, 1945; Marco *et al.*, 1961) and an alteration in the cerebral mitochondrial function occurs in the course of increasing intake in dietary linolenate (Marco *et al.*, 1961).

Thus, fluctuation in levels of 18 : 2(ω6) due to endotoxin influences the pattern of fever, which in turn indicates whether fever is centrally or peripherally initiated. It also defines the existence of tolerance to endotoxin in animals rendered resistant to the pyrogenic action of a bacterial pyrogen within a short time by the combination of increased amounts of 18 : 3(ω3) and regular induction of small doses of endotoxin. Two different mechanisms can be differentiated: (1) initiation by peripheral arachidonic acid and its metabolites, which induce fever; and (2) hyperthermia induced by peripheral 18 : 2(ω6) and 18 : 3(ω3). These results can therefore be regarded as further evidence that polyunsaturates are actively involved in the induction of fever.

Linoleic and linolenic acids appear to be involved in thermoregulation. A change in the thermoregulatory process reflects the rise or fall in the body temperature and can therefore be considered a change in the life process accompanied by increased or decreased heat production (Benedict, 1915). In the present study, an increase in body temperature can therefore be equated with increased heat production induced by an increased concentration of 18 : 2(ω6), in turn induced by an increase of 18 : 3(ω3) in the diet. Under the stimulus of *S. thyphosa*, the fever had less effect on the body temperature.

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RESPONSES OF LIZARDS TO PYROGENS[1]

HELEN LABURN, D. MITCHELL, EVA KENEDI and G. N. LOUW

Department of Physiology, University of the Witwatersrand Medical School, Johannesburg, South Africa

The recent resurgence of interest in the survival value of fever originated with the observation that lizards develop fever in response to killed bacteria and to rabbit endogenous pyrogen (Bernheim and Kluger, 1976; Kluger, 1978). The fevers in the ectothermic lizards result from behavioural responses; the lizards select higher ambient temperatures following pyrogen injection. We noticed that all experiments on lizard fever have been conducted using iguanid lizards, and therefore attempted to repeat the experiments using lizards of a different family.

Cordylus cataphractus inhabit arid regions of Southern Africa, and is well known for its thermoregulatory sunbasking. Lizards were trapped in the wild and maintained in the laboratory for several months before experimentation. They were then exposed to a photothermal gradient with a sand base. The heat was supplied by tungsten lamps. The temperature at the cool end of the gradient was measured using exposed thermocouples, and the hot end by a thermocouple in a blackened copper tube of approximately the dimensions of the lizards.

Lizard body temperatures were measured using in-dwelling 36-gauge thermocouples inserted via the cloaca to a depth of approximately 30mm. The lizard temperatures were measured at 15 minute intervals. Lizards were allowed 90 minutes in the gradient before any injections were made. All injections were intracardiac and, except in the case of rabbit leucocyte pyrogen, were made in a volume of 0.2ml.

In the first series of the experiments lizards were placed in a gradient where the ambient temperatures at the extremes were approximately 85°C and 25°C. In this environment injections of 0.5ml of rabbit leucocyte pyrogen, or injections of 0.2ml of a solution containing 4×10^9 organisms of killed Aeromonas hydrophila had no effect on body temperature of the lizards when compared with control injections of 0.2ml of sterile saline. During the seven hours following injection, the body temperatures of the lizards receiving either of the pyrogenic solutions did not differ from the body temperatures of the lizards receiving the control solution.

[1] This work is supported financially by the South African Council for Scientific and Industrial Research

Preferred body temperature lay between 30°C and 34°C. The pyrogenicity of the rabbit leucocyte pyrogen and of the A. hydrophila solution was verified by intravenous injection into rabbits.

In a second series of the experiments, the response of the cordylid lizards to pyrogens was investigated using a gradient in which the ambient temperature at the cooler end was 36°C and the warmer end 56°C. In this experimental situation the body temperature of the lizards immediately prior to injection of pyrogens was 36°C or higher. Injections of 0.2ml of a solution containing 4 X 10^9 organisms of killed A. hydrophila again failed to produce a rise in body temperature of the lizards. Lizards injected with 0.2ml of sterile saline showed identical body temperatures for the six hours following injection. When re-exposed to the gradient on the following day without further injection, both the controls and the experimental animals showed slightly higher body temperatures (about 37°C on the second day compared with about 36°C on the first day).

Our results indicate that C. cataphractus lizards do not develop fever in response to injections of rabbit leucocyte pyrogen nor to A. hydrophila, although both pyrogens produced fever in rabbits, and the bacterium is reported to be pathogenic in lizards (Kluger, 1978). In many respects our experiments are identical to the experiments performed by Kluger and his colleagues, and the lizards we used are very similar in mass (45 ± 3g) and habitat to the Dipsosaurus dorsalis most frequently used by Kluger. We now need to establish whether the ability to develop fever is confined to just one family of lizards, and if so, how the iguanid lizards differ from others.

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ROLE OF ARACHIDONIC ACID IN THE GENERATION OF FEVER.

Eva Kenedi, G. Norton, Helen Laburn, D. Mitchell and Olga Abrahams.

Department of Physiology, University of the Witwatersrand Medical School and Department of Chemical Pathology, University of the Witwatersrand, and South African Institute for Medical Research, Johannesburg, 2001.

In view of the role that fatty acids play as a constituent of cell membrane, in a cell to cell relation, in immunity, in vasodilatory reaction in thermoregulation, in fever, it is interesting that pyrogen fever in an essential fatty acid deficient diet was never investigated.

To investigate the relationship between fever response and nutritional status in animals, rabbits (4-5 weeks old) were subjected to a diet deficient in EFA and hyperthermic response to intravenous injection of human leucocyte pyrogen (HLP) and intraventricular injection of PGE_2 was studied. The data obtained indicate that fatty acid deficient rabbits have altered fever responses to intravenous injection of HLP, yet no changes were observed to intraventricular injection of PGE_2. The diagnosis of an essential fatty acid deficient state was made by observation of clinical signs combined with a determination of plasma and brain fatty acid content. Plasma unsaturated fatty acid (18:2) was significantly reduced, while 5, 8, 11 eicosatreonic acid (20:3ω9), the marker for EFA-deficient state, was found increased in the plasma. Brain fatty acid changes were observed in phospholipids of fatty acid deficient animals. In lecithine oleic acid (18:1) decreased, giving rise to 20:3ω9 while arachidonic acid (20:4 6) decreased. In phosphoethanolamine, palmitic (16:0) stearic (18:0) oleic (18:1) acids increased, and linoleic acid (18:2) decreased substantially ($P>0005$) but no changes were observed in the content of 20:3ω9 or 20:4ω6. Therefore we suggest the following hypothesis. Altered fever reponse can be due to changes in the plasma of unsaturated fatty acid resulting in reduced PGE_2 production, thus a delay in hypothalamic response resulting in reduced febrile response. In spite of the changes in brain fatty acid composition, each of the two phospholipid classes maintained its particular level of unsaturation during EFA deficiency. Thus response to PGE_2 was not impaired.

