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DEVELOPMENT OF THE FEBRILE RESPONSE TO PYROGENS IN THE FOETAL AND NEWBORN LAMB

by

QUENTIN J. PITTMAN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Development of the febrile response to pyrogens in the foetal and newborn lamb," submitted by Quentin J. Pittman in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. K.E. Cooper, Supervisor,
Division of Medical Physiology

Dr. W.L. Veale,
Division of Medical Physiology

Dr. G.R. Van Petten,
Division of Pharmacology and Therapeutics

Dr. N.E. Henderson,
Department of Biology

Dr. K. Lederis,
Division of Pharmacology and Therapeutics

Dr. R.D. Myers, External Examiner,
Laboratory of Neuropsychology,
Purdue University

June, 1976

ABSTRACT

During the last quarter of gestation, body temperature was measured in chronically-cannulated, unanesthetized sheep foetuses and was consistently found to be 0.3 - 0.8°C higher than the abdominal temperature of the ewe. When bacterial pyrogen was administered intravenously (iv) to the foetus, a fall in the number of circulating leucocytes was observed, but no change in foetal or maternal temperatures occurred. When bacterial pyrogen was given iv to the ewe, foetal temperature rose in parallel with the fever seen in the mother.

In newborn lambs less than 44-48 h age, fever did not usually develop after iv injection of endogenous pyrogen. Similarly, an initial iv injection of bacterial pyrogen in newborn lambs at either 4 h age or at 60 h age also failed to produce fever. However, lambs previously injected at 4 h age, and challenged a second time at 60 h age with bacterial pyrogen were able to develop a fever after the second injection. Thus, evidence would suggest that the febrile response to endogenous pyrogen develops over the first few days of life; superimposed on the development process is the requirement for a "sensitization" to bacterial pyrogen which may occur independently of the maturation of the response to endogenous pyrogen.

The "sensitization" process occurred between antigenically unrelated pyrogens, and fever could be produced at 60 h by bacterial pyrogen if the lambs had been infused with maternal or foetal plasma at birth. "Sensitization" could be induced by exposing the foetus

to iv pyrogen during the last few days of gestation but could not be demonstrated experimentally by exposure after birth to pyrogens administered into the gastrointestinal tract. Attempts to produce endogenous pyrogen, in vitro from neonatal or adult sheep leucocytes were generally unsuccessful, as were attempts to detect circulating pyrogen in febrile animals.

Newborn lambs did not respond with fever after microinjection of prostaglandin E_1 or E_2 into the hypothalamus or, in most instances, after intraventricular injection of these substances. The unresponsiveness to prostaglandins was shown to be independent of the ability to develop fever after iv pyrogen. Lambs responded to 5-hydroxytryptamine or noradrenaline injected intraventricularly with temperature changes similar to those seen in adult sheep. Thus, the central nervous system pathways involved in normal thermoregulation are functional at birth, but after "sensitization" the newborn lamb appears capable of developing fever without the central involvement of prostaglandins.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The body temperature of mammals remains relatively constant over a wide variety of environmental temperatures. For over 100 years it has been recognized that the thermal state of an animal is a result of a balance between the heat produced and that dissipated by the body (Wunderlich, 1871). An animal can achieve homeostasis of internal body temperature by regulating the amount of heat produced and the exchange of this heat with the environment. Heat is a by-product of the metabolism of the cells in the body, and differences in heat production are related to a number of factors including body size, age, sex, nutrition, endocrine activity and acclimatization. On a short term basis, shivering or nonshivering thermogenesis and voluntary muscular activity can greatly increase the amount of heat produced. Heat loss regulation can be brought about by behavioural means (e.g. choice of clothing, environment, change in posture), by alterations in skin blood flow and by variation in evaporative heat loss from respiration and sweating. In addition, the efficiency of heat exchange with the environment can be altered by varying the insulative value of subcutaneous fat and the thickness and composition of the fur.

A. The Role of the Hypothalamus in Temperature Regulation

1. Lesion studies

The idea that body temperature is a regulated function implies that there must be a regulating mechanism which can sense body temperature, compare it to a reference level and correct any deviations from the reference by appropriate behavioural or physiological action. Over the last half of the nineteenth century the concept arose that the brain was important in the control of body temperature (Bergmann, 1845; Tscheschichin,

1866; Richet, 1885; Ott, 1887). Subsequently, the importance of the hypothalamus was made apparent and it was demonstrated that thermoregulation was impaired after removal or isolation of portions of the hypothalamus (Isenschmid and Krehl, 1912; Bazett and Penfield, 1922; Keller and Hare, 1932; Bazett et al., 1933). The results of a number of lesion experiments led Meyer (1913) to propose that there are two brainstem centers for the regulation of body temperature, one responsible for heat loss and one for heat production. Indeed, the early evidence indicated that discrete lesions in the anterior hypothalamus appear to impair regulation against heat more so than against cold (Ranson and Ingram, 1935; Frazier et al., 1936; Ranson and Magoun, 1939; Clark et al., 1939). More recently, however, it has been shown that the loss of the anterior hypothalamic area (AH/POA) also impairs regulation against cold (Andersson et al., 1965; Squires and Jacobson, 1968; Carlisle, 1969; Satinoff and Rutstein, 1970). Lesions in the caudal hypothalamus also cause animals to become poikilothermic (Clark et al., 1939) and it is suggested that this area plays mainly a motor role in temperature regulation (Hardy, 1973; Satinoff, 1974).

Hypothalamic lesions also impair behavioural thermoregulation. In an experiment in which rats could bar-press for heat when the ambient temperature was below thermoneutrality, lesions of the anterior hypothalamic/preoptic area (AH/POA) impaired the accuracy of behavioural regulation (Lipton, 1968; Carlisle, 1969). That is, the fine control of body temperature appeared to have been lost. However, an increased rate of bar-pressing behaviour occurred, probably to compensate for the deficits in physiological regulation incurred from the lesions. Thus, as has been suggested by Bligh (1966a), destruction of the area responsible for fine,

or "narrow band" control still allows the animal a type of coarse "wide band" control which may reside in anatomical structures elsewhere than the AH/POA.

2. Electrical Stimulation

Thermoregulatory responses have been produced by electrical stimulation of the hypothalamus and related structures. In goats, stimulation of the preoptic area caused panting and cutaneous vasodilatation (Andersson et al., 1956). If these animals were shivering in the cold, when the stimulation began the shivering was promptly inhibited. Stimulation of this area and a number of other central nervous system (CNS) sites also inhibited shivering in cats (Hemingway et al., 1954). Andersson (1957) further found that, in goats, electrical stimulation of the septal areas caused shivering, vasoconstriction, pilo-erection and, in animals exposed to heat, an inhibition of polypneic panting. Hemingway (1963) observed that stimulation in both the septum and the dorsal medial posterior hypothalamus elicited shivering in cats.

3. Thermal Stimulation

While lesion and electrical stimulation experiments are important in localizing brain structures important in thermoregulatory control, they do not identify whether these structures are themselves thermosensitive. The existence of deep body thermosensitive structures is supported by experiments of Pickering (1932) which showed that occlusion of blood flow from a heated limb prevented vasodilatation elsewhere, and by Snell (1954), who infused warm saline into the bloodstream and found a linear relationship between degree of vasodilatation and the amount of heat infused.

The evidence for thermosensitive structures in the brain was obtained from experiments in which the carotid blood flow was heated or

cooled and thermoregulatory responses occurred (Kahn, 1904; Moorhouse, 1911; Hammouda, 1933; Newman and Wolstencroft, 1960). Downey et al. (1964) implanted water cooled cuffs around various blood vessels in rabbits. By measuring the heat extraction of the cuffs and by calculating heat production from oxygen consumption, they concluded that the rabbits produce significantly more heat when the internal carotids were cooled than when any other vessels were cooled. Thus they suggested that an important thermosensitive site lies within the distribution of the internal carotid artery. Similarly, when intravenous (iv) or intra-arterial infusions of warm isotonic saline were given to humans, the greatest alterations in finger heat elimination occurred after infusions into the internal carotid artery (Cooper, 1970).

Further support for a role for specific CNS areas in the sensing of body temperature comes from experiments in which thermodes were introduced into the brain in order to change the temperature of the surrounding tissue. It was found that heating in the area of the striatum and diencephalon caused body temperature to fall, whereas cooling activated heat production and conservation mechanisms so that body temperature rose (Barbour, 1921; Hashimoto, 1915; Prince and Hahn, 1918). Magoun et al. (1938) showed that heating of the medial hypothalamic nuclei was most effective in activating temperature loss, and Hemingway et al. (1940) further localized the thermosensitive area as the anterior hypothalamus. There is now considerable evidence to show that heating the anterior hypothalamus of a variety of species causes a fall in body temperature accompanied by panting, sweating and cutaneous vasodilatation (Beaton et al., 1941; Folkow et al., 1949; Strom, 1950; Ingram and Whittow, 1962; Ingram et al., 1963; Hammel et al., 1963; Proppe and Gale, 1970;

Phillips and Jennings, 1973; Calvert and Findlay, 1975). Similarly, cooling this area causes shivering, vasoconstriction, reduction in respiratory frequency and a rise in body temperature (Strom, 1950; Hensel and Kruger, 1958; Freeman and Davis, 1959; Andersen et al., 1962; Hammel et al., 1960, 1963; Jacobson and Squires, 1970; McEwen and Heath, 1974; Calvert and Findlay, 1975). Heating or cooling the anterior hypothalamus of goats (Andersson et al., 1962; Andersson, 1970), baboons (Proppe and Gale, 1970; Gale et al., 1970), and rats (Szczlepanska-Sadowska, 1974) causes changes in endocrine activity that complements the physiological mechanisms already discussed. Brück and Schwennicke (1971) were able to elicit nonshivering thermogenesis in guinea pigs by cooling the anterior hypothalamus.

Temperature changes in the anterior hypothalamus also influence behavioural temperature regulation. Satinoff (1964) and Carlisle (1966) cooled the hypothalamus of rats and found that the animals increased the frequency with which they bar-pressed for heat. Also, rats can respond for cool air when the hypothalamus is warmed (Corbit, 1970). Other species which have been found to alter thermoregulatory behaviour after hypothalamic temperature displacements are pigs (Baldwin and Ingram, 1967), opossums (Roberts et al., 1969), baboons (Gale, et al., 1970) and squirrel monkeys (Adair et al., 1970; Stitt et al., 1971).

Sites within the CNS other than the AH/POA have also been found to be thermosensitive. Temperature displacements of the medulla (Holmes et al., 1960; Lipton, 1971; Tabatabai, 1972; Chai and Lin, 1973; Lin and Chai, 1974) and the posterior hypothalamus (Adair, 1974) have been shown to be effective in activating both physiological and behavioural thermoregulatory responses. There is a considerable body of evidence to indicate that warming the cervical and thoracic regions of the spinal cord activates

heat loss mechanisms, whereas cooling produces heat conservation and production (reviewed by Thauer, 1970; Thauer and Simon, 1972; Bligh, 1973; Hensel et al., 1973). Klussman and Pierau (1972) have provided evidence that spinal motoneurons themselves may be thermosensitive. Temperature sensors may also exist outside of the CNS along major blood vessels (Blatteis, 1960; Bligh, 1961, 1963; Thompson and Barnes, 1970) and in the abdomen (Rawson and Quick, 1970, 1971; Adair, 1971; Riedel et al., 1973).

A major input for thermal sensation comes from cutaneous thermoreceptors. Heating and, in particular, cooling the skin can be shown to cause appropriate thermoregulatory responses in the absence of any observable changes in the temperature of the body core (reviewed by Benzinger, 1969; Hensel, 1970, 1973, 1974). Certain areas of the skin, for example, the face in man (Nadel et al., 1973; Stevens et al., 1974), and the scrotum in rams (Waites, 1962; Hales and Hutchinson, 1971) and pigs (Ingram and Legge, 1972), are particularly sensitive to ambient temperature changes. Using electrophysiological recording techniques, it has been possible to identify warm or cold fibers arising from the rat scrotum (Iggo, 1969; Hellon et al., 1975), and face of cats (Hensel and Wurster, 1970), and dogs (Iggo, 1969).

4. Single Unit Studies

The search for thermosensitive areas within the brain has led to studies in which the firing patterns of individual neurons were observed during hypothalamic or skin temperature displacements (reviewed by Hellon, 1970; Nakayama, 1972; Eisenman, 1972; Bligh, 1973). Neurons have been found within the AH/POA which respond to local heating (Nakayama et al., 1961) or cooling (Eisenmann and Jackson, 1967) and to changes in ambient

(Wit and Wang, 1968a; Hellon, 1970), midbrain (Nakayama and Hardy, 1969) and spinal cord temperatures (Guieu and Hardy, 1970). There is also evidence that posterior hypothalamic neurons can sense their own temperature (Edinger and Eisenmann, 1970; Wunnenberg and Hardy, 1972) and can also respond to changes in anterior hypothalamic and cutaneous temperatures (Nutik, 1973a, 1973b). Neurons which change their firing characteristics in response to local temperature changes have also been found in the sensorimotor cortex (Barker and Carpenter, 1970) and medulla (Lee and Chai, 1976), but it is uncertain what role, if any, these neurons play in thermoregulation.

The evidence from lesion, thermal stimulation and single unit studies would suggest that there is a complex inter-relationship between various CNS and peripheral thermosensitive areas. It would appear that the principal thermosensitive area of the brain is the AH/POA. Neurons from this, and other areas of the body, project to the posterior hypothalamus where the integration is completed and the effector signal is developed (Hardy, 1973; Satinoff, 1974). In the absence of the AH/POA it is possible that other thermosensitive areas (i.e. midbrain, posterior hypothalamus, spinal cord) can provide a subsidiary, or backup control over thermoregulatory function. Thus the evidence is not in favour of a specific "thermoregulation center" as such, but rather for an integration of various anatomically connected temperature sensors each with a varied input and control over thermoregulatory function. Nevertheless, it is in the anterior and posterior hypothalamus where, in the intact animal, the principal control of thermoregulatory function resides. The integration of afferent inputs and the development of an effector signal is accomplished through the release of transmitter agents within the hypothalamus, and considerable

effort has gone into elucidation of the role of these substances in thermoregulation.

5. Hypothalamic Neurotransmitters and Thermoregulation

Pharmacological studies in thermoregulation have been directed mainly at the role of acetylcholine (Ach) and the monoamines noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in the control of body temperature. These substances occur in the hypothalamus in relatively high concentrations (Vogt, 1954; Amin et al., 1954) and can be observed with the use of fluorescence histology in nerve terminals within the AH/POA (Carlsson et al., 1962; Dahlstrom and Fuxe, 1964; Anden et al., 1965). In 1963, Feldberg and Myers suggested that the hypothalamus exerts its control over body temperature through the balanced release of NA and 5-HT. This theory was based on the findings that injections of NA and adrenaline into the cerebral ventricles of unanesthetized cats caused falls in body temperature whereas 5-HT, similarly administered, elevated body temperature (Feldberg and Myers, 1963, 1964). These observations provided the impetus for studies on the role of these and other putative neurotransmitters in a variety of species (reviewed by Feldberg, 1970; Myers, 1970a, 1974; Bligh et al., 1971; Veale and Cooper, 1973; Hellon, 1975). It soon became apparent that the effects of these monoamines on body temperature could be elicited best by microinjections directly into the AH/POA (Cooper et al., 1965; Feldberg and Myers, 1965). Furthermore, the temperature response to the intracerebral injection of a particular substance varied among species (Veale and Cooper, 1973). For example, cats, dogs and monkeys respond to 5-HT with a temperature rise (Feldberg and Myers, 1965; Feldberg et al., 1966), whereas sheep, goats and rabbits show a fall in body temperature after intraventricular injection of this amine (Cooper et

al., 1965; Bligh et al., 1971). Microinjection of Ach (or the cholinomimetic carbachol) into the hypothalamus also has varying effects on body temperature depending on the species and the injection locus (Hulst and De Wied, 1967; Myers and Yaksh, 1969; Bligh et al., 1971; Rudy and Wolf, 1972). The role of these putative neurotransmitters in thermoregulation was clarified somewhat by the observation that the temperature response after intracerebral introduction of an amine is highly dependent upon the environmental temperature (Findlay and Thompson, 1968; Bligh et al., 1971).

Attempts have been made to correlate the effects of micro-iontophoresis of these amines on the firing rates of individual hypothalamic neurons with those seen after displacements of local temperature. With one exception (Hori and Nakayama, 1973), these studies have shown little correlation between the responses expected on the basis of microinjection studies and the thermosensitive properties of these neurons (Cunningham et al., 1967; Beckman and Eisenman, 1970; Jell, 1973, 1974). However, if the amines are transmitters in afferent or efferent pathways and the thermosensitive neurons are interneurons within the hypothalamus, such discrepancies are not necessarily evidence against a transmitter role for these substances in thermoregulation.

Evidence in favor of a physiological role for the amines in thermoregulation is provided by the demonstration of a differential release of these substances under varying ambient conditions (Myers and Sharpe, 1968). For example, Myers and Chinn (1973) perfused the hypothalamus of unanesthetized cats with concentric push-pull cannulae (Myers, 1970) and demonstrated increased release of NA into the perfusate when the animals were placed in the heat. These results are in agreement with the idea that NA acts within the AH/POA of cats to lower body temperature.

Further evidence for the involvement of endogenous monoamines in thermoregulation is provided by pharmacological interference with the release, action or inactivation of the endogenous transmitters. When reserpine was administered to rabbits, rectal temperature increased, and this effect was attributed to the known action of reserpine in releasing endogenous catecholamines (Cooper et al., 1967; Bannerjee et al., 1968; Cooper et al, 1976b). It is possible to affect body temperature by interfering with the action of endogenously released transmitters by intracerebral injection of specific blocking agents. Thus, Feldberg and Saxena (1971a) observed that intraventricular injection of the α-adrenergic blocking agent phenoxybenzamine into rabbits and rats causes hypothermia, whereas in cats it causes hyperthermia. Similarly, Preston (1973) showed that intraventricular injections of phenoxybenzamine and phentolamine impairs shivering in rabbits. Complementing these observations are the findings that intraventricular injection of imipramine and desipramine into cats and rabbits causes changes in body temperature, presumably through synaptic accumulation of endogenous catecholamines (Cranston et al., 1972).