With thanks to Professor Mendelsohn for help with biochemical determination, to Upjohn for the provision of PGE_2, and to M.R.C. for financial support.

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Name and Address of 1st Author Mrs. Eva Kenedi, Department of Physiology, University of the Witwetersrand Medical School, York Road, Parktown, Johannesburg, 2001.

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ABSTRACTS

BIOMEDICAL ADVANCES IN AGING '88



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EFFECT OF DIETARY LINOLENATE ON THE PATHOGENESIS OF FEVER

E. Kenedi, D. Mendelsohn

Department of Chemical Pathology, School of Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg

The pathophysiological consequences of the reduction in the level of arachidonic acid 20:4 (n-6) by increases in linoleic 18:2 (n-6) and/or linolenic acid 18:3 (n-3) in a feverish condition induced by bacterial pyrogen are unkown, yet it is accepted that endotoxin fever is arachidonate dependent and it is also recognised that an increasing amount of linolate or linolenate reduces the formation of arachidonate irreversibly.

In order to investigate the effect of increased dietary linolenate on the generation of fever and on the plasma lecithin fatty acid composition in animals, 5 rabbits were fed on a generous diet supplemented with hay one week prior to and during the fever experiment. Their hyperthermic responses to intravenous injections of 2,0 ug/kg endotoxin S. Thyphosa were measured. Blood was collected from their ear arteries before they were fed their supplementary diet, after one week on the supplementary diet and thereafter at 60 and 180 minutes after the first and seventh endotoxin injections and was analysed on a gas chromatog raph. The blood analyses were compared with the lecithin fatty acid pattern of the first samples taken before they were fed their supplementary diet.

The results indicate that within one week increased dietary linolenate affects the interrelationship between polyunsaturates. The 20:4 (n-6) decreased and 22:5 (n-6) remained unchanged and the absence of 20:5 (n-20:5 (n-6) and 22:4 (n-6) was observed. Administration of a single dose of endotoxin 2,0 ug/kg led to a reduction in the 18:3 (n-3) and elevation in 20:4 (n-6) and 18:2 (n-6). The degree of this alteration directly influenced the generation fever. However, prolonged intake of linolenate, combined with chronic induction of fever, affected plasma unsaturation, thus influencing the fever response and the body temperature. Increasing 18:2 (n-6) and 18:3 (n-3) reduced the biosynthesis of 20:4 (n-6); therefore, arachidonate dependent fever response had been curtailed by the seventh injection.

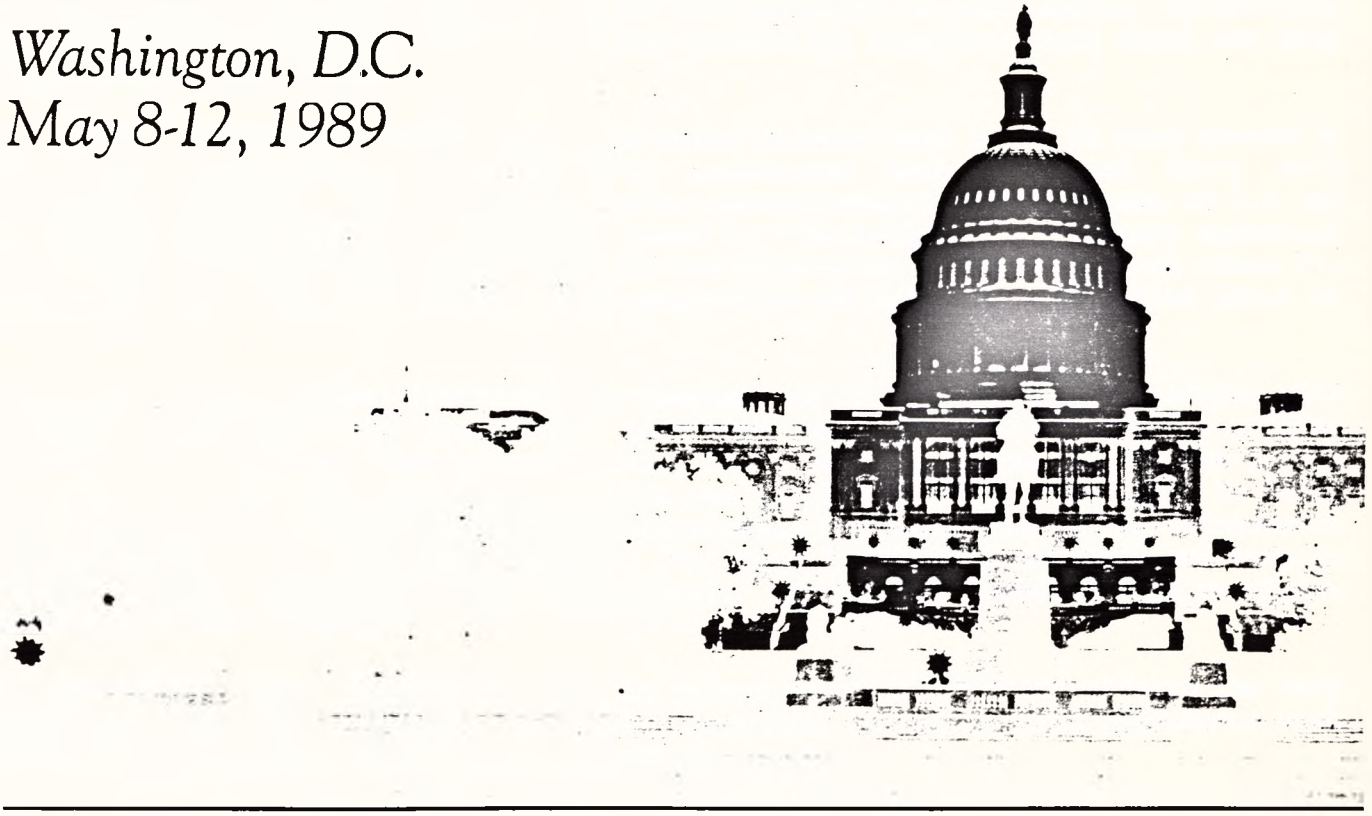
This suggests that deficiency in the prostaglandin E_2 precursor leads to no generation of fever but to tolerance to endotoxin, while an increase in the substrates indicates the peripheral involvement of 18:2 (n-6) and 18:3 (n-3) in increasing the hypothalamic "set point" to a higher level. This diminishes the effect of fever on the temperature.

Name and Address of 1st Author: Mrs Eva Kenedi, Department of Chemical Pathology, School of Pathology, University of the Witwatersrand, P.O. Box 3873, Johannesburg, 2000

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CHRONIC IMBALANCE IN EICOSANOIDS A COMMON FEATURE OF HYPERTHERMIA IN RABBITS.

Eva Kenedi, D.Mendelsohn

Department of Chemical Pathology, School of Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg

Although chronic mobilisation in eicosanoids has been observed in various diseases, the cause of this disorder has not been defined, nor has been associated with altered metabolic rate (respiratory quotient). Yet R.Q. serve as an indicator for the kind of combustion occuring in the body and also serve as the index of the body temperature. The consistency in the body temperature has been shown in our previous studies to be controlled by serum unsaturation. Shift in lecithin fatty acid unsaturation has been found to alter the formation of eicosanoids. Alterations in the latter modifies the biochemical tranducing signals around which the body temperature is regulated.

This study examines several cases in which rabbits were found to have unusually high body temperature (39,7-40,40°C) associated with essential fatty acid deficiency symptoms. This prompted us to investigate; their responses to 4,0 and 6,0 ug/kg (body mass) of endotoxin (Case 1), the influence of dietary essential fatty acid on fatty acid composition and its effects on body temperature (Case 2). Furthermore to analyse the fatty acid composition of hyperthermic rabbits prior to death (Case 3).

Results in Case 1 shows; the high body temperature of the host in conjunction with depressed eicosanoids in β-position prevented the rabbits to develop a fever response to increased dose of endotoxin, hence tolerance occured. In Case 2; the diet rich in 18:2(n6) and 18:3(n3) acids was unable to rectify the shift in fatty acid configuration, thus endogenous hyperthermia has been defended by increase in the pentaeonic acid 20:5(n6) content. In Case 3; the fall in oleic and rise in linoleic acids, suppressed the biosynthesis of each eicosanoids, the resultant hyperthermia led to death.

The definition that alteration in the formation of eicosanoids affects the body temperature and the response of fever provides a utilitarian hypothesis with research implications, as well as, a diagnostic tool.

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Name and Address of Presenting Author: Eva Kenedi Department of Chemical Pathology, University of Witwatersrand, P.O.Box. 3873 Johannesburg 2000. RSA.

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ABSTRACT FORM

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and Molecular Biology
The George Washington Univers.
School of Medicine
2300 Eye Street, N.W.
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SERUM EICOSONOIDS IN FEVER. HYPERTHERMIA AND TOLERANCE

Eva Kenedi
Department of Chemical Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg,

Previous studies from our laboratory have suggested that thermoregulation is closely related to the stability in linoleic acid, while fever is dependent on eicosonoids. Based upon this hypothesis the present work further examine the above findings.

5 rabbits (4-5 weeks old) fed on a standard diet (containing 50% linoleic acid) were subjected; i) single i.v. injection of increasing dose (2,0-6,0 ug/kg) of S.thyphosa (LPS), ii) systemic daily injections of 2,0 ug/kg LPS (till tolerance occurred). Blood collected from the ear artery before and during hyperthermic response, was analysed on gas chromatograph. The blood analyses were compared with the lecithin fatty acid pattern of the first sample taken before experiment has begun.

Results shows; increasing dose of endotoxin effect fever curve and provoke dose dependent rise in the proportion of lecithin 18:2(w6). The return to normal level in all fatty acids coincided with the termination of the fever. Chronic induction of fever however not only alter the pattern but increases the duration of the fever beyond 180 min. Rise in the fever plateau elevates the body temperature and the concentration of serum 18:2(w6), while diminishing the 18:3(w6)-> 20:3(w6)-> 20:4(w6) content. Arachidonate dependent fever thus has been curtailed.

This suggest that shift in lecithin configuration under the action of endotoxin, affects the genesis of fever, while rise in the substrata elevates the hypothalamic "set point" to higher level. This condition leads to hyperthermia and tolerance to pyrogen.

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Name and Address of Presenting Author: EVA KENEDI DEPARTMENT OF CHEMICAL PATHOLOGY
UNIVERSITY OF WITWATERSRAND AND THE S.A. INSTITUTE FOR MEDICAL RESEARCH

Area Code and Phone Number: P.O.BOX 3873 JOHANNESBURG 2000 R.S.A. 27-11-942 2704

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In the hyperthermia of heat stress, thermoregulatory processes attempt to lower body temperature. In fever, the body temperature is actively maintained at an elevated level. Procedures which set out to lower the temperatures of febrile patients by external cooling, such as sponging, are therefore physiologically inappropriate.

We thank the Medical Research Council for financial support.

The Pyrogenic Moeity of Endotoxin

E. Keneck, H. Laburn, D. Mitchell and F. P. Ross*
*Departments of Physiology and *Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg 2001.*

It is widely accepted that the pyrogenic action of the endotoxins results from their common lipid moeity, lipid A. Evidence for the pyrogenicity of lipid A derives largely from a single paper [Dey, Feldberg, Gupta and Wendlandt (1975). *J. Physiol.*, 253, 103 – 119]. In the experiments described in Dey *et al.*'s paper, lipid A was dissolved either in bovine serum albumin or in triethylamine. We have reported recently that bovine serum albumin itself is pyrogenic when injected into rabbits [Hattingh, Laburn and Mitchell (1979). *J. Physiol.*, 290, 76 – 77]. Is it possible that the putative pyrogenic action of lipid A actually arises from the vehicles in which it was dissolved?

We have retested the action of intravenous lipid A on the body temperatures of conscious restrained rabbits. Not only bovine serum albumin but also triethylamine, in the doses required to dissolve lipid A, is inherently pyrogenic in rabbits; the experiments of Dey *et al.* therefore provide no evidence that lipid A is pyrogenic. However, rabbit serum albumin is not pyrogenic in rabbits, and lipid A derived from the endotoxin of *Salmonella typhosa* and dissolved in rabbit serum albumin is markedly pyrogenic in rabbits. The polysaccharide residue of the endotoxin had no effect on the body temperature of the rabbits. Though earlier evidence on the pyrogenicity of lipid A is demonstrably inconclusive, lipid A indeed appears to be the pyrogenic moeity of endotoxin.

We thank the Medical Research Council for support.