The evidence that has accumulated from microinjection studies in the cat and monkey has provided the basis for an amine model of thermoregulation (Myers and Yaksh, 1969; Myers, 1974). According to this model, there is a cholinergic heat production pathway within the anterior and posterior hypothalamus which is activated by 5-HT within the AH/POA. The release of NA in this area inhibits the cholinergic heat production pathway but does not activate a heat-loss pathway which is thought to exist only within the posterior hypothalamus (Myers and Yaksh, 1969; Myers and Waller, 1973, 1975). However, there is evidence that the NA may also activate heat loss directly (Myers and Chinn, 1973; Cooper et al., 1976).

Another model (Bligh et al., 1971) based on studies in sheep, goats and rabbits also depicts a cholinergically mediated heat production pathway. Heat loss is under control of a serotonergic pathway and both heat loss and heat production pathways receive crossed inhibitory inputs which release NA. This model is based mainly on studies involving intraventricular injections of agonists and antagonists under different ambient temperatures, and awaits confirmation of microinjection and release studies.

6. Ions in the Hypothalamus

As previously discussed, the controlled release of amines within the anterior and posterior hypothalamus activates heat loss or heat production mechanisms to maintain a constant body temperature. Though the need to postulate a 'set-point' mechanism has been questioned (Hammel, 1972), it would appear that there is an optimum temperature about which body temperature is regulated. An anatomical and physiological basis for a set-point mechanism has been proposed by Myers and Veale (1970, 1971). They were able to alter body temperature in cats by perfusing the cerebral ventricles or the area of the posterior hypothalamus with solutions in which the sodium-calcium ratio was disturbed. Solutions with a high sodium-calcium ratio caused shivering and a rise in body temperature (Feldberg et al., 1970; Myers and Veale, 1970, 1971). Conversely, solutions in which the physiological ratio of sodium to calcium was altered to increase relatively the calcium concentration caused body temperature to fall. Consequently, Myers and Veale (1970) proposed that the ratio of sodium to calcium within the posterior hypothalamus determines the firing rate of cells concerned with body temperature and sets the temperature at a specific point. Since the original observations in cats, a number of other species including the monkey, rabbit, rat and golden hamster have been shown to respond in a

similar manner to shifts in the ionic ratio in the hypothalamus (reviewed by Myers, 1974a). Indeed, in monkeys it has been possible to reset the 'set-point' for extended periods of time by repeatedly perfusing the posterior hypothalamus with solutions containing an altered sodium-calcium ratio (Myers and Yaksh, 1971). Furthermore, these animals thermoregulated about this new reference level in response to body heating or cooling. Additional evidence in support of the ionic set-point theory came from the observations that the elevation in body temperature that occurred during fever was accompanied by an efflux of ^{45}Ca from the brain into the cerebral ventricles (Myers and Tytell, 1972). During the falling phase of the fever, $^{45}Ca^{++}$ was retained and ^{22}Na was detected in the ventricle.

These and other experiments (Myers, 1974b) provide evidence that the temperature changes produced by altering the Na^{+}/Ca^{++} ratio in the posterior hypothalamus may indeed have a physiological basis. The theory that the posterior hypothalamus contains an independent set-point mechanism represents the only biological basis for a reference level in a regulated system suggested to date.

B. Fever

Fever is a condition in which deep body temperature is elevated and is characteristic of disease in mammals. Wunderlich (1871) and Liebermeister (1875) appeared to have recognized that body temperature was regulated at an elevated level during fever and this observation was supported by Lefèvre (1911) and Barbour (1921). A large number of experimental observations confirm this concept. Febrile human subjects respond to an exercise-induced increase in body temperature in a similar manner to non-febrile subjects, but activation of heat loss occurs at a higher

body temperature (Fox and MacPherson, 1954; MacPherson, 1959; Grimby, 1962). When cats (Weiss et al., 1967), and dogs (Cabanac et al., 1970) were given the opportunity to regulate their environmental temperature, febrile animals selected a warmer ambient temperature than when they were non-febrile.

Though fever may be explained as a state in which temperature increases because warm sensors become less responsive and cold sensors more sensitive (Mitchell et al., 1970), most evidence suggests that fever is an actual resetting of the 'set-point' about which body temperature is regulated (Cooper, 1972a). For example, Cooper et al. (1964a) infused warm saline into febrile humans, and found that the heat load evoked the same amount of cutaneous vasodilatation as it did in the non-febrile subject. Hockaday et al. (1962) observed that hypothermic subjects which regulated at a low body temperature responded with the same temperature increase to intravenous pyrogen as did normal subjects. When Cabanac and Massonet (1974) had humans evaluate the thermal comfort of a temperature-controlled glove in both the febrile and non-febrile states, the results were consistent with the concept of a change in set-point during fever.

Further support for this concept came from the experiments of Belyavsky (1965), who heated the hypothalamus of rabbits by diathermy, and observed that the responses to hypothalamic heating were diminished during the rising phase of the fever. In a similar type of experiment, Andersen et al. (1961) were able to modify the febrile response to intravenous pyrogen by hypothalamic heating and cooling in the unanesthetized dog.

The shift in set-point to a higher temperature is brought about by increased heat production and conservation and decreased heat loss (Palmes and Park, 1965; Atkins and Bodel, 1972). It is of interest that the

temperature increase after a standard dose of pyrogen is the same over a broad range of environmental temperatures (Grant, 1949; Thompson et al., 1959; Kerpal-Fronius et al., 1966; Cooper, 1972a; Cooper and Veale, 1974). The mechanism employed to elevate temperature, however, may change under different ambient conditions (Atkins and Bodel, 1972). In a cold environment, fever may be brought about by increased thermogenesis (e.g. shivering and increased metabolism), whereas in a hot environment an inhibition of heat loss may suffice to raise body temperature an equal amount. Animals may utilize different ways to elevate temperature even when the environmental temperature does not vary. For example, Wells and Rall (1948) injected pyrogens into curarized dogs, and found that they developed fevers of the same intensity as did animals which were not curarized, in spite of a lack of shivering. The curarized animals elevated their temperatures largely by limitations in heat loss. Also, cold-acclimated guinea pigs were found to exchange shivering for non-shivering thermogenesis during fever (Blatteis, 1976a).

1. Bacterial Pyrogens

Fever may occur from a variety of agents including fungi, viruses, steroids, non-microbial antigens and bacteria. It is recognized that the pyrogenic action of bacteria is derived from bacterial by-products (Seibert, 1925) known as endotoxins or bacterial pyrogens. Much of the experimental work on fever, including that to be presented in this thesis, has dealt with fevers caused by experimental introduction of these pyrogens into the body. In brief, bacterial pyrogens are large, lipopolysaccharide molecules (molecular weight of about 10^6) which are derived from the cell walls of Gram-negative bacteria (Bennett and Beeson, 1970; Luderitz et al., 1966; Nowotny, 1969). The lipopolysaccharide molecule is composed of three

regions: 1) a core region containing sugars, 2) an O-specific side chain containing immunological specificities, and 3) a lipid A region (Work, 1971). The pyrogenic part of the molecule is the lipid A, but the presence of the KDO sugar (2-keto-3-deoxyoctonic acid) is necessary for full activity. Further physical and chemical properties of bacterial pyrogens have been well reviewed (Bennett and Beeson, 1950; Atkins, 1960; Ribi et al., 1964). It is of interest that bacterial pyrogens are relatively resistant to heat, requiring a temperature of about 150°C for 2 h to destroy their activity. Thus, normal autoclaving for sterilization will not destroy these molecules.

Pyrogens have a multitude of effects on the body. They have been shown to affect cell structure, change enzyme levels, modify the metabolism of carbohydrates, fats and protein, increase or decrease resistance to bacterial and viral infections, raise or lower body temperature, cause hemorrhage, increase blood coagulation, modify hemodynamics, cause shock, affect gastric secretion and motility, destroy tumours and modify endocrine function (Thomas, 1954; Bennett and Beeson, 1950; Bennett, 1964). Fever is one of the most sensitive indices of endotoxin action, with dosages of as little as 0.0001 µg/kg producing detectable temperature increases in rabbits (Landy and Johnson, 1955; van Miert and Frens, 1968). The production of fever by bacterial pyrogen in mammals appears to involve a sequence of steps, as outlined in Fig. 1. Bacterial pyrogens, released into the body, interact with leucocytes and other cells of the reticuloendothelial system and cause them to synthesize and release an endogenous pyrogen. This endogenous pyrogen, which is often called leucocyte pyrogen if the leucocyte has been involved, makes its way to the central nervous system and acts within the AH/POA to produce fever. Its action there is believed

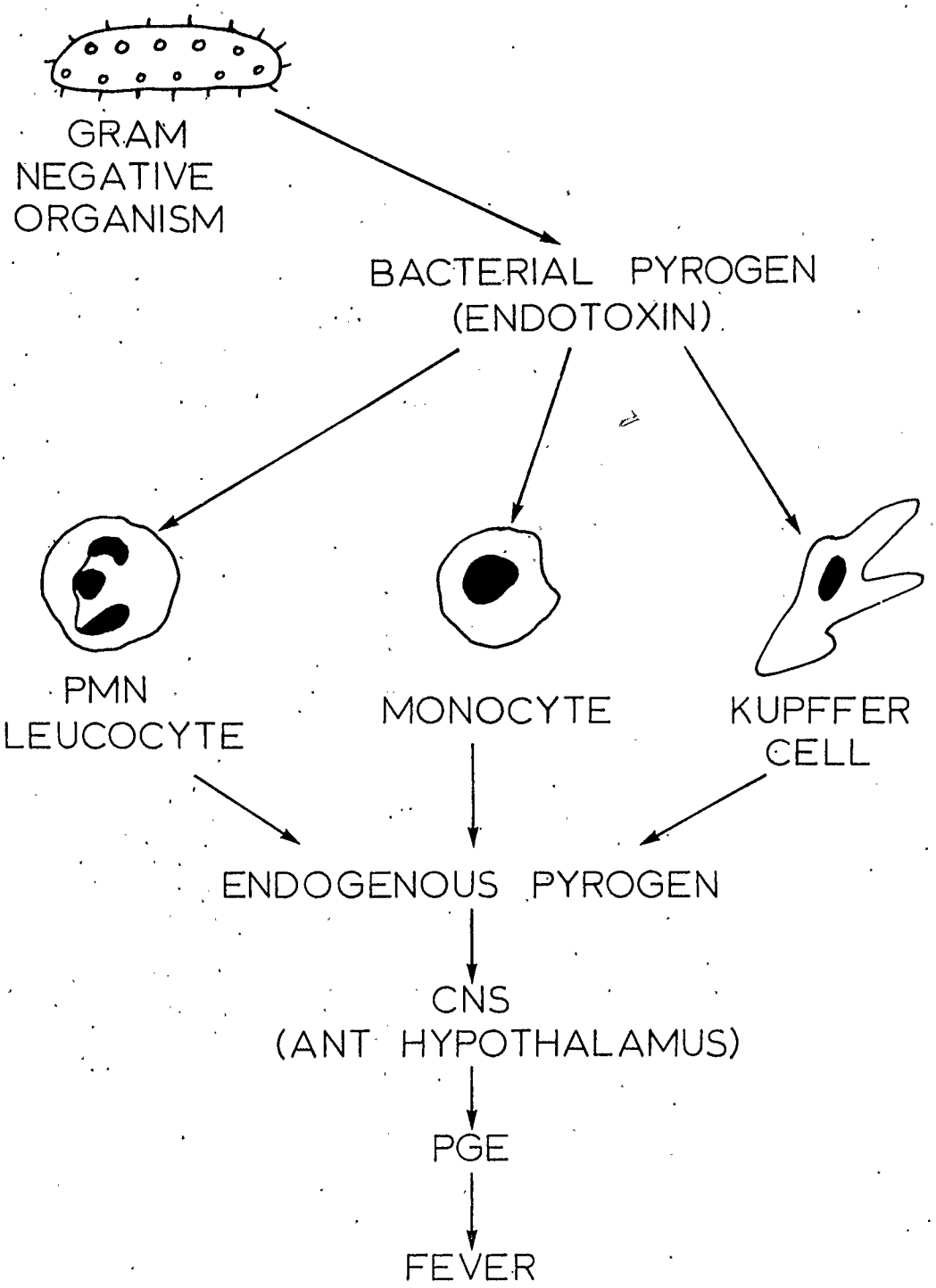

Figure 1. Schema of the current concept of the mechanism of action of bacterial pyrogens in producing fever in mammals.

to be mediated through the synthesis and release of prostaglandins of the E series, which activate heat production and conservation mechanisms to elevate body temperature. This model of fever is based on considerable experimental evidence.

When labelled endotoxin is administered iv to experimental animals, the radioactivity-tagged pyrogen is detected in liver, lung, spleen, bone marrow, leucocytes, monocytes and platelets, but not in the brain (Rowley et al., 1956; Braude et al., 1958; Herring et al., 1963; Cooper and Cranston, 1963; Herion et al., 1964; Brunning et al., 1964). Clearance from the circulation is rapid and is thought to be associated with transport of the lipopolysaccharide by the white cells to the reticuloendothelial system. This is reflected in a drop in the number of circulating leucocytes (Sundelin, 1939; Eichenberger et al., 1955; Herion et al., 1960; Atkins and Snell, 1964; Schofield et al., 1968).

There is considerable evidence to suggest that bacterial pyrogen exerts its febrile action through the release of an endogenous mediator:

1. A 20-60 min latency is observed between iv bacterial pyrogen injection and the onset of fever (Atkins, 1960).

2. Incubation of leucocytes and other body tissue with small amounts of bacterial pyrogen causes the production of a new pyrogenic factor which is characterized by a much shorter latency of action and different physical properties than bacterial pyrogen (Gerbrandy et al., 1954; Atkins and Snell, 1964).

3. Febrile responses to endogenous pyrogen occur most quickly following infusion into the carotid artery, whereas the route of administration of endotoxin does not affect its latency of action (King and Wood,

1958). This would suggest that endogenous pyrogen acts directly on the brain, but that the action of bacterial pyrogen is indirect.

4. When plasma from febrile animals is infused into other animals, a short latency fever occurs in the recipient animal (Grant and Whalen, 1953; Atkins and Wood, 1955; Petersdorf and Bennett, 1957).

2. Endogenous Pyrogens

Bennett (1948) and Bennett and Beeson (1953) demonstrated that polymorphonuclear leucocytes contained a fever-inducing substance. Subsequently, evidence was obtained that the interaction of blood with bacterial pyrogen produces a pyrogen with a short latency of action (Grant and Whalen, 1953; Gerbrandy et al., 1954). Though the early evidence suggested that endogenous pyrogen could only be produced by leucocytes (hence its alternate description, leucocyte pyrogen) there is now considerable evidence to suggest that a variety of body tissues are capable of elaborating this substance. Atkins and Snell (1964) demonstrated the production of endogenous pyrogen from heart, lung, spleen, kidney and skeletal muscle and, in addition, monocytes (Bodel and Atkins, 1967), peritoneal macrophages (Hahn et al., 1967) and Kupffer cells of the liver (Dinarello et al., 1968) are excellent sources of endogenous pyrogen.

The chemical composition of endogenous pyrogen has been studied, but the elucidation of structure has been hampered because of problems in isolating a homogenous substance (Cooper et al., 1960; Rafter et al., 1966; Kozak et al., 1968; Murphy et al., 1971, 1974). It would appear that the molecule is a lipoprotein with a reported molecular weight of 10-20,000. There is some evidence that different tissues may produce pyrogens of different molecular weights (Dinarello et al., 1974), with pyrogen obtained from monocyte preparations having a molecular weight of

38,000. Chemically, endogenous pyrogen can be differentiated from endotoxins on the basis of its molecular weight, heat lability and susceptibility to peptide-cleavage. Following intravenous injection, the latency of response to endogenous pyrogen is generally shorter than after bacterial pyrogen. Bacterial pyrogen injections also produce tolerance, whereas febrile responses to endogenous pyrogen do not decline over subsequent injections (Murphy et al., 1967; Atkins and Bodel, 1974).

A number of studies have been carried out in which the requirements for synthesis and release of endogenous pyrogen have been reported (Fessler et al., 1961; Kaiser and Wood, 1962a; Berlin and Wood, 1964; Bodel et al., 1968; Hahn et al., 1970; Moore et al., 1970; Nordlund et al., 1970). The majority of these studies have been carried out using leucocytes as the pyrogen source, and Atkins and Bodel (1971) have discussed the probable cellular events leading to the production of leucocyte pyrogen. Step 1, or cell activation, requires phagocytosis or attachment of a stimulating agent (micro-organism or endotoxin molecule) to the leucocyte in the presence of serum or plasma. This step is associated with increased glycolysis and activation of the hexose monophosphate pathway. Step 2 involves the production of new RNA and protein, but no new pyrogen is produced. Step 3, the late period of pyrogen production, is characterized by the presence of pyrogenically active material within the cell, and requires intact cell structure. The final step involves the release of the newly-formed pyrogen. It must be cautioned that this sequence has been identified from an in vitro system and it is uncertain how closely it resembles what occurs in the body after pyrogen injection.

3. Tolerance

Previous exposure to endotoxins can lead to a tolerance to their febrile actions (reviewed by Bennett and Beeson, 1950). Tolerance can take two forms. One type occurs about 18-48 h after injection of a large dose of endotoxin (Atkins and Snell, 1964) and is believed to result from a refractoriness on the part of the cells of the body to produce endogenous pyrogen (Snell and Atkins, 1967). A second type of tolerance occurs after animals are given daily injections of endotoxin and is characterized by a progressively diminished febrile response (Beeson, 1946). This state of tolerance has been shown to be transferable in serum (Greisman and Hornick, 1975), may be associated with humoral antibodies (Ritts et al., 1964; Kim and Watson, 1965; Greisman et al., 1975) and is characterized by a rapid clearance of injected endotoxin from the blood (Beeson, 1947; Herring et al., 1963; Cooper and Cranston, 1963) mainly by the liver (Carey et al., 1958). Dinarello et al. (1968) have provided evidence that this stage is associated with increased uptake of the endotoxin into Kupffer cells, coupled with a reduction in the release of endogenous pyrogen by these cells.

4. The Role of the Hypothalamus in Fever

There is considerable evidence that the rise in body temperature that occurs in response to iv pyrogen is mediated within the brain (Cooper, 1965, 1972b). In dogs and cats (Chambers et al., 1949), and humans (Cooper et al., 1964b) with cervical spinal cord transections, injection of pyrogen fails to cause fever, but shivering may occur in muscles innervated from above the lesion. Secondly, fever occurs after a shorter latency when infusions of endogenous pyrogen are given into

the carotid artery than into other vessels (King and Wood, 1958). Finally, injections of both bacterial pyrogen and endogenous pyrogen in amounts that are ineffective if administered into the circulation cause fever if injected into the cerebral ventricles (Bennett et al., 1957; du Buy, 1966; Adler and Joy, 1965; Sheth and Borison, 1960).

Though the lesion studies of Bard and co-workers (Bard and Woods 1962; Bard et al., 1970) do suggest that the integrity of the hypothalamus is important for the genesis of fever, a number of studies would indicate that it is not necessary to have an intact hypothalamus for the development of fever. There is evidence that cats (Ranson et al., 1939; Chambers et al., 1949), and rabbits (Veale and Cooper, 1975; Cooper et al., 1976c)with large anterior hypothalamic lesions have the ability to develop fever even though normal thermoregulation is impaired.

Microinjection of either bacterial or endogenous pyrogen into discrete brain loci indicate that the anterior hypothalamus is the area most responsive to the febrile action of both types of pyrogens (Villablanca and Myers, 1965; Myers et al., 1974; Cooper et al., 1967; Repin and Kratskin, 1967; Jackson, 1967; Lipton et al., 1973). Some additional evidence suggests that there may also be pyrogen sensitive sites in the midbrain (Rosendorff and Mooney, 1971). It is uncertain as to whether the action of endotoxin following administration directly into the brain is an effect of the endotoxin itself on the pyrogen-sensitive sites, or occurs through the mediation of an endogenous pyrogen. There is some evidence for the latter suggestion, since endotoxin directly applied to the AH/POA causes fever after a longer latency than is observed after similar injections of endogenous pyrogen (Cooper et al.,

1967). Histological examination of the brains of rabbits used in these studies revealed leucocyte infiltration of the injection site after bacterial pyrogen, suggesting that these cells could have released endogenous pyrogen. It is of interest that tolerance does not develop to endotoxin applied directly to the brain (Myers et al., 1974).

5. Mode of Action of Pyrogens in the Brain

a) Single Unit Studies:

Studies on the activities of neurons in the AH/POA after pyrogen administration suggest that pyrogens modify neuronal activity. Following administration of bacterial pyrogen iv, warm sensitive neurons showed a reduction in activity while cold-sensitive types showed an activation, and no effect was seen on thermally insensitive neurons (Wit and Wang, 1968; Cabanac et al., 1968; Eisenman, 1969; Nakayama and Hori, 1973). Acetylsalicylate, an antipyretic, appeared to reverse the pyrogen effects on firing rates. Similar effects were seen in urethane-anesthetized cats in which leucocyte pyrogen and sodium-salicylate were microinjected directly into the AH/POA (Schoener and Wang, 1974). It is of interest that Cabanac et al. (1968) also recorded from temperature-sensitive neurons in the midbrain which responded in a similar manner after iv pyrogen. Thus, it may be that the fevers resulting from microinjection of endogenous pyrogen into this area of the brain (Rosendorff and Mooney, 1971) may occur as a result of an action of pyrogen on this population of neurons.

b) Ions and Fever:

If fever is indeed a change in set-point (Cooper, 1972a) and the set-point mechanism is based on the sodium-calcium ratio of the poster-

ior hypothalamus (Myers and Veale, 1970), it should be possible to show an effect of pyrogen in this area of the brain. Confirmatory evidence in this respect is that radio-labelled endogenous pyrogen given iv can be detected by autoradiography in the posterior hypothalamus (Allen, 1965). Furthermore, posterior hypothalamic lesions abolish fever (Cooper and Veale, 1974), suggesting that the efferent pathways involved in elevating body temperature course through the posterior hypothalamus.

A possible effect of pyrogen on the Na^{+} /Ca^{++} ratio in the posterior hypothalamus has been demonstrated by Myers and co-workers. Myers and Tytell (1972) labelled the tissue and fluid of the brain of cats with $^{45}Ca^{++}$ or $^{22}Na^{+}$. During the rising phase of a pyrogen-induced fever, the $^{45}Ca^{++}$ efflux into the third ventricle increased, whereas during defervescence, increased amounts of $^{22}Na^{+}$ were detected in the CSF and $^{45}Ca^{++}$ was retained in the tissue (Myers, 1974). These results are interpreted as supporting the theory that the pyrogen-induced change in set-point involves an alteration in the ratio of Na^{+} to Ca^{++} in the posterior hypothalamus. It is difficult to reconcile the idea of a posterior hypothalamic site of action of pyrogen when its febrile action, as shown by microinjection experiments, appears to reside in the AH/POA. However, it may be that pyrogen exerts a dual action on the CNS involving a disturbance in both the anterior and posterior hypothalamus (Myers, 1970a).

6. The Role of Prostaglandins in Fever

When endogenous pyrogen was injected into the AH/POA of rabbits fever occurred, but a latency averaging 7.8 min was observed before the onset of fever (Cooper et al., 1967). These observations suggested

that the action of endogenous pyrogen within the AH/POA involved an intermediary step. There is now considerable evidence to indicate that prostaglandins of the E series are mediators, within the AH/POA, of the fever response to pyrogen. This evidence has been summarized and discussed in several comprehensive reviews (Feldberg and Milton, 1973; Veale and Cooper, 1974; Veale et al., 1976). There are three lines of evidence that probably provide most weight for the theory of prostaglandin involvement in fever. The first stems from the original observation of Milton and Wendlandt (1970) that microinjection of prostaglandin E_1 (PGE_1) into the third ventricle of unanesthetized cats causes a prompt fever after a very short latency. Prostaglandins have since been injected into the CNS of a number of animals and their pyrogenic actions confirmed in many species. Secondly, antipyretics which lower pyrogen fever interfere with the synthesis of prostaglandins (Vane, 1971). Furthermore, antipyretics lower pyrogen fever, but have no effect on a fever caused by direct application of prostaglandin to the brain (Milton and Wendlandt, 1971). Thus, the long period of controversy and uncertainty with respect to the mechanism of action of antipyretics in lowering fever appears to have been resolved. Thirdly, prostaglandin levels in the cerebrospinal fluid rise during a pyrogen-induced fever, and are reduced again when fever is lowered by administration of an antipyretic (Feldberg and Gupta, 1973). A survey of these and other data lead to the conclusion that the prostaglandin theory for the mediation of pyrogen-fever is an attractive one. However, some evidence has been brought forth for a component of fever not involving the central action of prostaglandins.

It is generally accepted that the AH/POA is the only area of the brain into which localized injections of prostaglandins cause fever. Indeed, in rabbits in which large lesions were made in the AH/POA, injections of prostaglandins either into the lateral cerebral ventricle or directly into the area of the lesion were without effect on body temperature (Veale and Cooper, 1975; Cooper et al., 1976c). Nevertheless, iv injections of endogenous pyrogen caused fever which was slower in onset and took longer to reach maximum but was of approximately the same magnitude as that observed before the lesions. Thus, there would appear to be a region of the brain sensitive to the hyperthermic action of pyrogens where fever is produced without the involvement of prostaglandins.

The effects of pyrogens on the firing rates of neurons in the AH/POA were previously discussed. If pyrogens do exert their action through the mediation of prostaglandins, it should be possible to obtain similar results from direct application of prostaglandins to the AH/POA. However, in an extensive study on rabbits (Stitt and Hardy, 1975), in which 138 AH/POA units were studied following microiontrophoresis of PGE_1, less than 9.0% of the total population tested showed any effect due to the prostaglandin. These effects differ from those observed by other investigators following pyrogen administration and would appear to dissociate the actions of pyrogens and prostaglandins.

Finally, Cranston et al. (1975) were able to administer salicylate to rabbits in a dosage which lowered the prostaglandin level in the cerebral spinal fluid but did not lower fever. In addition, they injected a prostaglandin antagonist, SC 19220, into the lateral ven-

tricle, which blocked the pyrexia due to PGE_2 but failed to block a pyrexia of similar magnitude caused by endogenous pyrogen (Cranston et al., 1976). It would appear, therefore, that the role of prostaglandins in fever may again be open to question.

C. Foetal and Neonatal Physiology

1. The Environment of the Foetal Lamb

The foetus lives in an environment protected from the extremes of the external world (e.g. temperature) but totally dependent on the mother for provision of nutrients and oxygen supply. Within the uterus the foetus rests in the amniotic fluid, which is initially secreted by the mother and has a composition similar to plasma, but is eventually composed mainly of foetal urine (Alexander et al., 1958).

Foetal and maternal tissues lie in contact with each other at the placenta, and nutrients and oxygen must pass through it to reach the foetus. The placenta is a tissue with high metabolic and enzymatic activity (Juchau and Dyer, 1972). In the sheep, it is formed of cotyledons (30-80 in number) which are dispersed over a wide area of the uterus (Dawes, 1968). The histological classification of placentas is based on the number of layers of tissues separating foetal and maternal blood, and considerable variation is seen among the mammalian species. The sheep placenta has been classified for years as a syndesmochorial type placenta (Amoroso, 1961), but more recent evidence would suggest it should be classified as epitheliochorial (Wynn, 1968). This classification indicates that the foetal and maternal circulations are separated by three layers of maternal tissue, namely endothelium, connective tissue and epithelium, and three layers of foetal tissue - trophoblast, connec-

tive tissue and endothelium. In the sheep placenta, blood on each side of these tissue layers appears to flow in a manner which is intermediate between concurrent and countercurrent flow and has been described as cross-current (Metcalfe et al., 1965; Stevens, 1966; Silver et al., 1973). The anatomical study of Stevens (1966) suggests that maternal flow appears to be parallel to the long axis of the placental villus, whereas the foetal blood flow is at right angles to this. (Makowsky et al., 1968) however, suggest the reverse arrangement, wherein maternal flow is at right angles to the villus and foetal flow is parallel. Either arrangement will show a cross-current type of flow. Placental flow in the sheep foetus approximates 175 ml/kg/min (Dawes, 1968).

2. Thermal Aspects of Life In Utero

Adamsons and Towel (1965) have outlined three factors that will affect the temperature of the foetus relative to the mother. They are 1) the caloric output of the foetus and placenta, 2) diffusion capacity of sites available for heat exchange, and 3) rate and direction of blood flow through the donor and acceptor systems. Meschia et al. (1967) and Crenshaw et al. (1968) have measured the oxygen consumption of sheep foetuses during the last third of gestation, and found that the O_2 consumption of the foetus was greater per kg than that of the ewe. From their data, Abrams et al. (1970) have calculated heat production of the foetus and have found it to be approximately 1.6-2 times that of the adult sheep. Consequently, the foetus must lose heat to the mother or its temperature would increase. Since steady-state conditions do exist in utero, the facts would suggest that foetal temperature must be higher than the average internal temperature of the mother. Indeed,

data from rabbit (Hart and Faber, 1965) and human foetuses at delivery (Wood and Beard, 1964; Adamsons, 1966; Mann, 1968; Walker and Wood, 1970) and chronic recordings from animals in utero (Abrams et al., 1969; Morishima et al., 1975) show that foetal temperature is consistently about 0.3-0.8°C higher than maternal temperature. Furthermore, in both chronic and acute experiments on sheep, the temperature of the umbilical artery, carrying blood from foetus to the placenta, is 0.1-0.4°C higher than the umbilical vein temperature (Abrams et al., 1970). This is evidence that the foetus does lose heat via the placenta but it is likely that heat exchange also takes place between the external body surface of the foetus and the amniotic fluid. It is of interest that when maternal temperature was raised during external heating (Morishima et al., 1975), or during pyrogen fever (Abrams et al., 1969) the foetal-maternal gradient was maintained. In contrast, when the foetus was dead, the temperature gradient was absent or even reversed (Abrams et al., 1969). When pregnant bitches were cooled, so that body temperature fell, foetal temperature fell in parallel with maternal temperature (Assali and Westin, 1962).

In view of the parallel shift in foetal temperatures with maternal temperature changes, it is unlikely that the foetus, in utero, is actively regulating its temperature. However, the high thermal diffusion capacity of the placenta and the fluid environment of the foetus may exchange heat so efficiently that the foetus is unable to effect any change in its temperature with respect to that of the mother. It is a common observation that foetal lambs, exteriorized during the last third of gestation, can shiver when exposed to the laboratory air. In addi-

tion, Alexander et al. (1972, 1973b) measured metabolic rates in prematurely delivered lambs and suggested that the ability of foetal lambs to increase metabolic rate in response to cold is already developing some 20 days prior to full term. Whether a similar response would occur in utero is not known.

3. Temperature Regulation in the Newborn

Thermal stability in the newborn depends on a balance between heat production and heat loss as it does in the adult. However, there are certain factors, both physical and physiological, which are of particular importance in the newborn with respect to heat production and heat loss.

The size of an animal is very important in determining the amount of heat that will be lost to the environment. Since newborns are smaller than their adult counterparts, they will be much more susceptible to heat loss problem. Brück (1961) has calculated that, as a result of the high surface to mass ratio of newborns, in full-term infants the expected heat loss will be 2.7 times that of an adult per weight unit. Also, because of their small size, the capacity for heat storage will be much less in the newborn.

A second factor which often affects heat loss differently in the newborn than in the adult is the amount of insulation. Newborns often lack a significant subcutaneous fat layer and have a higher heat conductivity of the tissues due to increased water content (Hill, 1961; Hensel et al., 1973). In addition, many neonates are born without hair. Heat loss can also be greatly increased at birth due to evaporative cooling of the foetal amniotic fluids. Alexander (1975) has noted that

convective heat losses can be especially great from lambs born in windy outdoor conditions.

It is apparent, therefore, that in some small newborn animals the capacity to limit heat loss may be small. This would have the effect of raising the lower threshold of the thermal neutral zone of the foetus above that of the adult. To partially combat this problem, newborns may behaviourally limit heat loss. For instance, newborn piglets will huddle together and thus lower their effective surface area (Mount, 1959).

Though newborns are not often likely to be exposed to ambient temperatures that necessitate activation of heat loss mechanisms, in the few studies done this capacity appears to be present. Newborn human infants can sweat (Foster et al., 1969) and increase blood flow to the skin in response to an increase in ambient temperature to 32-34^{o}C (Brück, 1961). Thermal tachypnea in response to high environmental temperatures has been observed in a number of neonates including lambs (Alexander and Brook, 1960), guinea pigs (Hensel et al., 1973), dogs (Jensen and Ederstrom, 1955), and calves (Hales et al., 1968; Bianca and Hales, 1970).

On the basis of their ability to increase heat production in response to cooling, newborns can be divided into two groups. In the first group, characterized by the vole, ground squirrel and mouse, energy metabolism does not rise after cooling (Gelineo and Gelineo, 1951). Only after a period of 3-4 weeks is cooling countered by increased heat production. The second group of neonates show thermoregulatory metabolic reactions to cold and most mammals including rats, rabbits,

guinea pigs, dogs, cats and humans are included in this group (Brück, 1961). In newborn lambs, the maximal metabolic response to cold exposure can be as much as 5 times the minimal metabolic rate of 1 1 O_2/kg/h (Alexander and Williams, 1968). Only about 60% of this is derived from shivering thermogenesis; the remaining 40% apparently comes from non-shivering thermogenesis (NST). It is believed that this metabolic response to cold is mediated by the local release of noradrenaline from the sympathetic nerves in brown fat. The subsequent oxidation of the brown adipose tissue is an excellent source of heat (Hull, 1966). As animals mature, the ability to utilize non-shivering thermogenesis is lost, unless they are reared in a cold environment (Zeisberger et al., 1967).

During exposure to cold, neonates can also increase the insulative properties of their skin by increased vasomotor activity. Brück (1961) showed that constriction of skin blood vessels occurred in response to body cooling in both full size and low birth weight infants. Alexander et al. (1973a) used the radioactive microsphere technique to obtain measurements of blood flow to most tissues of the body and concluded that in lambs there was a marked decrease in flow to the skin during cold exposure. Blood was redistributed to the brown fat tissues and deep skeletal and cardiac muscle.

The deep-body temperatures of the non-precocious newborns are similar to the adults of the species (e.g. humans, (Brück, 1961), lambs (Alexander, 1961), and it would appear that they regulate at this temperature. However, there is evidence that the newborn of small species such as cats, dogs and rats may regulate at a temperature several

degrees lower than the adult of the species (Hensel et al., 1973). Thus the set-point in this case may be set at a lower level. Nevertheless, the immature hamster pup shows thermal sensitivity in that it will show thermotaxis to a source of warmth (Leonard, 1974).

Neonates often show more tolerance to cooling than do adults. For instance, Adolph (1951) showed that hearts of newborn cats stopped beating when cooled to 5-10°C, whereas those of adult cats stopped at 15-20°C. It would appear that the less mature an animal is at birth, the more resistant it is to hypothermia. In lambs, death occurs at a rectal temperature of about 20°C, whereas the more immature neonatal rat survives rectal temperatures as low as 1°C (Alexander, 1975).

In neonatal animals, temperature often fluctuates far more than is seen in adults exposed to the same ambient conditions. Hensel et al. (1973) have pointed out that, contrary to the common conclusion that this represents an immaturity of the thermoregulatory system at birth, it rather represents a discrepancy of the efficiency of the effector system in the fact of considerable heat loss problems.