Central Neurogenic Mediation of the Cerebrovascular Response to Inhaled CO_2

K. P. Klugman, G. Mitchell and C. Rosendorff
MRC/University Circulation Research Unit, Department of Physiology, University of the Witwatersrand Medical School, Johannesburg 2001.

The inhalation of carbon dioxide causes a cerebrovascular vasodilation and thus an increase in cerebral flood flow. Controversy exists as to the role of an intrinsic (metabolic) as opposed to extrinsic (neurogenic) control of this response. Evidence favouring a neural component in the vasodilatory response to CO_2 rests largely on two points. First, destruction of vasomotor nerves affects the response of cerebral vessels to CO_2. In this regard, brain stem lesions have been shown to decrease the vasodilatory response to CO_2 [e.g. Shalit *et al.* (1967). *Arch. Neurol.*, 17, 342 – 353]. Secondly, both β-adrenoceptor and cholinoceptor blockade will reduce the vasodilatory effect of CO_2 on cerebral blood vessels [e.g. Aoyagi *et al.* (1976). *Stroke*, 7, 291 – 295; Scremin *et al.* (1978), *Stroke*, 9, 160 – 165]. We have recently shown that both β-adrenoceptor and cholinoceptor blockade will abolish the vasodilator effects of stimulation of the intracerebral noradrenergic pathway (INP) (Klugman *et al.*, *Stroke*, in press). The INP is an ascending noradrenergic pathway that arises in the brain stem and passes through the hypothalamus to the cortex. We have thus investigated the role of the INP in mediating the vasodilation in hypothalamic resistance vessels due to inhaled CO_2.

Hypothalamic blod flow (HBF) was measured in conscious rabbits using a ^{133}Xe technique described previously [Cranston and Rosendorff (1971). *J. Physiol.*, 215, 577 – 590]. The inhalation of 2%, 4% or 8% CO_2 significantly increased HBF. β-adrenoceptor blockade with 70 nmol propranolol significantly attenuated the increase in HBF due to 4% CO_2, while phenoxybenzamine (150 nmol), an α-adrenoceptor blocking agent, was without effect on the vasodilation. Cholinoceptor blockade with atropine (1 nmol) similarly attenuated the vasodilator effect of 4% CO_2. Finally, chemical sympathectomy of the INP either at the level of the hypothalamus or brain stem, significantly reduced the increase in HBF due to CO_2. Chemical sympathectomy was carried out using 6-hydroxydopamine (1.2 μmol).

Our results are consistent with the idea that an adrenergic-cholinergic interaction in the intracerebral noradrenergic pathway may play a role in the cerebrovascular dilatory action of inhaled CO_2.

Effect of Morphine on the Quantitative Responses of Thalamic Neurones to Graded Noxious Stimulation in Rat

D. M. Ford, Linda Gelgor and D. Mitchell
Department of Physiology, University of the Witwatersrand Medical School, Johannesburg 2001.

Transmission of nociceptive information through the spinal cord and brain may be attenuated by various analgesic procedures including opiate administration. We have examined the effect of I.V. morphine (6 μmol/kg) on the responses of single ventrobasal thalamic neurones to graded noxious thermal stimulation of the tail in urethane-anaesthetized rats. The tail was immersed in a tube perfused with water, the temperature of which was controlled in the range of 40 – 50°C.

We recorded 24 single neurones which were excited when the temperature was raised above a threshold (about 43°C). These neurones were unaffected by innocuous mechanical manipulation of the tail, hind limbs and lower abdomen. Morphine suppressed the activity of 23 neurones. Four neurones responded only when the tail was heated to 47 or 48°C, though the maximum firing rate attained was identical to that recorded before morphine administration. The threshold temperatures of 19 neurones were raised above 50°C. The effect of morphine was rapid in onset on all neurones (within 20 s), but of variable duration (5 – 30 min). Morphine had no effect on neurones responsive to non-noxious stimuli.

We attempted to reverse the effects of morphine on 8 neurones by I.V. naloxone (3 μmol/kg). The responses of 6 neurones to noxious stimulation were restored within 2 min. In 3 neurones, the nociceptive response was greater than that observed prior to morphine.

Our results show that I.V. morphine (6 μmol/kg) raises the threshold of thalamic neurones to noxious thermal stimulation by 5°C or more. This effect is mediated by an action on opiate receptors, since it is reversed by naloxone.

We thank the MRC for support.

Effect of Substance P on Intracerebral Blood Flow in Conscious Rabbits

S. Markowitz, K. P. Klugman, G. Mitchell, C. Rosendorff and F. Lembeck*
*MRC/University Circulation Research Unit, Department of Physiology, University of the Witwatersrand Medical School, Johannesburg, South Africa, and *University Institute for Experimental and Clinical Pharmacology, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria.*

The undecapeptide, substance P, has been demonstrated to cause a vasodilation in numerous vascular beds [e.g. von Euler and Gaddum (1931), *J. Physiol.*, 72, 74 – 87]. Despite its high concentration in the brain, and the hypothalamus in particular, its effect on intracerebral blood flow is unknown. In addition, substance P has been postulated to interact with acetylcholine and noradrenaline, both of which we have demonstrated to affect intracerebral blood flow [Klugman, Mitchell and Rosendorff (1979). *Br. J. Pharmacol.*, 66, 217 – 221]. We have thus investigated the effect of exogenous substance P on hypothalamic blood flow (HBF) and the role of endogenous noradrenaline and acetylcholine in modulating the cerebrovascular effect of injected substance P.

HBF was measured in conscious rabbits, using a ^{133}Xe washout technique. Two weeks prior to experimentation, perspex headplates were fitted to the rabbits' skulls so that stereotaxic injection could be made at the time of experimentation. As blood flow in the hypothalamus on either side of the midline is identical, one side may be designated the control side and the other, the test side. Thus the microinjection (5 μl) of a substance having vasoactive properties may be detected as a change in flow on the test side relative to a control injection on the other side. While 3.3 pmol of substance P was without effect on HBF, 33 pmol or 330 pmol of substance P significantly ($P < 0.001$) increased HBF.

The role of a cholinergic mechanism in the substance P-induced vasodilation was tested by the local hypothalamic injection of the cholinoceptor antagonist atropine (1 nmol) and mecamylamine (25 nmol). Cholinoceptor blockade with either drug abolished the vasodilation due to substance P. Local injection of the adrenoceptor antagonist prepranolol (70 nmol) or the I.V. injection of 7 μmol phenoxybenzamine also abolished the vasodilation induced by substance P. In addition, chemical sympathectomy of the hypothalamus was carried out by injecting 1.2 μmol 6-hydroxydopamine into the hypothalamus in a series of rabbits, four days prior to experimentation; 330 pmol of substance P was without effect on HBF in these animals.