Though regulation does appear to exist in most species at birth, very little attention has been paid to the aspects of CNS control of thermoregulation in neonates. One notable exception is the observation of Brück and Wunnenberg (1970) on the inter-related control of shivering and non-shivering thermogenesis in neonatal guinea pigs. They found that, when newborn guinea pigs are exposed to a cold environment, NST is initiated first, and shivering occurs only after more severe cooling. They concluded that, whereas the stimulation for NST is a function of cooling of both body surface and hypothalamus, shivering occurs only

after spinal cord cooling. Consequently, heat produced by NST in the interscapular and cervical brown adipose tissue warms the cervical spinal cord and suppresses shivering. As the ability to warm this area by NST is reduced in the presence of more severe cooling, shivering is initiated. Thus shivering and non-shivering thermogenesis are controlled by the product of the temperature deviations in the two receptor regions.

The effects of temperature change on the firing rates of neurons in the brains of newborns have been studied. Henderston et al. (1971) recorded from neurons in the hypothalamus and thalamus of 5-12 day old rabbits, and obtained results from neurons which had characteristics which resumbled those found by others in adults. Despite the fact that they had considerable difficulty in recording due to lack of rigidity in the skulls of the newborns, they nevertheless concluded that the AH/POA region is not as rich in temperature response units in young animals as it is in adults. Rather, their results seemed to point to the posterior area as containing the greater proportion of temperature sensitive units in developing newborn rabbits.

4. Development of the Brain

Since the major control of thermoregulatory functions rests in the hypothalamus, it would appear that the development of this part of the brain will be a determining factor in the ability of a neonate to thermoregulate. Himwich (1962) has pointed out that the developmental anatomy, biochemistry and neurophysiology of the brain changes markedly among species. For example, guinea pigs appear to have a relatively mature brain at birth, whereas the rat brain does not attain adult

characteristics until 20-25 days postnatal. When evaluating brain maturity, the question also arises as to what characteristics will give a reasonable index of maturity, the electroencephalogram, the neurotransmitter levels, the ionic concentrations in the extracellular fluid? One aspect that has received considerable study is the development of the biogenic amine systems in the foetal and neonatal brain. It is necessary to distinguish between different brain regions, for Pscheidt and Himwich (1966) have shown clearly in cats that each region has a characteristic and often different developmental pattern.

Several studies have looked at developmental patterns in foetal and neonatal rat brains. The consensus from these studies is that, even though brain levels of NE, 5-HT, and dopamine are low in foetal brains, it is likely that monoaminergic neurons are functional at about the time of birth. However, enzyme and transmitter levels increase for an additional 20 days (Nachmias, 1960; Karki et al., 1962; Hyyppa, 1969; McGeer et al., 1971; Loizou, 1972; Coyle and Henry, 1973; Nomura et al., 1976.)

In the mouse, fluorescent axon terminals are seen in the hypothalamus at birth (Golden, 1973). Millard and Gal (1972) concluded that, in human foetus, mono-oxygenases for the synthesis of the monoamines are present and functioning by the end of the first trimester. Hyyppa (1972) measured levels of DA, NE and 5-HT in human foetal hypothalamus and found high DA levels, but low NE and 5-HT. He concluded that these neurotransmitters were probably functional at birth. Widdowson and Dickerson (1960) determined the chemical and ionic composition of whole human brain and found that values had reached adult levels by

about 1/3 to 1/2 term. Similarly, Saunders and Bradbury (1973) found that levels of potassium, sodium chloride and calcium in term lambs were not very different from the adult levels. This could be of significance if the ionic constituents of the posterior hypothalamus are indeed determinants of the 'set-point' temperature (Myers and Veale, 1970).

The biochemical development of the foetal sheep brain has received little study. However, since the newborn lamb is relatively mature at birth, it is probably that brain development would resemble that of the guinea pig, another precocious newborn. Evidence from guinea pigs would indicate that major brain development is completed at birth (Himwich, 1962; Dobbing and Sands, 1970). Developmental studies on sheep brain have been carried out which would indicate that the blood brain barrier is functionally similar to that of the adult by 125 days gestation (20 days before term) (Reynold et al., 1973; Evans et al., 1974). These studies have evaluated CSF secretion in foetal lambs and have found the level to be higher in the foetus than in the adult.

5. Effect of Pyrogens in Newborns

a) Clinical Studies

There are numerous reports in the literature of newborn and premature infants suffering severe infection, for example, meningitis, enteritis and septicaemia, without the expected accompaniment of a marked rise in body temperature. Epstein et al. (1951) reported a study of 26 cases of salmonella infections in neonates in which, of 18 babies suffering Salmonella oranienburg infections, only 4 were febrile

and none of the 8 babies infected with S. bareilly became febrile. In another study, Smith et al. (1956) reviewed a large number of case histories of neonatal septicaemia. Of 87 newborn infants (mean postnatal age 9.2 days), 51% experienced fever as defined by a rise in rectal temperature above 38.3°C. Many of the febrile babies appeared to be at the older end of the age distribution. Similarly, Bergstrom et al. (1972) noted that fever was significantly more common in 11-30 day old babies suffering urinary tract infections than in those becoming ill during the first 10 days of life.

However, the definition of fever in the premature and newborn infant is not easy as Moncrieff (1953) pointed out. In the early hours of life when the central body temperature may not yet be regulating effectively, or when the body temperature set-point mechanism may not have adjusted to a stable level of control, the infant may have a lower than expected body temperature in a cool nursery. A rise in temperature due to infection superimposed on a sub-normal temperature related to an immature thermoregulatory system might not reach some arbitrarily defined febrile level. It is known that in the adult with chronic episodic hypothermia, a fever reaction can occur at sub-normal temperatures in response to intravenous bacterial pyrogen (Duff et al., 1961; Hockaday et al., 1962). Nevertheless, bearing in mind this possibility, a careful analysis of available epidemiological data seems to indicate a significant incidence of severe infection without fever in the first 1-2 days of postnatal life (Parmalee, 1948; Epstein et al., 1951; Smith et al., 1956; Sanford and Grulee, 1961; Craig, 1963; Bergstrom et al., 1972; Marzetti et al., 1973). In the

few animal studies that have been carried out, there is evidence that newborn guinea pigs (Uhr, 1962) and young rabbits (Watson and Kim, 1963) show smaller febrile responses after intravenous endotoxin than do adults of the species. Blatteis (1975) observed that pyrogenic sensitivity was not apparent in guinea pigs during the first postnatal week and that even up to a month postnatal, febrile responses may not occur in guinea pigs raised at thermoneutral temperatures. Thus, the fever response in neonatal guinea pigs differs from that of the adult, in that it is influenced by ambient temperature.

b) Endotoxin Shock

Other evidence of neonatal refractoriness to endotoxins has been published. Young rabbits are more resistant to the lethal effect of large doses of endotoxin or other bacterial toxins than adults (Parish and Okell, 1930; Burky, 1932; Smith and Thomas, 1954; Brunson et al., 1955; Sterzl et al., 1961; Watson and Kim, 1963). The same is true for neonatal rats (Miler, 1962) but curiously, newborn guinea pigs are more susceptible to the lethal effect of endotoxin (Uhr, 1962). Age effects in the Schwartzmann reaction have been studied in rabbits, and Witebsky and Neter (1936) observed that a high percentage of young rabbits were resistant to the elicitation of the Schwartzmann reaction. Finally, newborns appear to be much more resistant to the hemodynamic effects of large dosages of endotoxins. This has been shown in puppies (Hinshaw et al., 1962; Reddin et al., 1966) and in the sheep foetus and lamb, which, up to 10 days or more postnatally, could tolerate more than 10 times the dose of endotoxin which would be lethal in the adult sheep without exhibiting shock (Bech-Jansen et al.,

1972).

The lack of response on the part of newborns to endotoxin is not likely due to more rapid detoxification of the endotoxin in the circulation. It is well known that serum incubation will inactivate endotoxin (Skarnes et al., 1958) but Ainbender et al. (1972) found that serum from full-term infant was less than 1/10 - 1/100 as effective as adult serum in inactivating endotoxin in vitro. It would appear more likely that the greater response to bacterial pyrogens in adults is associated with a state of hypersensitivity as suggested by Stetson (1961, 1964) and Watson and Kim (1963). However, it is unlikely that lack of, or reduced febrile response in neonates is due to lack of circulating gammaglobulins. Van Miert and Atmakusuma (1971) found that there was no difference in the pyrogenic response to bacterial pyrogen between goat kids that had been fed colostrum, and therefore had high gammaglobulin levels, and colostrum-deprived kids with undetectable gammaglobulin levels. There is also evidence that patients with gammaglobulinaemia or hypoglobulinaemia have normal fever reactions to bacterial endotoxins (Good and Varco, 1956). The development of the response to endotoxins, however, may depend on the sensitivity of some cellular system.

c) Neonatal Leucocytes

The febrile response appears to involve the interactions of white cells, and other components of the RES with the injected pyrogens. In leucocytes this involves phagocytosis and associated metabolic changes including increased glycolysis and lactate production, increased oxygen consumption, an increase in hydrogen peroxide formation and

activation of the hexose monophosphate shunt (Karnovsky, 1962). Humoral factors known as opsonins enhance the efficiency of phagocytosis (Winklestein, 1973).

There is considerable evidence to indicate that leucocytes in neonatal humans are functionally and metabolically different than those of adults, and this may have an effect on the febrile process. Leucocytes from newborns appear to be deficient with respect to phagocytosis (Matoth, 1952; Gluck and Silverman, 1957; Miller, 1969) and in addition show decreased migration to a chemotactic factor generated from S. aureus and E. coli (Miller, 1971). In addition, there appears to be a deficiency in intracellular killing of ingested bacteria in newborn leucocytes (Cocchi and Marianelli, 1967; Coen et al., 1969). It also appears that the serum of neonates is deficient in the generation of chemotactic factors such as opsonins (Miller, 1969).

Other work would also suggest differences in the metabolic state of the newborn's leucocytes. When nitroblue-tetrazolium (NBT) dye is added to in vitro preparations of white cells engaged in phagocytosis, it is taken up and reduced to formazen, which can be identified histologically (Feigin, 1971). Leucocytes from newborns show increased NBT reduction, which suggests an activated metabolic state with increased oxygen consumption and hexose monophosphate pathway activity (Park et al., 1970; Humbert et al., 1970; Cocchi et al., 1971). Whether or not these differences between neonatal and adult sera would affect the febrile response to injected pyrogen, or indeed, if they even extend to other species is unknown.

D. Rationale for this Research

The previous discussion has pointed out that newborns appear to respond to pyrogens in a somewhat different manner than do adults of the species. In particular, clinical and experimental evidence would indicate a reduction in the incidence or intensity of fevers in newborns. The work reported in this thesis was undertaken to investigate the development of the fever response in the newborn lamb. Experiments were designed to achieve the following objectives:

1. To determine the effect on the foetus of bacterial pyrogen injected either into the maternal circulation or directly into the foetus.

2. To determine if newborn lambs respond with fever after intravenous endotoxin or endogenous pyrogen.

3. To determine the mechanisms involved in the maturation of the response to endotoxins in the neonatal lamb.

4. To determine if newborn lambs can respond with fever after injections of prostaglandins and pyrogens into either the cerebral ventricles or the tissues of the hypothalamus.

5. To determine the temperature response of newborn lambs to injections of monoamines into the cerebral ventricles or the tissue of the hypothalamus and compare these responses with those of adult sheep.

The lamb was chosen as an experimental model for this study because it fulfilled several criteria:

1. There was an established sheep herd at the medical school and the foetal lamb model was in current use.

2. The large size of the lamb foetus makes it an ideal prepar-

ation for chronic recording of physiological variables.

3. Newborn lambs are relatively mature at birth and can maintain a stable body temperature.

4. Thermoregulation in the non-febrile lamb and the adult sheep has received considerable study.

II. FEVER IN UTERO

In order to study developmental aspects of the febrile response, the effect of fever in the mother on foetal temperature was observed in the unanesthetized sheep foetus. Further to this, foetuses in utero were injected intravenously with bacterial pyrogen and temperature was monitored. In addition, white cell counts were carried out with foetal blood after administration of pyrogen either into the ewe or directly into the foetus. To determine if the fever observed in the foetus during maternal fever was passive or was a response of the foetus to pyrogen (endogenous?) crossing the placenta, the antipyretic sodium salicylate was infused directly into the foetal circulation.

Methods

Sheep were kept for the greater part of the year in outdoor paddocks. Breeding dates were recorded and pregnant ewes were brought into the vivarium within the Medical School several days before surgery or expected lambing dates. They were shorn and housed in groups at an ambient temperature of 18-20°C. Food and water was available ad libitum. Sheep were of mixed breeds, but showed characteristics of Suffolk, Finn, Columbia and Dorset breeds.

Surgical Procedure - Chronic Preparation

For the monitoring of foetal heart rate, blood pressure, blood gas values, and temperature, the foetus was implanted with vascular cannulae, electrocardiogram (ECG) leads and a thermistor. A thermistor was also placed in the abdomen of the ewe adjacent to the uterus. Surgery was performed under rigid aseptic conditions at a foetal age of

approximately 110 days (term for the sheep is approximately 145 days). After the ewe was thoroughly scrubbed and shaved over the left flank, abdomen and neck, a non-occluding jugular cannula was inserted. Halothane anesthesia was induced with the use of an anesthetic machine (Fluotec) and respiration was controlled by a Bird Mark 7 respirator and a Bird Mark IV anesthetic assister/controller.

Cannulae and other leads were introduced into the abdomen of the ewe through a small incision on the left side. Externally, the cannulae were attached to stopcocks which were kept in small bags filled with tincture of Zephiran (Zephiran chloride, Winthrop Laboratories, Aurora, Ontario, Canada). The cannulae, ECG and thermistor leads were enclosed in a cloth bag sutured to the side of the ewe. A mid-ventral abdominal incision was then made to exposure the uterus and the cannulae and leads were retrieved from the abdomen. The uterus was opened and the foetus manipulated to expose the appropriate areas for cannulation. For most of the experiments outlined in the thesis, the foetal saphenous and femoral vessels were cannulated and the tips of the cannulae advanced into the vena cava and dorsal aorta respectively. In other operations, cannulae were introduced into the common carotid artery and external jugular vein and advanced towards the heart. Medical grade tubing (Silastic; Dow Corning Corporation, Midland, Michigan, U.S.A) with an internal diameter of 7.6×10^{-4}m and 1.65×10^{-3}m external diameter was used for the iv cannulae. The foetus was then manipulated to expose the thorax, and ECG leads were inserted subcutaneously on the thorax and leg. A thermistor (type 402) (Yellow Springs Instruments Co., Inc., Yellow Springs, Ohio) was then introduced either into

the abdomen or into the neck next to the carotid artery in order to measure deep body temperature. At all times, care was taken to minimize loss of amniotic fluid, and, when necessary, this was replenished with warm sterile saline. The placental membranes and uterus were closed with surgical gut, the linea alba was drawn together with mersilene suture and the skin was closed with wound clips. While the foetal surgery was in progress, a non-occluding cannula was introduced into the common carotid artery of the ewe.

The ewe was given 1.2×10^6 international units of penicillin (Benzathine Penicillin G and Procain Penicillin G-Derapen, Ayerst Laboratories, Montreal, Canada) the day before, the day after and 5 days after surgery. The animals were allowed a minimum of 5 days post-operative recovery before studies commenced. Cannulae were kept patent by daily flushing with sterile saline and throughout the remainder of gestation the condition of the foetus was periodically evaluated by monitoring heart rate, body temperature, pH and partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$).

In order to evaluate such variables, potentials from thermistors, ECG leads and pressure transducers were recorded on paper in a Beckman Dynograph Recorder. The dynograph was interfaced with an analog to digital converter and multiplexer to a PDP-11 computer. The computer provided on-line calculation and digital output of mean and standard deviation of the mean heart rate, diastolic, systolic and pulse pressure and mean arterial blood pressure. Blood pressure was transduced with the use of a Statham pressure transducer which was placed at mid-flank level of the ewe. Foetal ECG was recorded from

the electrodes implanted during surgery.

For determination of foetal PaO_2, $PaCO_2$ and pH, blood samples (1.5 ml) were collected via the indwelling arterial catheters into heparinized syringes and immediately analysed using a blood gas/pH analyzer (Instrumentation Laboratories).

Administration of pyrogen to the ewe

Foetal and maternal temperatures were recorded on the dynograph for a minimum of 30 min but usually for 1 h prior to drug injection. Then, 0.2 - 1.0 µg of bacterial pyrogen, in 1.0 ml of sterile, pyrogen-free saline, was administered to the ewe via the indwelling jugular cannula and flushed in with a further 2.0 ml of saline. The endotoxin used (SAE pyrogen) was a lipopolysaccharide extracted from Salmonella abortus equi (Difco Laboratories, Detroit, Michigan, U.S.A). Temperature was recorded for a further 140-170 min after injection until the fever had subsided and temperature had returned to baseline level.

Administration of pyrogen in utero

Three foetuses were prepared for chronic injection and recording procedures as described in the preceding section. At various foetal ages following recovery from surgery, 0.3 µg of SAE pyrogen in 3.0 ml of sterile pyrogen-free saline was administered directly to the foetus through the venous cannula, and temperature was recorded for at least 90 min after the injection.

Four other foetuses were challenged with intravenous bacterial pyrogen, in utero, with the ewe under spinal anesthesia. To do this, the ewe was given a spinal anesthetic (40 g/l xylocaine hydrochloride, Astra Ltd.) and a mid-ventral incision was made to allow access to the

uterus and the hindquarters of the foetus. Under local anesthesia (xylocaine hydrochloride), the foetal saphenous vein was cannulated with sterile medical grade tubing that passed into the inferior vena cava. A thermistor was inserted into the rectum of the foetus and the opening in the uterus was closed. Each experiment was continued with the mother still under spinal anesthetic, though in some cases it was necessary to supplement this with general halothane anesthesia as the spinal anesthesia began to lose effect. At least 45 min of observation was possible before general anesthesia began. After administration of 0.3 µg of SAE pyrogen in 3.0 ml of sterile, pyrogen-free saline to the foetus through the saphenous cannula, rectal temperature was monitored for at least 90 min.

In order to evaluate the effects of the injected pyrogen on circulating white cells, 0.5 ml blood samples were taken from the venous cannula into heparinized syringes. Samples were taken immediately before the injection, and then at 10 min intervals thereafter over a 90 min period. They were placed on ice and the white cells were counted within 4 h either in a standard hemocytometer or a Coulter counter.

Administration of salicylate to foetus

In this series of experiments, three pregnant ewes which had been used in the chronic cannulation experiments described above were given 0.3 µg SAE pyrogen intravenously and temperature was recorded. At approximately 40-45 min post-injection, as the maternal and foetal temperatures began to rise, 100 mg/kg of sodium salicylate, in 2-3 ml of sterile pyrogen-free saline, was administered to the foetus via the

venous cannula. This was followed by a continuous infusion of 3 mg of sodium salicylate/30 µl saline/min. The salicylate solution was sterilized by passage through a sterile 0.22 µ bacterial filter (Millipore). The dosage of salicylate was calculated from the expected weight of the foetus as derived from the data of Pipkin and Kirkpatrick (1973).

In one foetus the salicylate content of the blood 30 min after the initial injection of sodium salicylate was determined by the spectrophoto-fluorometeric method (Saltzman, 1948). A 3 ml blood sample was collected into a heparinized syringe, and following centrifugation, the supernatant was withdrawn and a 0.5 ml sample of plasma was mixed with 9.5 ml tungstic acid. Following filtration and treatment with 10 N sodium hydroxide, fluorescence was measured with a Turner Model 110 Fluorometer (G.K. Turner Associates, Palo Alto, California) with filters set at 365 mµ for activation and 455 mµ for emission spectra.

These experiments were carried out in the laboratory at an ambient temperature of 19-21^{o}C. All drug solutions were sterilized by passage through a sterile 0.22 µ bacterial filter (Millipore) before injection, and all syringes used were sterile and pyrogen-free.

Results

Administration of pyrogen to ewe

For this series of experiments, 5 pregnant ewes were given pyrogen injections on 10 separate occasions at foetal ages of between 116 and 142 days. On all occasions during the control period, foetal temperature was higher by 0.3 - 0.9^{o}C than that of the ewe. If the maternal temperature fluctuated, foetal temperature showed a similar

change. Approximately 40-50 min after the injection of pyrogen, maternal temperature began to rise, reaching a peak of 0.6 - 1.0°C after 70-100 min post-injection and returning to normal within 140-170 min after the injection. The rise in temperature was associated with a decrease in ear skin temperature (subjectively assessed by feeling the ears), shivering and a decreased respiratory rate. During defervescence, the ears became hot and respiratory rates increased from 30-40/min to 150-200/min.

The increase in temperature seen in the ewe after pyrogen injection was paralleled by a similar increase in the foetal temperature (Fig. 2). On occasion, foetal temperature would lag slightly behind that of the ewe, but the temperature difference between foetus and ewe was always present. On two separate occasions, however, foetuses were found to have died in utero, and when temperature was recorded under these circumstances, the foetal and maternal temperatures were approximately the same, and no consistent temperature differential was observed.

Administration of pyrogen to foetus

SAE pyrogen (0.3 μg) was given to each of three chronically cannulated foetuses at foetal ages of 117, 118 and 123 days. In each of these experiments, foetal and maternal temperatures remained essentially unaltered during the 90 min following pyrogen injection. Fig.3 shows the temperature records of a 123-day old foetus and its mother after intravenous injection of bacterial pyrogen. Similar flat temperature curves were seen after pyrogen injection in the other two foetuses. One foetus was re-injected with pyrogen twice over a 13-day

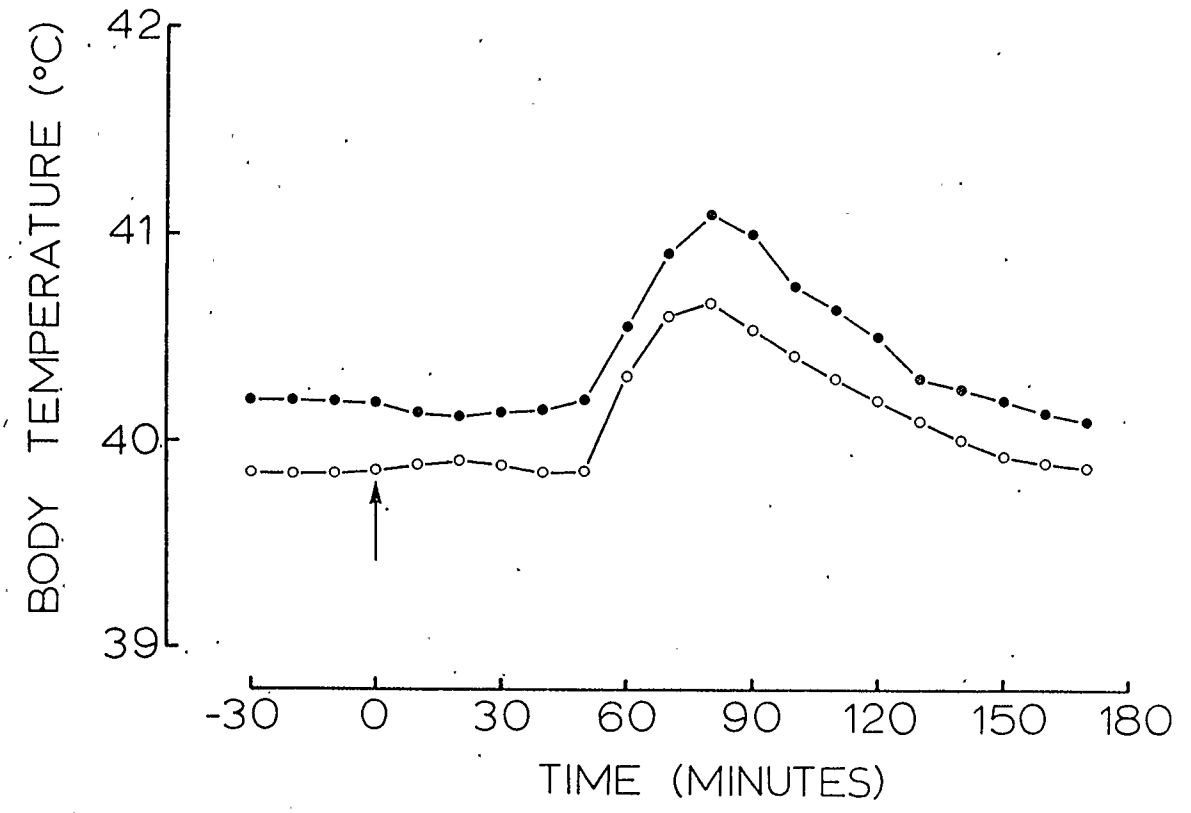

Figure 2. Records of foetal (filled circles) and material (open circles) temperatures after intravenous administration of SAE pyrogen to the ewe, at time 0, as indicated by the arrow.

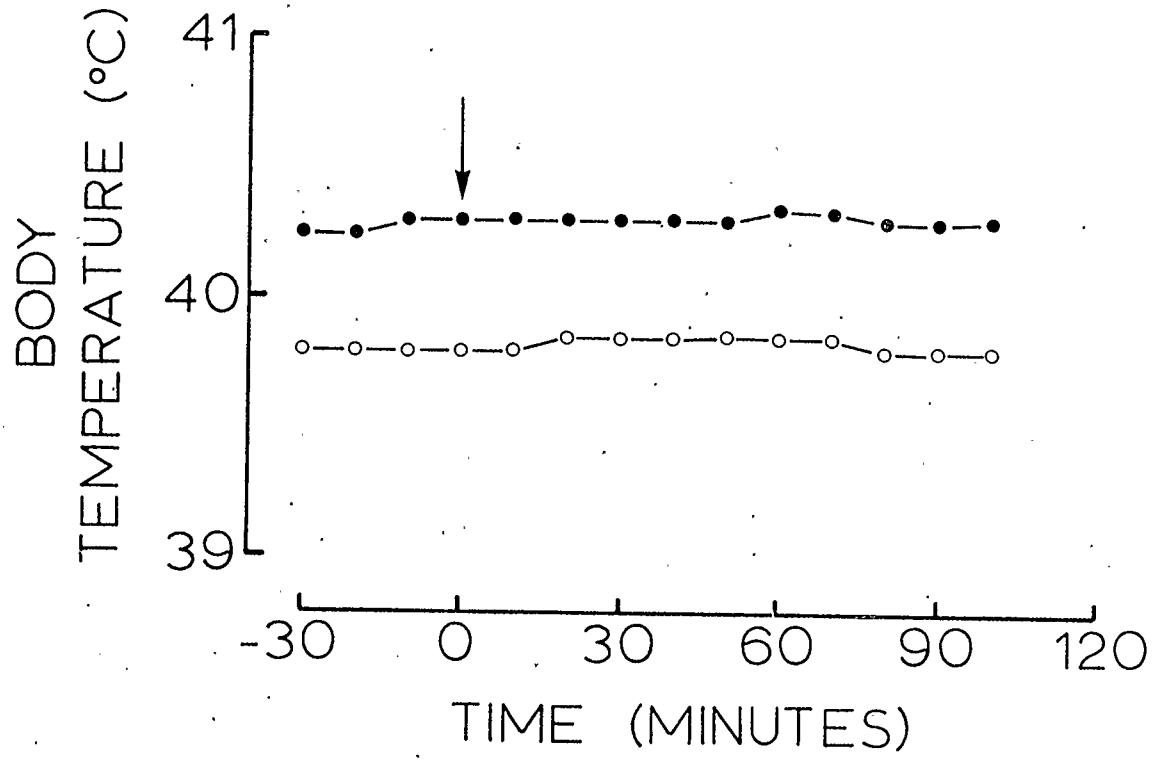

Figure 3. Records of foetal (filled circles) and maternal (open circles) temperatures after intravenous administration of SAE pyrogen to the foetus, at time 0, as indicated by the arrow.

period, and on none of these occasions was a fever observed.

With the ewe under spinal anesthesia, each of 5 foetuses was given 0.3 µg of SAE pyrogen at gestational ages of 101, 115(2), 130 and 135 days. None of these foetuses developed fever in the 90 min period after the injection. However, in all these animals, the white cell count was sharply reduced shortly after the pyrogen was injected. Fig.4 shows that white cell numbers were reduced to nearly one half the pre-injection levels in a 115-day old foetus, even though no increase in body temperature was observed.

White cell counts were also taken on two occasions following endotoxin administration to two chronically cannulated foetuses. One foetus was at a gestational age of 138 days and the other was 123 days, and in each instance the number of circulating white cells decreased shortly after the pyrogen injection (Fig.5, lower). An injection of sterile, pyrogen-free saline to a foetus on another day did not cause a similar change in the white cell count.

White cell counts were also done on a chronically cannulated 124-day old foetus following administration of 1 µg SAE pyrogen to the ewe. Even though a fever of 1.1°C was produced, the white cell count did not drop significantly from that observed prior to the injection.

Administration of salicylate to foetus

In three pregnant ewes, each given 0.3 µg of SAE pyrogen, body temperature rose by 0.75, 1.00 and 1.05°C. Their foetuses were given intravenous sodium salicylate, but nevertheless, foetal temperatures rose a comparable amount (0.75, 1.05 and 0.95°C respectively). The temperature records for one such experiment are shown in Fig.6, and it

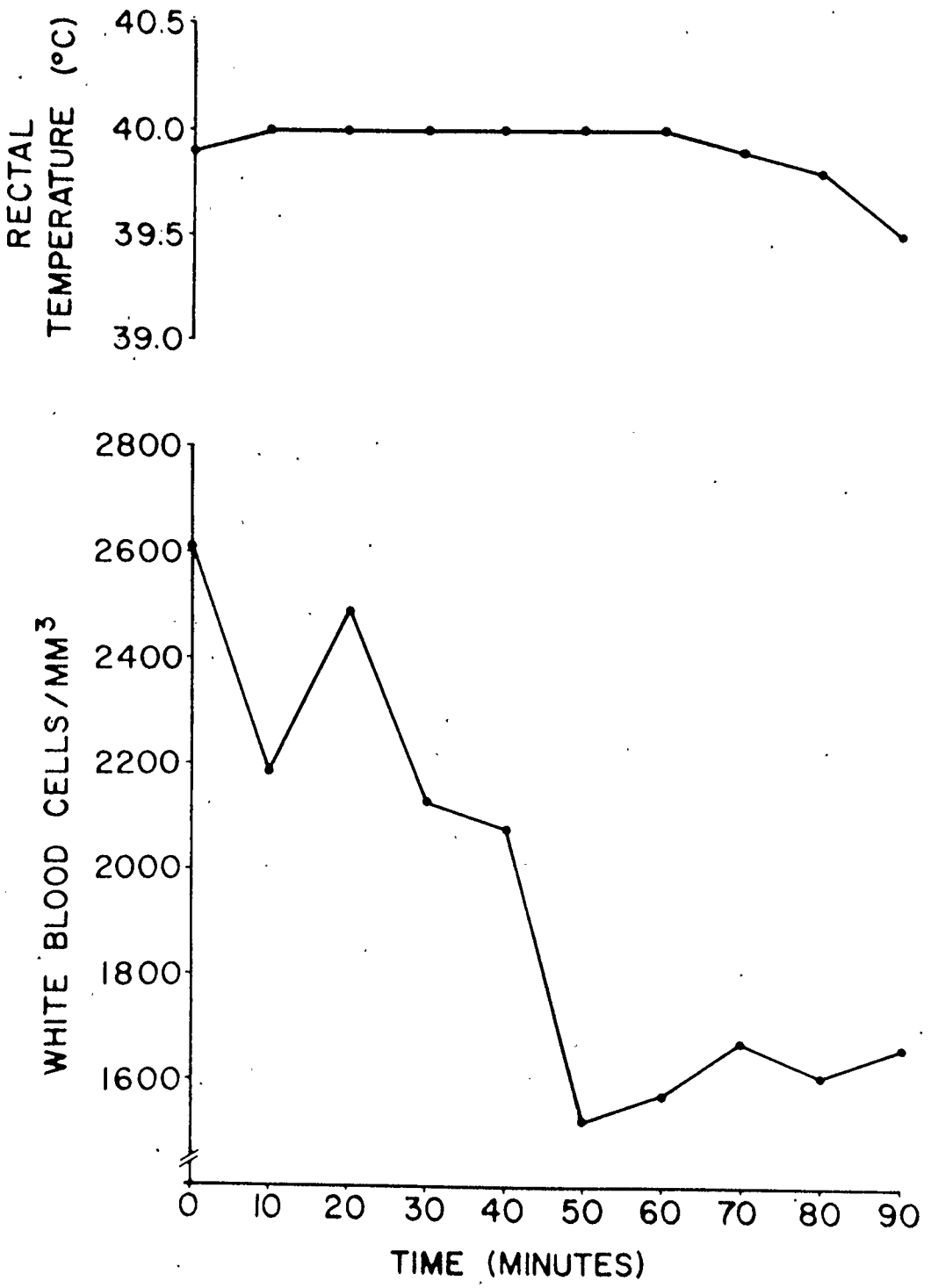

Figure 4. Number of white blood cells in blood samples from a 115-day-old unanesthetized foetus and the body temperature of the foetus after intravenous injection of 0.3 µg SAE pyrogen.

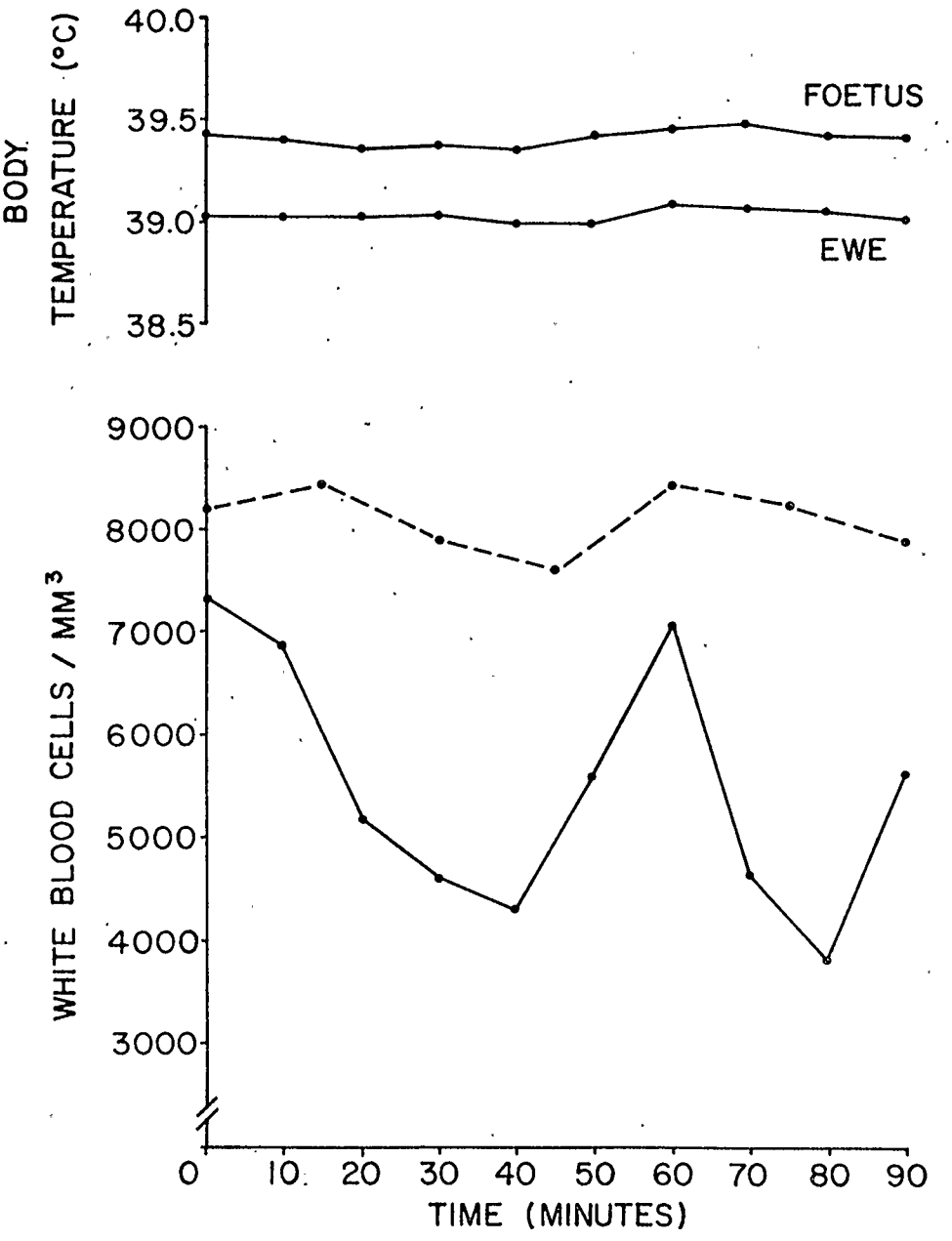

Figure 5. Number of white blood cells in blood samples from an unanesthetized 123-day-old foetus after intravenous injection of 0.3 µg of SAE pyrogen (solid lines) and during a control injection of the same volume of pyrogen-free saline (broken lines) at time 0. Body temperatures of the foetus and ewe after intravenous injection of pyrogen into the foetus are also shown.