However, there were no significant differences in coronary perfusion responses to exercise between any of the groups.

The limitation of exercise tolerance often reported by patients on β-blockers is, therefore, probably due to a limitation of the cardiac output response to exercise, with an associated relatively high peripheral (but not coronary) resistance.

Anaesthetic Effects of Low Temperature

D. M. Ford*, L. Geigor*, G. Mitchell*, B. Isaacs†, and W. Walwyn†
*Departments of *Physiology and †Physiotherapy, University of the Witwatersrand, Johannesburg 2001.*

Therapeutic effects of low temperature may result from vasodilation, local anaesthesia or central analgesia. We have shown that significant vasodilation will not occur as a result of superficial cooling. We have therefore examined whether circumscribed superficial cooling produces a transient local anaesthesia or induces prolonged central analgesia.

In these experiments ischaemic pain was produced in rats by occluding blood supply to their tails, using a pressure cuff. Pain was taken to be present when the rats showed characteristic signs of distress. Anaesthesia/analgesia was assumed if test rats showed a significant increase in time to reaction compared to control rats.

The first experiments established a cooling/warming curve for rat tail temperature. The tails of the rats were cooled by placing the tails in a waterbath at 0°C. Tail temperature was measured using a thermocouple. The results indicated that a minimum temperature of 5.7 ± 0.3°C was obtained 10 minutes after submersion. Tail temperature was not significantly different from starting values (24.3 ± 2.7°C) 60 minutes after the tail was removed from the icebath. A second series of experiments revealed that anaesthesia/analgesia appeared between 10.5 and 11.3°C on cooling and disappeared between those temperatures on warming. As it took about six minutes for tails to reach 5.7°C from 11°C and about six minutes to reach 11°C on warming, anaesthesia/analgesia lasted for about 12 minutes. A third series of experiments attempted to distinguish local anaesthetic effects from central analgesic effects. In these experiments the tails were kept at minimum temperature for one minute, eight minutes, and 22 minutes on the assumption that prolonged exposure to low temperature might extend the duration of anaesthesia/analgesia. If pain was absent after tail temperature had returned to 11°C then analgesia and not local anaesthesia was present. In all three experiments pain reappeared as soon as tail temperature reached 11°C and pain was absent for 13.5, 19.5 and 37 minutes in each case. This period of anaesthesia was not different from predicted values of 13, 20 and 34 minutes.

We conclude that cooling inhibits nerve function to produce local anaesthesia and does not induce central anaesthesia.

Heat-stress in Rabbits: Responses to Circulating Endotoxin and Administration of Indomethacin

A. Blumsohn, N. Butkow and Helen Laburn
Department of Physiology, University of the Witwatersrand, Johannesburg 2001.

We have performed a series of experiments to test the hypothesis that endotoxin (LPS) absorption from the intestine is a major factor operating in the physiological response to heat stress.

Conscious rabbits were heated in a climatic chamber (41°C), until a rectal temperature of about 43°C was reached. The rabbits were then allowed to cool passively at ambient temperature (18 – 20°C). Using a Limulus Lysate assay, detectable serum levels of endotoxin were found in all of 12 heat-stressed rabbits. Endotoxaemia was prevented by the suppression of the gut flora by intestinal antibiotics. Despite the high titres of circulating endotoxin (0.02 – 500 ng/ml), rectal temperatures within three hours of cooling reached levels only slightly higher than preheat temperatures.

High doses of indomethacin (4 mg kg^{-1} h^{-1}) had no effect on the initial rate of cooling. However, after three hours of cooling, rectal temperature plateaued at levels significantly below pre-heat-stress temperatures ($P = 0.05$, t-test). This dose of indomethacin did not affect rectal temperatures of normothermic rabbits.

The lack of a sustained rise in rectal temperature despite the presence of circulating endotoxin, and the hypothermia which results from indomethacin administration, may suggest that there is an altered sensitivity to the pyretic effects of endotoxin and leucocyte pyrogen in hyperthermic rabbits.

Antipyretic Effects of Zomepirac

N. Butkow, Helen Laburn and D. Mitchell
Department of Physiology, University of the Witwatersrand, Johannesburg 2001.

Zomepirac (Zomax) is marketed commercially as a non-steroidal, non-narcotic drug with potent anti-inflammatory and analgesic properties [Pruss *et al.* (1980). *J. clin. Pharmacol.* 20, 216 – 222]. These actions have been attributed to inhibition of prostaglandin synthesis at the site of injury. As fever is thought to be mediated, at least in part, by synthesis of postaglandins in the hypothalamus [Hellon R.F. (1975). *Pharmacol. Rev.* 26, 289 – 321], we decided to investigate the antipyretic efficacy of zomepirac when injected peripherally and centrally into rabbits during fever.

Fever was induced by an intravenous infusion of leucocyte pyrogen synthesized from human buffy coat cells or by intravenous injection of 1 μg of the purified lipopolysaccharide of *Salmonella typhosa* endotoxin. Three hours after the start of leucocyte pyrogen infusion and during steady-state fever, zomepirac, or its solvent, was injected intravenously. Doses of 10 mg, 5 mg, 2 mg and 1 mg zomepirac caused a significant dose-related reduction of the leucocyte pyrogen fever. Endotoxin (1 μg) was injected simultaneously (I.V.) with a central injection of 100 μg zomepirac or vehicle into the lateral ventricles of rabbits. Again the endotoxin fever was significantly inhibited when zomepirac was injected compared to the fever response when the solvent was injected. Zomepirac injected peripherally or centrally into afebrile rabbits had no effect on rectal temperature.

We conclude that zomepirac is a potent antipyretic agent as well as being analgesic and anti-inflammatory. We believe its anti-pyretic potency would compare favourably with that of indomethacin. Moreover, because zomepirac lacks the adverse side effects of indomethacin, zomepirac may be of considerable value clinically.

We thank Ethnor for the kind gift of zomepirac and the CSIR for financial assistance.

Evidence Against an Essential Role for Arachidonic Acid in the Generation of Fever

Eva Kenedi, G. Norton, H. Laburn and D. Mitchell
Department of Physiology, University of the Witwatersrand, Johannesburg 2001.

Fever is thought to result from actions, in the hypothalamus, of derivatives of arachidonic acid, the predominant essential fatty acid found in animal tissues. Pyrogens are thought to stimulate, and antipyretic aspirin-like drugs inhibit, the metabolism of arachidonic acid [Laburn, Mitchell and Rosendorff (1977). *J. Physiol.* 267, 559 – 570]. We have investigated the possibility that rabbits fed a diet deficient in essential fatty acids show an altered fever response.

Young rabbits were placed on a three month diet in which 6.8% of the fatty acid content was unsaturated fatty acids in comparison with 46.2% in normal rabbit pellets. This diet was known from previous experiments to induce essential fatty acid deficiency in rats [Infante and Kinsella (1978). *Biochem. J.* 176, 631 – 634]. Rabbits of similar age, fed the normal rabbit diet, were used as controls.

The fatty acid deficient diet induced a significant fatty acid deficiency in the rabbits. The serum level of linoleic acid was significantly less than in control animals and there were substantial increases in palmitic and stearic acids. Moreover, the concentration of eicosatetraenoic acid, a fatty acid known to be a reliable marker of essential fatty acid deficiency [Farthing, Jarrett, Williams and Crawford (1980). *Lancet*, 1088 – 1089] was found to be elevated in the plasma of the rabbits fed the fatty acid deficient diet.

The fever responses of the control and deficient rabbits were tested by injecting intravenously 2 ml of a 1:10 dilution of leucocyte pyrogen synthesized from human buffy coat cells. There was no difference in the fever responses of control and fatty acid deficient rabbits. In addition the rabbits were tested for their reaction to 4 nmol (in μl distilled water) prostaglandin E_2 injected into the cerebral ventricles. Again, no difference in fever response was observed between normal and fatty acid deficient rabbits.

We suggest that arachidonic acid may not be essential for the generation of fever; alternatively, leucocyte pyrogen may act independently of arachidonic acid derivatives to induce fever. We await determinations of brain fatty acid concentration in the groups of rabbits to substantiate our conclusions.

We are grateful to Epol for assisting us in making up the fatty acid deficient diet; to Professor D. Mendelsohn, Dr O. Abrahams and Dr I. Fin-

nigan for help with the biochemical determinations; to Upjohn for the provision of PGE_2, and to the Medical Research Council for financial support.

Ballistic Technique for Analysis of Knee Stability

M. C. Siff and H. Isaacs
Communication Studies Division and Department of Physiology, Neuromuscular Research Laboratory, University of the Witwatersrand, Johannesburg 2001.

The knee joint is one of the most commonly injured structures of the body, particularly in sportsmen, among whom it accounts for approximately one quarter of all injuries. This susceptibility to injury is largely a consequence of stability relying on soft tissue structures which permit a large number of degrees of freedom ranging from flexion/extension and pure rotation to two distinct modes of gliding. A non-invasive technique which enables the physical characteristics of the musculo-tendonous structures of the knee to be determined relies on constraining the subject to oscillate freely in the low squat position on a force-plate attached to appropriate recording apparatus. The ensuing damped simple harmonic motion displays a specific resonant frequency and damping ratio for each subject.

This method was used to examine the knees of males and females of different ages and body masses. It was discovered that the mechanical stiffness of the knee tissues increases with increasing body mass, but that the damping ratio increases significantly only for subjects of body mass greater than approximately 75 kg. The stiffness of the knee tissues is of the same magnitude for males and females of the same body mass, but the damping ratio for the female knee is significantly greater than that of the male knee. This suggests that the female knee may be less susceptible to damage by impulsive longitudinal stresses produced, for instance, by running and jumping.

The observation that subjects with a history of musculo-tendonous injuries of the knee tend to exhibit both high knee stiffness and low damping ratio indicates that measurement of these parameters may be useful in establishing appropriate training routines for sportsmen or soldiers. In addition, this research confirms the potentially harmful nature of certain traditional exercises performed with the knees in the fully flexed position.

Proteins in Crevicular Fluid

E. R. Marcus, C. P. Jooste and J. Hattingh
Department of General Physiology, University of the Witwatersrand, Johannesburg 2001.

The amounts of fluid produced in the crevicular sulcus increases with inflammation and periodontal disease. The fluid is thought to be an inflammatory exudate or an extracellular transudate [Hattingh and Ho (1980). *J. periodont. Res.* **15**, 90 – 95]. The volume of crevicular fluid is very small and has a flow rate of 0.03 µl/min, making it difficult to examine its composition. The concentration of plasma proteins in crevicular fluid was found to be one-tenth their concentration in plasma by Brill and Bronnestam [(1960). *Acta Odont. Scand.* **18**, 95 – 100], whereas Bang and Cimasoni [(1971). *J. dent. Res.* **50**, 1683] reported them as being equal to their plasma concentrations.

We have devised an immunological method for examining the protein content of this fluid. The volume of fluid collected is measured on a Harco Electronics Periotron (No. 60032) digital fluid meter which is capable of detecting volumes as small as 0.005 µl. The crevicular fluid is collected on standard periodontal papers and these are placed in an electrical field on antibody-containing agarose gels. Proteins migrate forming precipitin peaks and specific proteins are then compared with known concentrations of standards and plasma. We found that the three proteins we examined in crevicular fluid were at similar relative concentrations to values reported for human plasma but at a lower total concentration.

Application of Catastrophe Theory in Analysing Joint Stability

M. C. Siff and H. Isaacs
Communication Studies Division and Department of Physiology, Neuromuscular Research Laboratory, University of the Witwatersrand, Johannesburg 2001.

Numerous physical injuries are a consequence of impaired joint stability. Understanding of traumatic injuries, which are usually associated with short-term impulsive forces applied to a joint, is more straightforward than an analysis of injuries related to the gradual degeneration of soft tissues in the region of a joint. The latter situation is frequently encountered in the case of the so-called 'over-use syndrome'.

Theories which consider over-use to be a cumulative continuous change from stability to injury do not adequately describe the abrupt, discontinuous onset of some over-use injuries. Catastrophe theory, invented by French mathematician R. Thom, in a broad sense concerns any situation involving a discontinuous transition that occurs when a system has more than one stable state, or can follow more than one stable pathway of change.

Catastrophe theory uses abstract multi-dimensional surfaces to describe sudden changes of state. It examines a behaviour surface whose characteristics are determined by one or more control factors or dimensions. In the case of joint mechanics the behaviour surface describes the state of stability of the joint and the control dimensions are features such as structural predisposition to injury and magnitude of physical loading. It emerges that the multi-factorial nature of the injury process can be usefully modelled by catastrophe theory, which can also be extended to offer a deeper understanding of other complex, discontinuous processes in physiological systems.