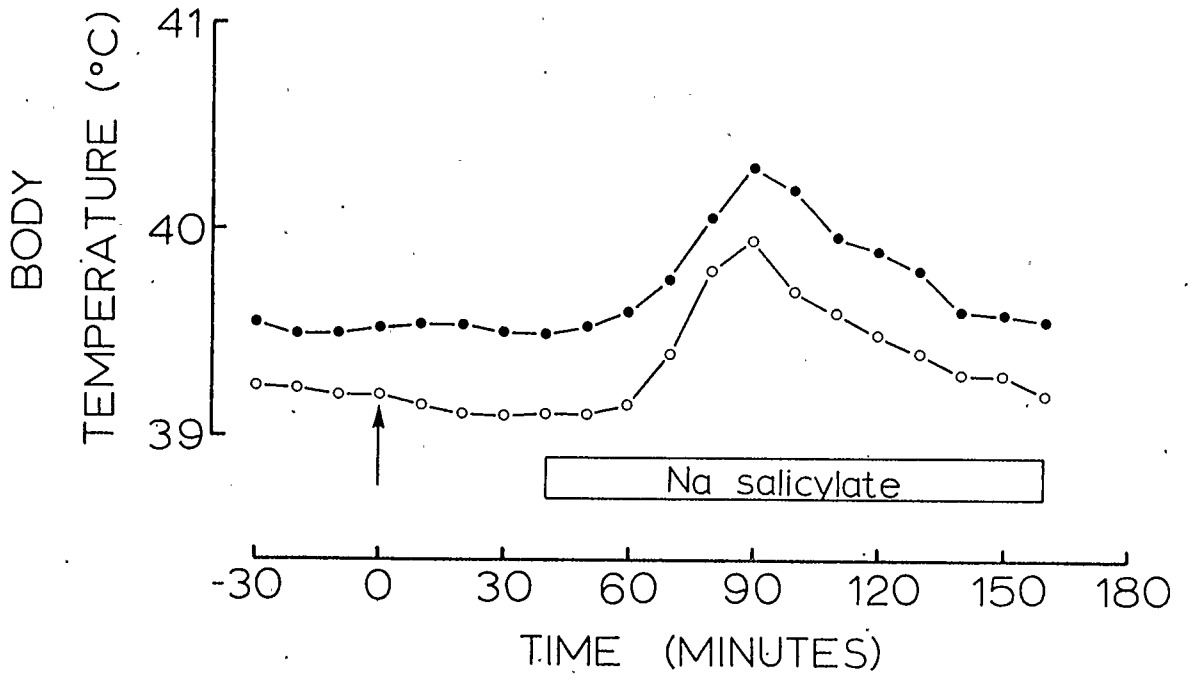

Figure 6. Records of foetal (filled circles) and maternal (open circles) temperatures after intravenous administration of SAE pyrogen to the ewe, at time 0, as indicated by the arrow. The bar indicates the onset and duration of an injection of 300 mg of Na salicylate followed by 3 mg Na salicylate/min.

can be seen that foetal temperature rose in parallel with that of the ewe's, in an identical manner to that previously observed during maternal fever and when salicylate was not administered. In the foetus, the salicylate content of the blood, 30 min after the onset of the salicylate infusion, was 20 mg/100 ml.

Discussion

Our results confirm that foetal temperature, measured *in utero*, is consistently higher than maternal temperature (Adamsons and Towel, 1965; Abrams *et al.*, 1969; Morishima *et al.*, 1975). As has been discussed earlier, this temperature differential can be accounted for because the foetus has a higher metabolic rate, and therefore, higher heat production than the surrounding maternal tissues. In support of this, was the observation that the temperature of a dead foetus was the same as the maternal temperature.

The rise in temperature that has been observed in the foetus in the present experiments, and by others (Abrams *et al.*, 1969), after intravenous bacterial pyrogen injection into the ewe may be a response of the foetus to endotoxin, or to maternally produced endogenous pyrogen that crosses the placenta; alternatively, it may be a result of a build-up and storage of heat in the foetus owing to the increase in maternal temperature. One observation would suggest that the latter explanation is most likely. That antibodies do not cross from maternal to foetal circulations in sheep explains a lack of antibodies in the newborn lamb (Vahlquist, 1958; Landy and Weidanz, 1964). If these molecules do not cross to the foetus, it is unlikely that the larger

bacterial endotoxins would cross. However, the possibility remains that the maternally produced endogenous pyrogen could cross to the foetal circulation and elevate set-point for body temperature in the foetus.

One component of the sequence of events in the pathogenesis of fever appears to be present in the foetus during the last one-third of gestation. That is, the foetus appears to be capable of recognizing as foreign the injected lipopolysaccharide to which it is first exposed since there was a fall in the number of circulating white cells. The disappearance of the white cells from the circulation is a phenomenon common to the adults of many species after administration of endotoxin (Sundelin, 1939; Eichenberger et al., 1955; Atkins and Snell, 1964; Ritts et al., 1964; Schofield et al., 1968) and is thought to be associated with transport of the lipopolysaccharide to the cells of the reticulo-endothelial system (Rowley et al., 1956; Braude et al., 1958; Cooper and Cranston, 1963).

If a similar foetal white cell response should occur after a maternal injection of pyrogen, this would be good evidence that the endotoxin is crossing the placenta and directly affecting the foetus. However, when pyrogen was administered to the ewe so that both the ewe and the foetus developed a fever, no change in the number of circulating white cells was observed. This could be considered as evidence that endotoxins do not cross the placenta. They do not cross from foetal to maternal circulations to any significant extent, since pyrogen injected into the foetus does not cause fever in the ewe.

Finally, it would appear that, following endotoxin injection

into the ewe, maternally produced endogenous pyrogen does not cross the placenta and stimulate heat production and conservation in the foetus. The evidence that this does not occur to any great extent is that infusion of salicylate into the foetus during maternal fever did not change the temperature gradient between ewe and foetus. It has been shown that the reduction in febrile body temperature after salicylate is administered is brought about through the inhibition of heat production and an increase in heat loss (Pittman et al., 1976). If the lamb foetus was indeed febrile as a result of the action of endogenous pyrogen on its thermoregulatory system, the presence of salicylate should have reduced heat production and increased heat loss. The decreased heat production, in particular, should have been reflected in a reduction in the temperature difference between foetus and ewe. Since no such change was observed, one may conclude that the fever seen in the foetus after maternally administered endotoxins is not due to endogenous pyrogen crossing the placenta and directly elevating foetal temperature. Rather, maternal fever causes a passive fever in the foetus due to a build-up and storage of heat in the foetus.

It is still uncertain as to whether the lack of fever in the foetus after direct endotoxin administration is due to the inability of the foetus to overcome the 'heat sink' of the ewe's body or is due to an immaturity in the response to pyrogen. If such an immaturity does exist, it does not appear to be in the ability of the foetal white cells to recognize the injected pyrogen.

III. NEWBORN STUDIES

A. Effect of Bacterial Pyrogen and Endogenous Pyrogen on Temperature Regulation in the Newborn Lamb.

It would appear that the newborn lamb is naive with respect to bacterial endotoxins (Landy and Weidanz, 1964). Consequently, if the response to bacterial pyrogens is associated with a state of hypersensitivity (Stetson, 1961; Watson and Kim, 1963) the newborn lamb might not develop a fever when first exposed to bacterial pyrogens. The following experiments were carried out to determine if newborn lambs respond with fever after intravenous endotoxin or endogenous pyrogen.

Methods

Lambs were born within the medical vivarium or, in some of the initial experiments, on a nearby sheep farm. Lambs were of mixed breeds, but showed characteristics of Suffolk, Finn, Columbia and Dorset breeds. They were allowed to suckle immediately after birth and remained with the ewe in small lambing pens during the experimental period.

Pyrogen

The bacterial pyrogen used in the present study was SAE pyrogen. In adult sheep, 0.3 μg of this pyrogen caused a fever of 0.7-1.0° C.

Endogenous pyrogen (leucocyte pyrogen) was prepared from adult sheep blood collected from the external jugular vein into sterile, pyrogen-free, heparinized plastic syringes. The blood was centrifuged

at 2000 g, the supernatant plasma withdrawn and the cellular constituents were resuspended in 0.9% saline. A white cell count was carried out and SAE pyrogen was added in a concentration of 1 µg/25 x 10^6 white cells. This mixture was incubated for 3 h at 37° C, and then centrifuged, and the supernatant fluid containing the leucocyte pyrogen was removed and stored in ampoules. Its pyrogenicity was tested in adult sheep and a standard cross-over procedure (Murphy et al. 1971) was utilized to compare the leucocyte pyrogen fever with that produced by bacterial pyrogen.

Experimental Procedure

Measurement of rectal temperature. Rectal temperature was measured by means of a thermistor probe (YSI, Type 401) inserted into the rectum to a depth of 10 cm and held in place by adhesive tape wrapped around the tail. The potential from the thermistor bridges was either read directly off a Yellow Springs Telethermometer or recorded on a Beckman type R411 Dynograph Recorder.

Administration of pyrogen to newborn animals. In newborn lambs, injections were given aseptically into the external jugular vein after 1 h of baseline temperature recordings. SAE pyrogen was given to some lambs in a dosage of 0.3 µg in 3 ml of sterile, pyrogen-free saline; other lambs received 1 ml of leucocyte pyrogen mixed with saline to a volume of 3 ml. Rectal temperature was recorded for 90 min after the injection.

Results

Fever in newborn lambs

When twenty-one lambs were challenged 4 h after birth, each with 0.3 μg SAE pyrogen, none became febrile (Fig. 7, Fig. 8, upper). Eight of these lambs received a second challenge when they were 48 h old and again they did not develop fevers. Seven of the lambs which had been challenged when 4 h old received second injections of endotoxin at 60 h, and they developed fevers averaging 0.79°C, and illustrated in Fig. 8 (lower record). In this study another group of seven lambs did not receive their first injection of bacterial pyrogen until 60 h after birth. As illustrated by the histogram in Fig. 7 and the temperature record in Fig. 8 (middle) they did not become febrile. Sterile pyrogen-free 0.9% NaCl solution did not cause fever when injected intravenously at any time after birth. In addition to the experiments reported in this initial study, a number of other lambs received a first injection of bacterial pyrogen at various ages after birth. The proportion of lambs which became febrile out of the total number injected with SAE pyrogen at ages 4 to 12 days is recorded in Table I. It can be seen that even up to as old as 12 days post-partum one lamb did not develop fever after intravenous SAE pyrogen. Often, in lambs that did develop fever in response to their first injection with bacterial pyrogen, the resulting fever was of considerably smaller magnitude than was that observed in lambs that had been previously injected with endotoxin.

When leucocyte pyrogen was injected 4 h after birth, into four lambs, only one developed fever. The dose of leucocyte pyrogen was such as would cause a fever of about 1°C in adult sheep. Of two 48 h

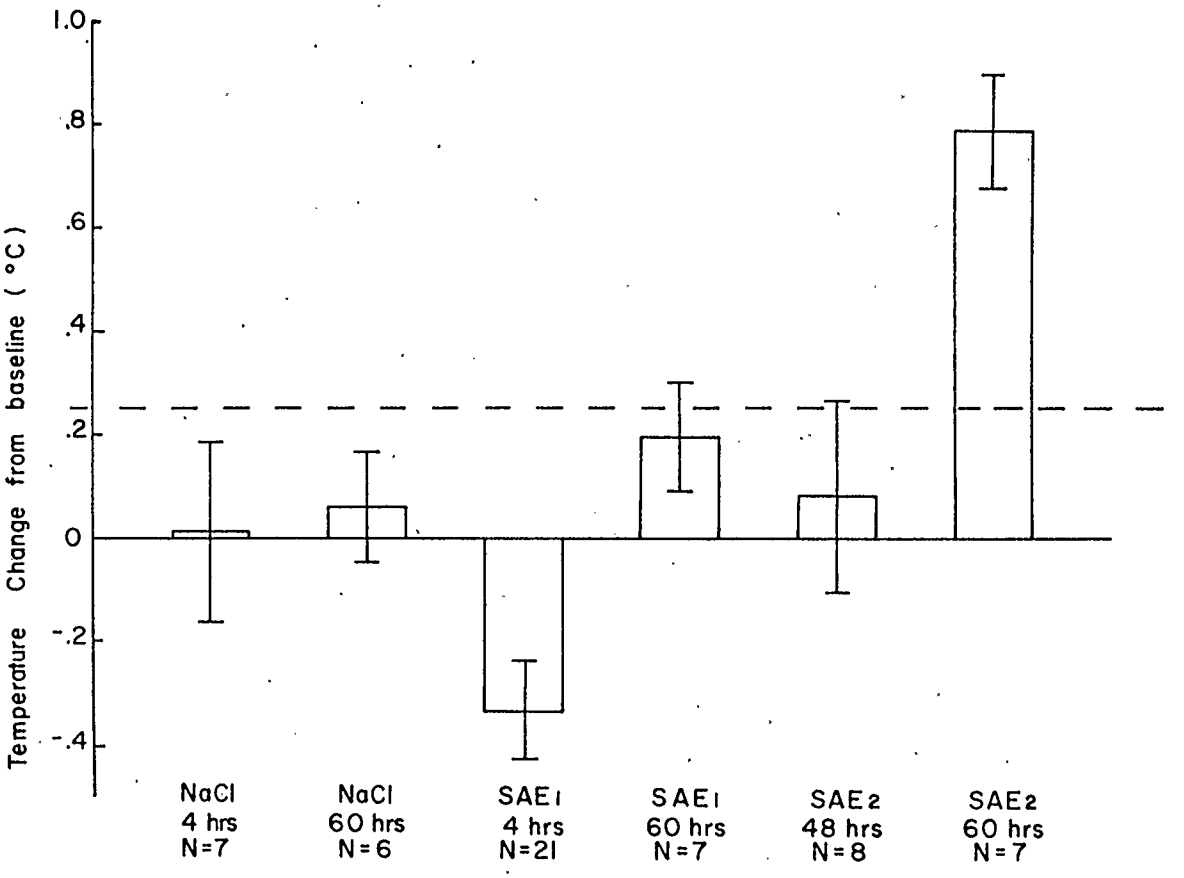

Figure 7. Responses of newborn lambs to intravenous injection of 0.3 µg SAE or 0.9% NaCl at various times after birth. The subscript (SAE_1) refers to first and SAE_2 to the second challenge. Bars and thin vertical lines represent means and twice the standard error of the means (SEM). The dashed line shows the upper limit of random temperature fluctuations. "N" denotes the numbers of animals.

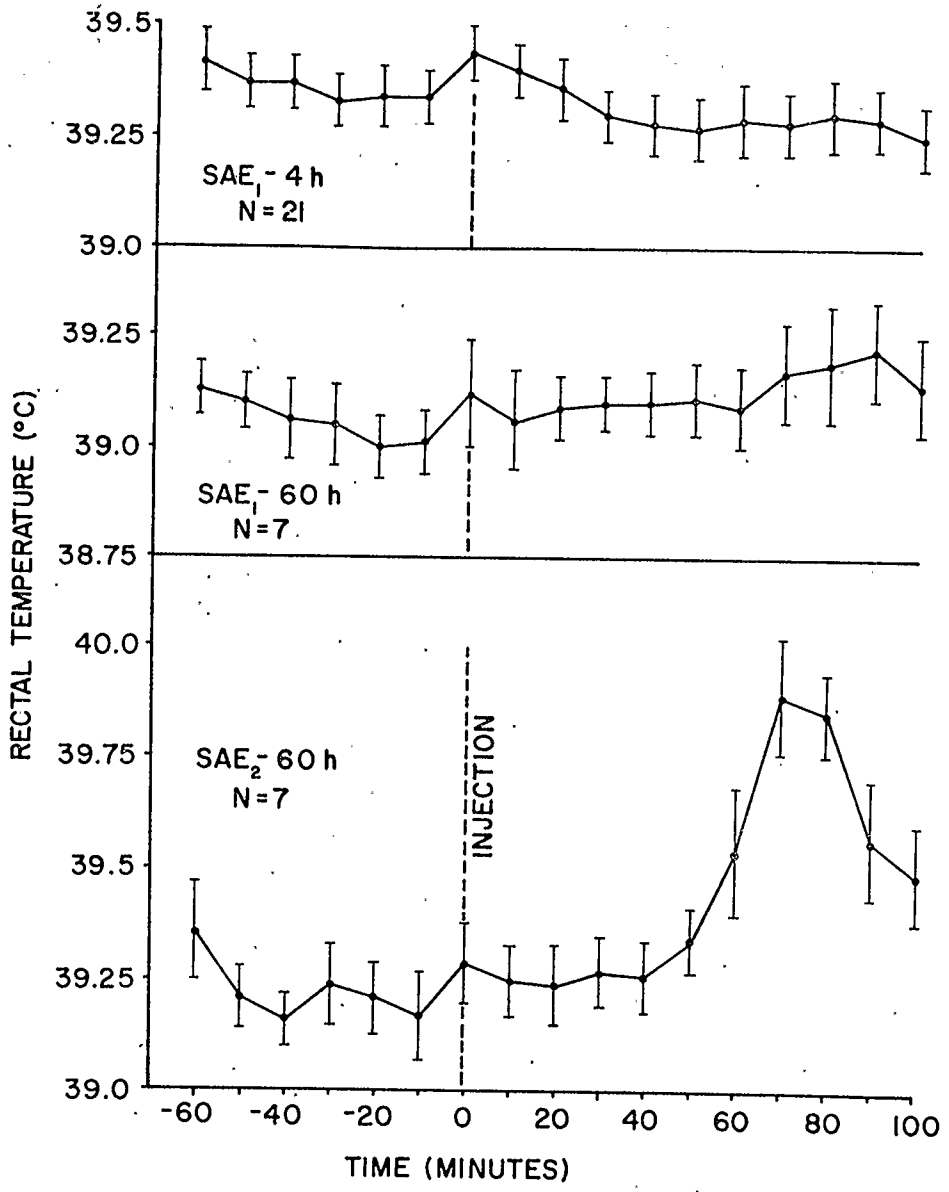

Figure 8. Records of means of body temperature of lambs after the intravenous administration of 0.3 µg SAE pyrogen at time 0, indicated by the broken line. The top record is the temperature response of twenty-one lambs injected with SAE pyrogen for the first time 4 h after birth. The middle record is the temperature response of seven lambs injected with SAE pyrogen for the first time at 60 h post partum. The bottom trace is the temperature response of seven lambs previously injected at 4 h and re-injected with SAE pyrogen at 60 h after birth. Vertical bars represent twice the SEM.