Wet Sheep and Histamine

J. Hattingh*, G. Mitchell† and M. F. Ganhao*.
**Department of General Physiology and †MRC Circulation Research Unit, University of the Witwatersrand, Johannesburg 2001.*

Carcasses from certain sheep originating in a variety of geographical areas in South Africa and South West Africa/Namibia show a wet, glistening appearance immediately after slaughter. They are called 'wet sheep'. Not all animals in a given batch are affected, the condition seems to be seasonal and both muscular and connective tissue from affected specimens is soft and watery to the touch.

Plasma and capsular interstitial fluid (C.I.F.) [Coetzee, Hattingh and Mitchell (1982). *Comp. biochem. Physiol.* **72A**, 173 – 178] were obtained from healthy sheep. Blood was taken from 'wet sheep' after death and subcutaneous connective tissue was centrifuged to obtain interstitial fluid (I.F.). Analysis of the fluids gave the following results.

Mean ± s.d.	Normal plasma	Normal C.I.F.	Wet plasma	Wet I.F.
n	20	18	22	15
Cl (mM)	107 ± 2.3	111 ± 2.3	116 ± 5.7	115 ± 11
Na (mM)	139 ± 9.4	133 ± 7.7	138 ± 3.3	128 ± 7
K (mM)	4.5 ± 0.5	4.2 ± 0.5	7.7 ± 0.8	5.1 ± 0.7
Osm. (mOsm/kg)	293 ± 18	277 ± 12	303 ± 8.2	290 ± 27
Protein (g/l)	78 ± 11	52 ± 12	83 ± 10.3	14 ± 3.7
C.O.P. mmHg)	29 ± 10	9.8 ± 1.1	30 ± 9.5	1.5 ± 0.1
A/G	1.15 ± 0.05	1.15 ± 0.14	1.09 ± 0.03	2.30 ± 0.6

The significant differences ($P < 0.05$) between total protein, colloid osmotic pressure and the A/G ratio in the interstitial fluid from 'wet sheep' may be ascribed to an increased capillary permeability [Guyton (1981) *Medical Physiology*. Saunders, London], and this may be the result of histamine or histamine-like action on capillaries caused by parasites, toxins, bacteria, and abnormal nutrients. The increased plasma K^+ in wet sheep may result from electrocution.

Insulin Secretion from Pancreatic Islets Prepared from Rats Reared on Low and High Protein Diets

A. Werner, F. Marcus, P. G. Wright and L. S. Levine
Department of General Physiology, University of the Witwatersran Johannesburg 2001.

We have compared the release of immunoreactive insulin (IRI) fro isolated pancreatic islets by rats reared from weaning to 14 weeks on a 4 or an 18% protein-containing diet. The islets were prepared by a slig modification of the method of Lacy and Kostianovsky [(1967). *Diabetes* 35 – 46] and incubated in a modified Krebs-Henseleit buffer maintained pH 7.4 with Hepes (20 mM). The incubations were carried out in sea glass vials and the reaction was terminated after 60 min by spinning do the islets and separating the supernatant, which was immediately frozen subsequent IRI assay.

Islets prepared from the rats fed the high protein diet released insulin response to a glucose challenge in a dose dependent manner. In contrast,

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PLASMA LIPID INVOLVEMENT IN THE PATHOGENESIS OF FEVER

E KENEDI, D MENDELSOHN

Department of Chemical Pathology, School of Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg

The biochemistry of fever is unknown. There are indications that fat metabolism might be involved. It is known that in stress situations e.g. starvation, the body obtains its energy requirements to a large extent from free fatty acids (FFA); in the rabbit subjected to trauma, cold stress or muscular inactivity, return to normal conditions is accompanied by the release of FFA mainly from the adipose tissue stores. At this stage the plasma level of phospholipid (PL) and triglycerides (TG) also increased. In heat stressed animals plasma linoleic acid (18:2(n-6)) increases and this was correlated with the ability of the animals to withstand the heat stress.

In order to investigate the relationship between plasma lipids and fever in animals, 4 rabbits (4-5 weeks old) were given a single i.v. injection of endotoxin S. thyphosa (LPS) and the following parameters measured before and during the hyperthermic response, (TG), (PL) and plasma lecithin fatty acid composition. Increasing the dose of endotoxin from 2 µg - 6 µg/kg caused an increase in the proportion of lecithin (18:2(n-6)) and a fall in the arachidonic acid (20:4(n-6)). In another series of experiments, daily injections of 2 µg/kg endotoxin for 6 weeks (the purpose of which was to determine whether tolerance to endotoxin could be produced) showed a distinct relationship between fever response and plasma levels of TG and PL, i.e. good fever response was accompanied by a rise of plasma TG and PL, and decreased fever response was accompanied by a fall in TG and PL. All animals became tolerant to endotoxin after about 3 weeks. During the fever response the lecithin 20:4(n-6) fell substantially, while 18:2(n-6) rose. This pattern remained constant throughout the course of the experiment.

The results indicate that endotoxin induced fever leads to elevated plasma TG and PL levels which are probably stress related. The alterations in the plasma lecithin fatty acid composition (↓20:4(n-6) and ↑ 18:2(n-6)) suggests that prostaglandins are being actively synthesized and these compounds might play an important role in the genesis of fever.

ROLE OF ARACHIDONIC ACID IN THE GENERATION OF FEVER

KENEDI E, MENDELSOHN D

Department of Chemical Pathology, School of Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg.

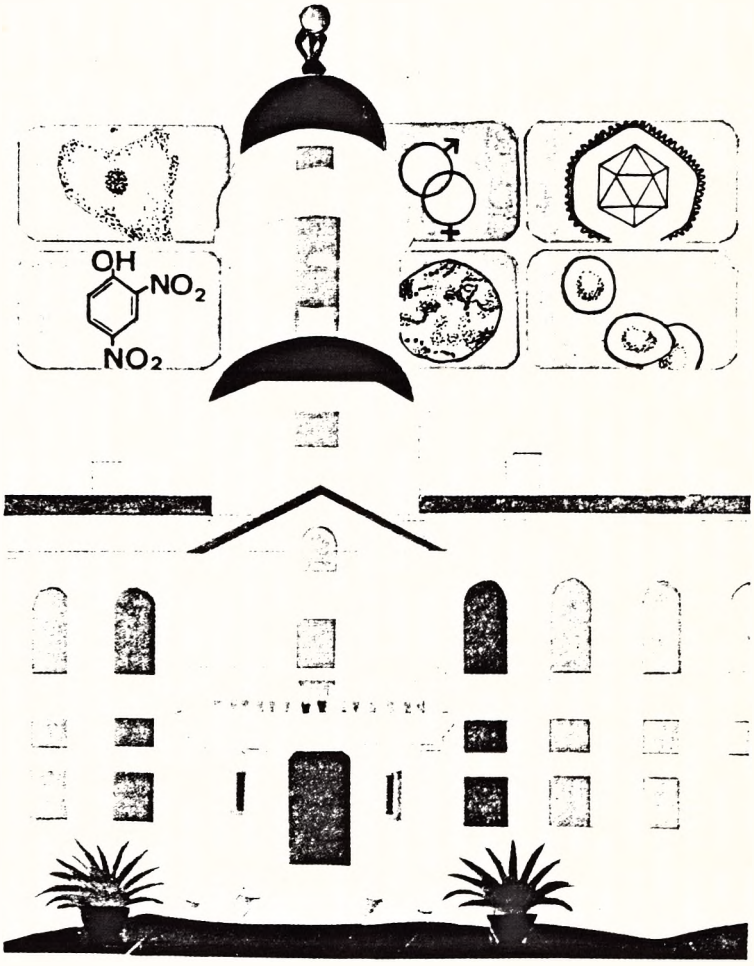
In order to study the possible role of polyunsaturated fatty acids (puf) in the genesis of fever, the relationship between fever response and nutritional status was investigated.