TABLE 1. Responses of newborn lambs at various ages after intravenous injection of bacterial pyrogen (SAE pyrogen) and endogenous pyrogen. The denominator indicates the total number of lambs at that age receiving their first injection. The numerator shows the number of these injected lambs which developed a fever of more than 0.3°C in the 90 min following the injection.

AGE	BACTERIAL PYROGEN	ENDOGENOUS PYROGEN
4 h	0/21	1/4
48 h	-	1/2
60 h	5/15	3/5
4 days	4/7	3/6
5 days	1/3	1/1
6 days	6*/11	-
12 days	0/1	1/1

*Tails had been amputated 3 days earlier on three of these lambs

old lambs given a first injection of leucocyte pyrogen, one developed fever and the other did not. As shown in Table I, a few lambs injected with leucocyte pyrogen at various times after birth became febrile, whereas others of the same age did not develop fevers in the 90 min period after the injection.

The temperature records of a group of lambs after leucocyte pyrogen injection are illustrated in Fig. 9 along with the temperature responses of a number of lambs receiving SAE pyrogen. In newborn lambs, both leucocyte pyrogen fever and bacterial pyrogen-fever resulting from the dosage we used are characterized by a steep rise in temperature and rapid defervescence. Over a range of ambient temperatures ($-2^{o}C$ to $+ 21^{o}C$) the increase in body temperature was accompanied by marked shivering and the lamb often assumed a tightly curled-up position. Defervescence was characterized by an increase in respiratory rate and the adoption of a relaxed, spread-out posture. Often the ear temperature increased at the onset of defervescence. There did not appear to be any obvious correlation between the height of a fever and ambient temperature or the conditions under which the experiments were performed.

The distribution of the times between injection and the onset of fever after both leucocyte and bacterial pyrogen are illustrated in Fig. 10. It can be seen that lambs responding to bacterial pyrogen have a fever with a longer latency of onset than do lambs receiving leucocyte pyrogen. The mean onset times of the fevers are distinct ($P < 0.01$), and this provides evidence that the injected material was most likely to be leucocyte pyrogen and not residual bacterial pyrogen in the supernatant

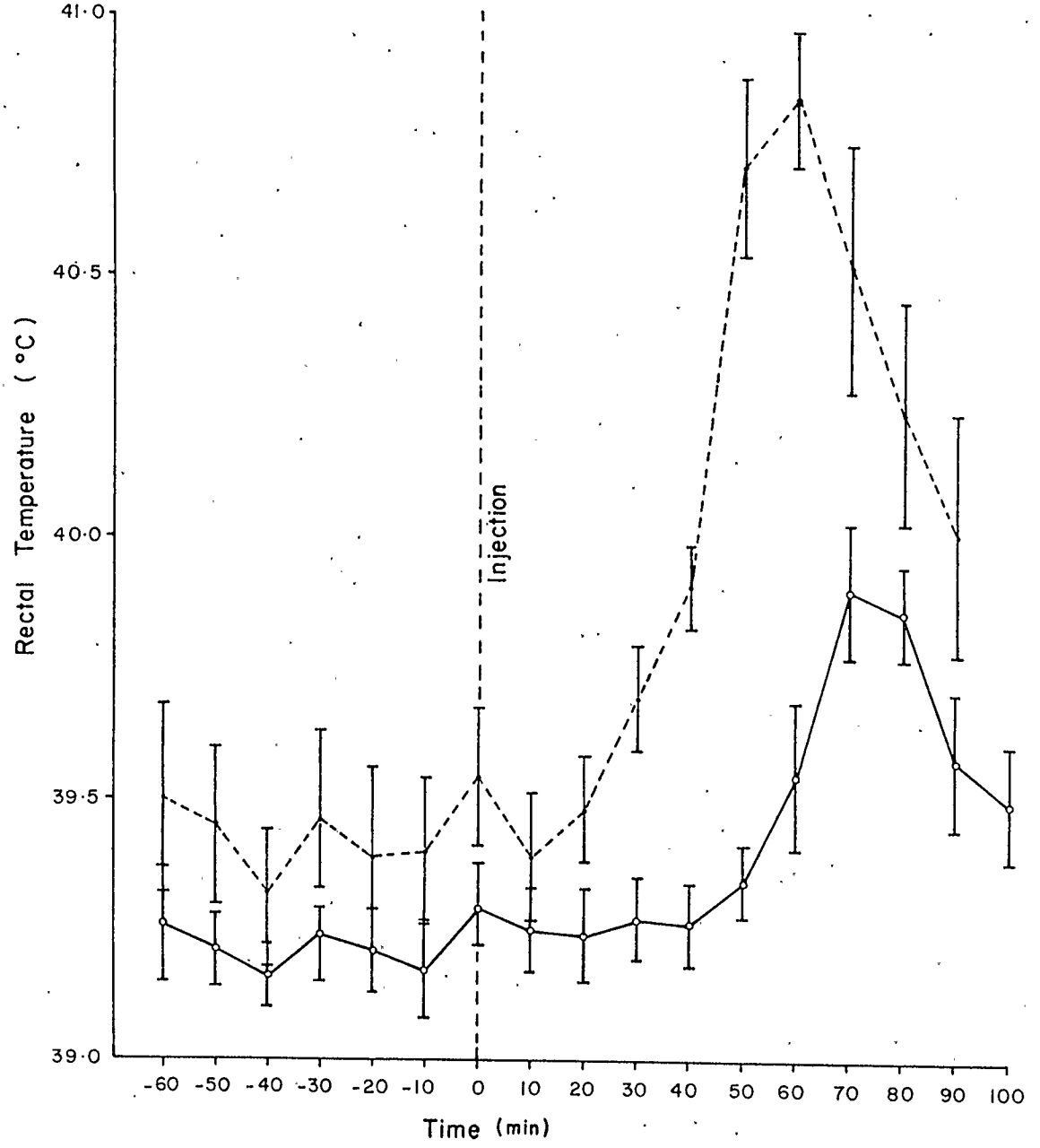

Figure 9. Record of mean body temperature response of ten lambs at 60 h to 12 days age receiving 0.3 µg SAE pyrogen (solid lines) and twelve lambs receiving endogenous pyrogen (broken lines) at time 0. Vertical lines represent twice the SEM..

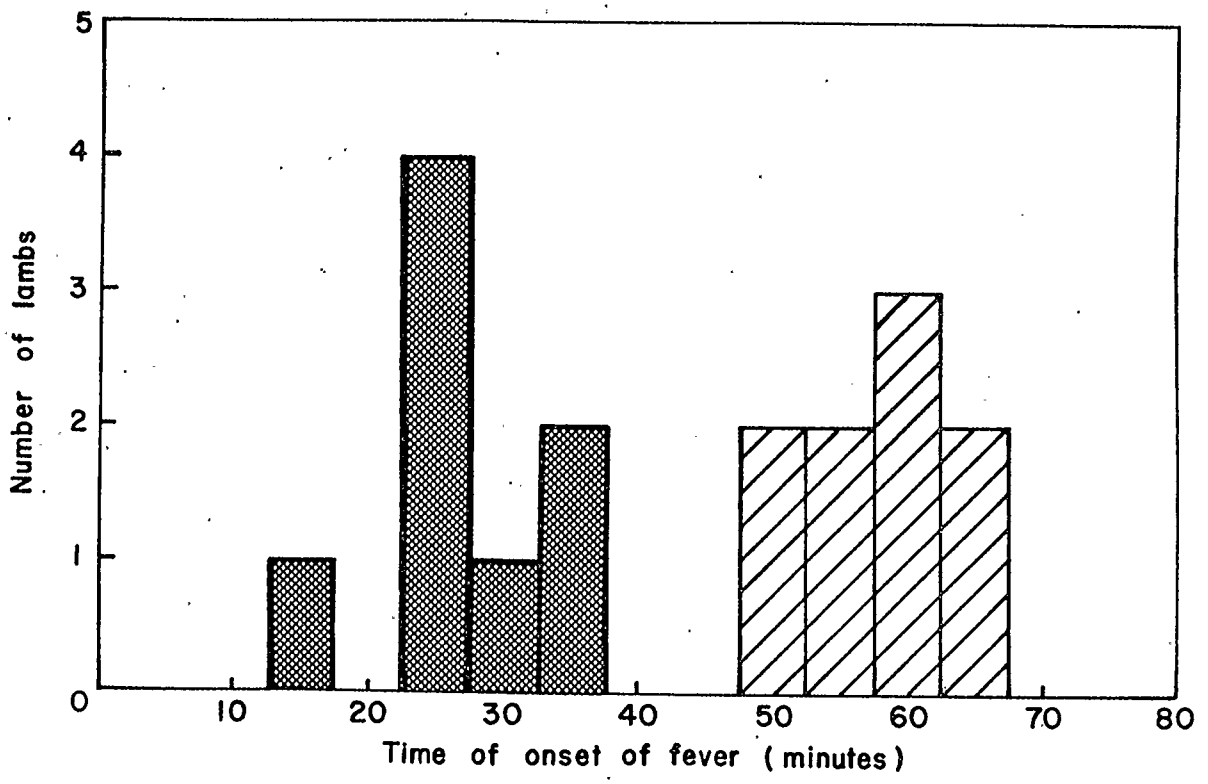

Figure 10. A histogram showing the times of onset of fever in lambs injected with endogenous pyrogen (dark blocks) or bacterial pyrogen (slanted lines).

fluid. Admittedly, the greater magnitude of the leucocyte pyrogen fevers may be related to their short latency periods, though in individual animals, bacterial and leucocyte pyrogen fevers still showed different latencies, even though fever heights were similar. To investigate this further, each of six adult sheep was given intravenous bacterial and leucocyte pyrogen. The bacterial pyrogen fever, with a mean height of 0.67°C (SEM 0.14, n = 6), and the leucocyte pyrogen fever (0.66°C, SEM 0.02, n = 6), were of nearly identical magnitudes, yet latency periods were similar to those observed in newborn lambs (Fig. 11). It appears, therefore, that in sheep, leucocyte pyrogen fevers can be differentiated from bacterial pyrogen fevers on the basis of the latency between injection time and onset of fever.

Discussion

It is evident that the newborn lamb at 4 h age does not respond with fever in response to an initial injection of bacterial pyrogen. As it becomes somewhat older (i.e. 60 h age) there is an increasing tendency for a fever to develop when first exposed to intravenous endotoxin. Blatteis (1975) has shown that guinea pigs up to eight days of age do not develop fever when first exposed to intravenous pyrogen. Failure of the neonatal lamb to develop a fever may be attributed to an inability of the cells of the body to produce an endogenous pyrogen, or to an immaturity of that part of the brain which in the adult mediates the response to pyrogen by activating heat-production pathways. It is doubtful that the heat-production pathways themselves are deficient since we have observed shivering in both the newborn lamb and the foetus, and the lamb can

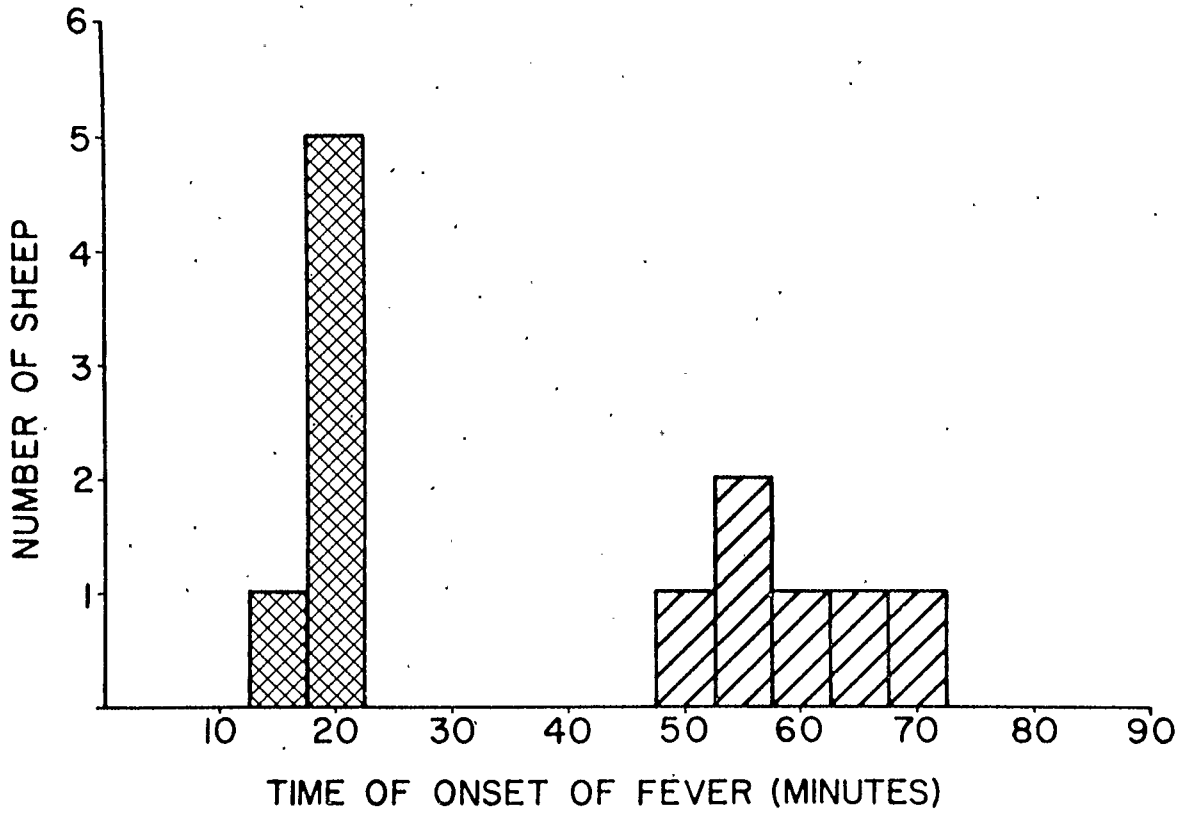

Figure 11. Time of onset of fever in six adult sheep injected with a standard dose of endogenous pyrogen (cross-hatched bars, left) and with 0.3 µg SAE pyrogen (diagonally-hatched bars, right).

maintain its temperature in a cold environment. When leucocyte pyrogen, which is reported to act directly on the hypothalamus (Cooper, 1965; King and Wood, 1958), was given by intravenous injection to the lamb 4 h post-partum, fever generally did not develop, suggesting that at this age the brain mechanisms for the febrile response are still immature. Perhaps a higher dosage of leucocyte pyrogen would have caused fever in these lambs, for Blatteis (1976b) has recently shown that fever could be induced in guinea pigs at birth, but the required dose of both bacterial and leucocyte pyrogen was greater. Nevertheless, his results and those presented in this thesis would suggest an immaturity of the hypothalamic mechanisms involved in fever production.

The results from 60 h old lambs, however, indicate that a sensitization process may be required for the maturation of the response to bacterial pyrogen. Many of the lambs injected for the first time with endotoxin at this age did not become febrile, whereas fever occurred in all instances if the lambs had been exposed to the pyrogen at 4 h age. It is to be expected that, as lambs grow older, they would be naturally sensitized by pyrogens released from bacterial populations which are known to inhabit the gut as early as the first day after birth (Smith, 1965). It has been suggested that endotoxins could also enter the bloodstream via the alveolar membranes in the lungs (Cooper, 1971b) and induce sensitization. Thus it is not inconsistent with the idea of a "sensitization" phenomenon that an increasing number of lambs should develop fever in response to an initial endotoxin injection in the

older age groups. Indeed, three of the lambs that had been injected at 6 days age (Table I) and developed fever had had their tails amputated. This procedure, occurring 3 days previously, may have been accompanied by a mild infection which could have sensitized the animals. It is of interest that this sensitization may not have occurred in lambs as old as 12 days, yet 3 h after they failed to develop fever to bacterial pyrogen, they were able to respond with a large fever after intravenous leucocyte pyrogen.

The results suggest that the part of the brain responsible for producing fever matures in the first few days of life. Even in lambs up to 4 days old, iv leucocyte pyrogen sometimes failed to produce fever. Thus lambs only 48 h old, even though sensitized when 4 h old, failed to develop a fever. There did not appear to be any correlation between the ability of the lamb to develop fever after leucocyte pyrogen and its apparent maturity as subjectively evaluated by weight, activity and alertness. Furthermore, Dawes and Parry (1965) have observed that lambs must usually be at a gestational age that is 95% of term in order to be viable. Thus, it would appear that the maturational process occurs over the first few days of life, and cannot be accounted for by a general prematurity of the lambs.