Rabbits (4 - 5 weeks old) were subjected to a diet deficient in essential fatty acids (EFA) and their hyperthermic response to an intravenous injection of human leucocyte pyrogen (HLP) as well as an intraventricular injection of prostaglandin E_2 (PGE_2) was measured. The diagnosis of EFA deficiency was made by observation of clinical signs combined with the determination of plasma and brain fatty acid changes. In the plasma both 18:2n6 and 20:4n6 were reduced, while 20:3n9 was increased giving an increased triene/tetraene ratio which is the chemical hallmark of EFA deficiency. Brain phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) fatty acid pattern showed similar changes, although the alterations in PE were not as marked as in PC.

The EFA deficient animals demonstrated a significantly reduced fever response to an intravenous injection of HLP compared to normal animals. The intraventricular injection of PGE_2 produced a similar fever response in both EFA deficient animals and controls. However, the intraventricular injection of PGE_2 did not correct the reduced fever response to HLP in the EFA deficient animals, nor did it alter the normal fever response in controls.

The data indicate that in EFA deficiency with a decreased production of prostaglandins, there is a reduced febrile response to HLP.

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IS CHRONIC IMBALANCE IN POLYUNSATURES A COMMON FEATURE OF HYPERTHERMIA IN RABBITS?

Eva Kenedi, D.Mendelsohn. Dept.of Chemical Pathology.School of Pathology, University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg

Although chronic mobilisation in polyunsaturates has been observed in various diseases, the cause of this disorder has not been elucidated nor associated with altered metabolic control such as respiratory quotient (R/Q/). Yet R//Q/ represents all metabolic events occuring within the body and also serves as the index of the body temperature. Consistency in the body temperature has been shown in our previous studies to be controlled by serum unsaturated fatty acids. A shift in lecithin fatty acid configuration has been found to alter the formation of polyunsaturates,and modify the biochemical transducing signals around which the body temperature is regulated

This study further examines the correlation between body temperature, serum lecithin fatty acid status,and febrile response to endotoxin in 15 rabbits showing unusually high body temperature (39.8-40.40°C) and EFA deficiency symptoms. Rabbits were divided into 3 groups(n=5) and subjected to; intravenous injection of endotoxin, fed on a hay supplemented diet, furthermore serum lipogram was assessed prior to death.

Results shows; high body temperature of the host depressed the formation of 18:3w6,20:3w6 fatty acids,this effected the response of the fever,hence tolerance occurred. The diet rich in EFA although corrected deficiency symptoms, was unable to abate the high body temperature , nor diminish the synthesis of 20:5w6. Both remains elevated while 18:3w 6 and 20:3w 6 acids remains suppressed. Serum lipogram prior to death shows altered lipid metabolism effected the balance between 18:1w9 and 18:2w6. The ensuing decay in the formation of polyunsaturates induced hyperthermia which led to death.

The data suggest,increased combustion in polyunsaturates became the pre-clinical signal by which hypothalamus readjust itself to higher metabolic level and consequently diminish the response of the fever.

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EFFECT OF DIETARY LINOLENATE ON THE PATHOGENESIS OF FEVER

E KENEDI, D MENDELSOHN

Department of Chemical Pathology, School of Pathology, University of the Witwatersrand and the South African Institute for Medical Research, Johannesburg.

The pathophysiological consequences of the reduction in the level of arachidonic acid 20:4(n-6) by increases in linoleic 18:2(n-6) and/or linolenic acid 18:3(n-3) in a feverish condition induced by bacterial pyrogen are unknown, yet it is accepted that endotoxin fever is arachidonate dependent and it is also recognised that an increasing amount of linoleate or linolenate reduces the formation of arachidonate irreversibly.

In order to investigate the effect of increased dietary linolenate on the generation of fever and on the plasma lecithin fatty acid composition in animals, 5 rabbits were fed on a generous diet supplemented with hay one week prior to and during the fever experiment. Their hypodermic responses to intravenous injections of 2,0 ug/kg endotoxin S. Thyphosa were measured. Blood was collected from their ear arteries before they were fed their supplementary diet, after one week on the supplementary diet and thereafter at 60 and 180 minutes after the first and seventh endotoxin injections and was analysed on a gas chromatograph. The blood analyses were compared with the lecithin fatty acid pattern on the first samples taken before they were fed their supplementary diet.

The results indicate that increased dietary linolenate affects the interrelationship between polyunsaturates. The level of 20:5(n-3) increased, while 20:4(n-6) lowered significantly followed by the absence in 22:4(n-6) and 22:5(n-6). Administration of a single dose of endotoxin 2,0 ug/kg led to a reduction in the 18:3(n-3) and elevation in 20:4(n-6) and 18:2(n-6). The degree of this alteration directly influenced the generation of fever. However, prolonged intake of linolenate, combined with chronic induction of fever, affected plasma unsaturation, thus influencing the fever response and the body temperature. Increasing 18:2(n-6) and 18:3(n-3) reduced the biosynthesis of 20:4(n-6); therefore, arachidonate dependent fever response had been curtailed by the seventh injection.

This suggests that deficiency in the prostaglandin E_2 precursor leads to no generation of fever but to tolerance to endotoxin, while an increase in the substrates indicates the peripheral involvement of 18:2(n-6) and 18:3(n-3) in increasing the hypothalamic "set point" to a higher level. This diminishes the effect of fever on the temperature.