Thus, evidence would suggest that the febrile process is immature at birth even to endogenous pyrogen; superimposed on the developmental process is the requirement for a "sensitization" to bacterial pyrogen which may occur independently of the maturation of the response to endogenous pyrogen. The results do not indicate

what is the component of the fever pathway that requires sensitization, whether it involves the white cells, the reticulo-endothelial system or some activating factor in the plasma. The results from the studies carried out on foetal lambs, *in utero*, in which a considerable decrease was observed in the number of circulating leucocytes after endotoxin administration, would suggest that the white cells appear to recognize the injected lipopolysaccharide. Evidence ought to be obtained on the ability of the tissues of the newborn lamb to generate endogenous pyrogen in response to a first exposure to endotoxin. However, it is known that leucocytes from human neonates (Dinarello, 1975) and newborn guinea pigs (Blatteis, 1976) are capable of elaborating this substance when incubated *in vitro* with bacterial pyrogen. Thus, in these species, this part of the fever pathway appears to be functional at birth.

B. Sensitization Studies

The development of the ability to produce a fever in response to bacterial pyrogen appears to require an activation, or sensitization of some component of the body. A series of studies were undertaken, therefore, to attempt to define some of the requirements for sensitization and also to determine what components of the febrile process are involved in sensitization.

Even though the newborn lamb does not develop fever after a first injection of bacterial pyrogen, there is circumstantial evidence that the white cells recognize the injected pyrogen and remove it from the circulation. It is of interest, therefore, to

find out if the white cells are capable of synthesizing and releasing endogenous pyrogen. Before any conclusions could be made with respect to the ability of neonatal leucocytes to generate endogenous pyrogen it was necessary to be able to reliably produce it from adult leucocytes. Consequently, a number of different methods were used in an attempt to stimulate leucocytes from both newborns and adult sheep to produce detectable amounts of endogenous pyrogen.

It was also of interest to develop an assay for the detection of circulating endogenous pyrogen in vivo as an approach to determine if lambs are capable of producing an endogenous pyrogen when first challenged with endotoxin. Circulating endogenous pyrogen has previously been demonstrated in rabbits made febrile with intravenous bacterial pyrogen (Atkins and Wood, 1955; Grant and Whalen, 1953). However, those studies involved the transfusions of large amounts of plasma, and in the present experiments it was preferable not to have to exsanguinate the lambs. The present studies represent an attempt to demonstrate a circulating endogenous pyrogen in sheep by plasma injection into the AH/POA of rabbits. It has been shown that rabbits become febrile after intrahypothalamic injection of human leucocyte pyrogen, prepared in vitro (Cooper and Roark, 1972). When injected in this manner, rabbits responded to 100-fold dilutions of human leucocyte pyrogen with prompt fevers, and the method has been used to detect circulating endogenous pyrogen in febrile humans (Cooper et al. 1976).

Bacterial pyrogens are strongly antigenic and have highly

specific side chains carrying the immunological specificities (Work, 1971). The anamnestic type response involved in the development of the febrile response to pyrogens implies that some moiety of the molecule must be recognized on second exposure. If this is the specific side chain part of the molecule, "sensitization" should not occur between different types of endotoxins. To test this hypothesis, lambs were sensitized with one type of pyrogen than challenged at a later date with an unrelated type of pyrogen.

The sensitization process was further investigated to determine if the factors involved in the recognition process are present in the plasma. An example of such a possible factor is a humoral antibody. If these factors do indeed appear in the bloodstream after exposure to pyrogens it may be possible to transfer the "sensitization" to a naive animal. This was attempted through the transfer of plasma from sensitized animals into lambs that were naive with respect to bacterial pyrogens.

Finally, some possible routes were examined through which newborn lambs could come into contact with endotoxins and become sensitized. In the non-experimental setting, lambs must become naturally sensitized; one possible route of sensitization in the alimentary tract and an attempt was made in newborn lambs to induce sensitization via this approach.

In a number of instances, foetal animals may become exposed to pyrogens <u>in utero</u> as a result of transplacental transmission or by ascending infection through the cervix (Benirshke, 1960; Klein and

Marcy, 1970; Davies, 1971; Gamsu, 1973). It is possible that such prenatal exposure to pyrogens may sensitize the foetus so as to permit it to respond with fever following intravenous pyrogen injection after birth. This hypothesis was tested by injecting pyrogen iv into foetuses, in utero then challenging them with pyrogen after birth.

Methods

1) Production of endogenous pyrogen

Initially, a modification of the procedure developed by Murphy (1967) was used to produce the endogenous pyrogen which was used in the studies reported in the previous section. Adult sheep blood was collected from the external jugular vein into sterile, pyrogen-free heparinized syringes. The blood was centrifuged and the cellular constituents were suspended in 0.9% NaCl and incubated for 3 h at 37°C with 1 µg SAE pyrogen/25 x 10^6 cells. The mixture was then centrifuged and the supernatant fluid containing the endogenous pyrogen was removed. All glassware, syringes and solutions were sterile and pyrogen-free. Pyrogen-free solutions were prepared by filtration through a molecular filter (Millipore, PSED 2510) which only passed molecules with a molecular weight of under 60,000. Glassware was made pyrogen-free by baking for 3 h at 200°C.

Blood was taken from 7 different lambs at 4 h age and the cells were incubated with either SAE pyrogen or typhoid-paratyphoid vaccine using the procedure just described. Supernatant from these mixtures was injected iv into adult sheep and temperatures were measured.

Because problems were encountered with the above procedure, a

number of modifications were tried. The cells were left suspended in plasma or pyrogen-free Krebs solution instead of saline during the incubation period. Secondly, the concentrations of bacterial pyrogen were varied between 0.001 µg and 1 µg SAE pyrogen/25 x 10^6 cells. Also, incubation times were lengthened to up to 24 h at 37^oC, and, after centrifugation, the supernatant was tested for pyrogenicity by intravenous injection of 5 or 10 ml aliquots in adult sheep. On other occasions, different pyrogens were used to stimulate the leucocytes. Typhoid-paratyphoid vaccine (Connaught, Toronto, Canada) or E.coli endotoxin (Difco) were incubated with the cells for varying lengths of time from 3-24 h.

In case the presence of heparin could be inhibitory to the production of endogenous pyrogen, one sample of adult sheep blood was drawn with ethylene diamine tetraacetic acid (EDTA) as anticoagulant instead of heparin and then incubated with pyrogen.

Fessler et al. (1963) provided evidence that administration of some antibiotics to rabbits made their leucocytes incapable of producing leucocyte pyrogen, in vitro. It was possible that the feed of the sheep may have been treated with an antibiotic which could inhibit the in vitro process of pyrogen formation. Therefore, the feed was tested for antibiotic activity. Ten grams of feed were crushed and let soak in about 40 ml of distilled water for 24 h. Two agar plates containing E.coli cultures were obtained from the bacteriology lab at the Foothills Hospital. E.coli are sensitive to the type of antibiotic that has previously been shown to inhibit leucocyte pyrogen production (Fessler et al., 1963). Tiny discs

of filter paper were soaked in the feed mixture then placed in the agar plates. Following incubation for 12 h or 24 h at 37°C, the plates were examined for antibiotic activity.

In further attempts to produce leucocyte pyrogen from adult sheep blood, a procedure based on that of Dinarello et al. (1974) was adopted. Heparinized sheep blood was centrifuged at 1500 g for 3 min and the supernatant containing the white cells was removed and a white cell count was done. The mixture was then spun at 4°C for 10 min at 180 g and the leucocytes were resuspended in Hanks Balanced Salt Solution (HBSS) with 10% AB human serum, 100 U of penicillin G and 2 U of heparin/ml (20×10^6 white cells/ml). The mixture was incubated at 37°C for 5 min, then E.coli endotoxin (0.003 µg/25×10^6 cells) was added and incubation at 37°C was continued for a further 30 min in a shaking water bath. This suspension was then centrifuged for 10 min at 180 g and the cells were resuspended in serum-free HBSS (5×10^6 cells/ml). This was incubated for a further 18 h at 37°C, then centrifuged and the supernatant tested for pyrogenicity in adult sheep.

Since the attempts to produce endogenous pyrogen from blood leucocytes were met with limited success, leucocytes were isolated from peritoneal exudates and stimulated for pyrogen production. This procedure was modified from that devised by Fessler et al. (1961) for collection of peritoneal exudates from rabbits. Exudates were produced by injecting sterile, pyrogen-free 3% starch in saline solution into the peritoneal cavity of a healthy sheep. Fifteen hours later, the

ewe was anesthetized with halothane and 2 liters of sterile, pyrogen-free saline, warmed to 37°C, was injected in the same manner. Then, the ventral surface of the abdominal wall was shaved and sterilized with tincture of Zephiran, and a hole was made with the use of a cannulation set, 6 cm lateral to the umbilicus. A sterile, pyrogen-free hollow tube was inserted in the wound and the liquid in the peritoneum allowed to flow by gravity into 1 liter flasks packed in ice. The hole in the abdomen was sutured shut with silk and the ewe was given penicillin intramuscularly. The sheep appeared in good health after this procedure.

The exudates were spun at 1500 g at 4°C for 20 min and the supernatant was removed and discarded. The cells were washed in cold saline, then centrifuged and suspended in 20 ml of saline. A white cell count was taken and leucocyte yield calculated. SAE pyrogen was added to the mixture in a concentration of 0.1 µg pyrogen/20 x 10^6 cells. This mixture was incubated in a waterbath for 6 h at 37° C. The cells were then spun off and the supernatant added to an equal volume of saline before testing for pyrogenicity in two adult sheep.

2) Detection of endogenous pyrogen

Preparation of assay animals - Twenty-three rabbits were prepared for microinjection studies after the method of Cooper et al. (1965). Under sodium pentobarbitone anaesthesia and aseptic conditions, the skin over the skull was incised and a stainless-steel headplate (Monnier and Gangloff, 1961) was fixed to the skull. After the plate was attached, the holes above the AH/POA region were drilled

out and the skin was sutured around the headplate. Following surgery, all animals were allowed a minimum of 5 days post-operative recovery before any experiments were begun. During this time period, the rabbits were trained to sit quietly in conventional restraining stocks.

Experimental Procedure - Rectal temperatures of the rabbits were measured with thermistors inserted 10 cm beyond the anus and taped to the tail. A continuous written record was maintained for 1 h prior to and 2 h after injection. For injection, a stainless-steel guide plate was affixed to the headplate and a modified push-pull cannula (Myers, 1970b) was lowered through the plate to the appropriate depth for the AH/POA (Veale, 1972). The injection cannula consisted of an inner (22 gauge) cannula that protruded 4 to 6 mm beyond the outer (20 gauge) guide cannula. The inner cannula was attached to a length of polyethylene tubing (PE-20) which was connected to a 10 µl Hamilton syringe mounted on a Harvard infusion withdrawal pump. The injection assembly was stored in tincture of Zephiran and before use was flushed with sterile, pyrogen-free saline and loaded with the injectate. For injection, a volume of 0.5, 1.0 or 2.0 µl was infused at a rate of 1.0 µl/min after which the cannula was left in place for an additional 30 secs. It was then removed, tested for flow, placed in the contralateral site and the procedure was repeated.

The plasma for injection was obtained from 3 adult sheep. Fevers were induced in these sheep with intravenous SAE pyrogen in amounts of 1.5 µg or 15.0 µg. Ten ml blood samples were withdrawn from the

external jugular vein into heparinized, pyrogen-free syringes during the rising phase of the fever, on two occasions, and on another occasion during a secondary temperature increase beginning approximately 2 h after the injection. The blood was centrifuged at 1500 g and the supernatant plasma immediately removed for injection into the rabbits.

3) Cross-sensitization

The pyrogens used in this series of experiments were the SAE lipopolysaccharide, typhoid-paratyphoid vaccine (Connaught) and a killed cell suspension of Yersinia enterocolitica prepared for the experiment. The dose of pyrogenic material injected was tested in adult sheep and, for each type of pyrogen, an amount was injected which was found to give approximately a 1^0 C fever in adult sheep. All injections were given in a volume of 3 ml of sterile, pyrogen-free saline into the external jugular vein of the lamb. Rectal temperature was measured as previously described for 60 min before, and 90 min after the injection.

4) Plasma transfer

At 4 h age each lamb was given an intravenous injection of either adult sheep or foetal lamb plasma. The quantity of plasma administered was either 50 ml or 20% of the circulating blood volume, which ever volume was the lesser. The relative blood volumes were estimated from the weight of the lambs after the method of Pipkin and Kirkpatrick (1973). The injection was given over a period of approximately 5 min. In most instances, the lambs were gently restrained for the injection procedure, however, in some cases, the more active lambs were briefly anesthetized with halothane gas. The 5 min period

under anesthesia did not appear to have any noticeable effect on the febrile responses of the lamb at a later date. At 60 h age, rectal temperature was recorded as previously described and the lambs were given an iv injection of SAE pyrogen (0.3 µg). Some lambs which had not been previously injected with plasma were given their first injection of pyrogen at 60 h age, and those served as controls. Where it was possible, one of a set of twins was given plasma, and its sibling served as the uninjected control.

The adult plasma used in this study was obtained from the mother of each lamb. An appropriate quantity of blood was withdrawn from the external jugular vein into heparinized syringes. It was immediately centrifuged at 1500 g, then the supernatant plasma was removed for injection into the lamb. At all times, the usual precautions were taken to avoid pyrogen contamination of the plasma.

The foetal plasma was obtained from lamb foetuses at ages 132 days to birth. In most cases, the source was a foetus which was removed because of the death, in utero, of its twin. The ewe was anesthetized with sodium pentobarbital, the uterus opened and the foetal lamb removed. If it was judged to be viable and healthy, it was exsanguinated by cardiac puncture. The heparinized blood was centrifuged and the plasma stored in pyrogen-free glass vials at -70^{0}C until needed. At this time, it was heated in a waterbath to 37^{0}C then injected into the 4 h old lamb.

5) Route of sensitization - gastrointestinal tract

Two lambs were each given 15 µg of SAE and another two were given 30 µg SAE pyrogen intragastrically at 4 h age. The pyrogen was

mixed in 3 ml of saline and given by intragastric tube to ensure that all of the pyrogen reached the stomach. At 60 h age, rectal temperature was recorded in these 4 lambs and 0.3 µg SAE pyrogen in 3 ml of sterile, pyrogen-free saline, was injected into the external jugular vein.

An additional four lambs were each given 50 µg of SAE pyrogen, in 1 ml of saline into the colon. The pyrogen was administered through a small tubing that was inserted 10 cm beyond the anus. These 4 lambs were then tested in the usual manner for their ability to develop fever at 60 h age following intravenous SAE pyrogen (0.3 µg).

6) Sensitization in utero

At a gestational age of 110 days, catheters were inserted into foetal carotid and jugular vessels. The surgical procedure involved for exposing the foetus was identical to that described earlier. Following recovery from surgery and at specific intervals before birth, each foetus was injected intravenously with SAE pyrogen (0.3 µg), and then allowed to continue undisturbed until birth. This study also utilized foetal preparations that had been involved in the studies reported earlier on the effects of direct administration of pyrogen on the foetus. At 60 h postnatal, the lambs were given a second intravenous injection of 0.3 µg SAE and body temperature was recorded. The age of 60 h was chosen because it had been shown that lambs sensitized at 4 h age could develop fever at 60 h age, whereas at younger ages, even if sensitized, the lambs often did not develop fever.

Results

1) Production of endogenous pyrogen

One attempt to produce endogenous pyrogen from sheep blood proved successful. The pyrogen was produced by the procedure developed by Murphy (1967) and when tested in adult sheep, 1 ml gave a fever with a magnitude of approximately $1^{0}C$ and a latency of onset of 15-20 min. This contrasted with a latency of onset of 50-70 min which was observed after iv endotoxin and is good evidence that the injected material contained an endogenous pyrogen, rather than residual bacterial pyrogen.

Subsequent attempts to produce endogenous pyrogen by this method proved unsuccessful and iv injections of as much as 10 ml of the supernatant were non-pyrogenic in adult sheep. It was thought that the heparin could have had an inhibitory effect on the formation or stability of the endogenous pyrogen. However, when EDTA was used as an anticoagulant instead of heparin, and the blood was then incubated with endotoxin, the supernatant again proved non-pyrogenic.

When the feed was tested for antibiotic activity by observing the effects of filter paper soaked in the feed mixture on an E.coli culture, no effect was seen. Thus, the inability to produce the endogenous pyrogen would not appear to be in the presence of antibiotics in the feed.

Other attempts to produce endogenous pyrogen by varying the method of Murphy (1967) also proved unsuccessful. These variations included changing the cell medium, the concentration and type of stimulating endotoxins and the length of incubation of the endotoxin-white cell mixtures.

The use of peritoneal exudate cells as a pyrogen source had been tested and found successful for rabbit endogenous pyrogen. However, when the procedure was tried with sheep exudates, the supernatant was non-pyrogenic in the adult sheep, even though large numbers of cells were collected.

Despite the lack of success in stimulating cells from adult sheep to produce endogenous pyrogen, an attempt was made to produce the substance from neonatal leucocytes. However, supernatants derived from 7 separate lamb cell preparations all were non-pyrogenic when injected in amounts of 5-20 ml into adult sheep.

2) Detection of endogenous pyrogen

In this series of experiments, all injection loci were initially tested for sensitivity to the hyperthermic effects of prostaglandin E_1 (Feldberg and Milton, 1973) injected in a quantity of 50 ng/side in 0.5 µl of sterile, pyrogen-free saline. In the 23 rabbits tested, this amount of prostaglandin caused a fever averaging 1.05 ± 0.09^0C (SE). When 9 rabbits were injected into these same loci with 1 µl of plasma taken from a non-febrile adult sheep, rectal temperatures rose by $0.38 \pm .07^0$C (SE). Thirteen rabbits then received 2 µl of plasma taken from a febrile sheep, and rectal temperature rose by 0.38 ± 0.11^0C. The blood was withdrawn from the sheep 40 min after intravenous injection of 15 µg of SAE pyrogen. At this time, rectal temperature of the animal had just begun to rise. On another occasion, a sheep was given 1.5 µg of endotoxin and fever was observed. This amount of pyrogen caused a fever which showed both an initial temperature

increase, then a secondary rise in temperature beginning 2 h after the injection. At this time, a blood sample was taken and the plasma injected in a 1 µl volume into the AH/POA of four rabbits. In the following 2 h, their temperatures rose by 0.43 ± 0.12^{0}C. Thus, the temperature change observed after injection of febrile sheep plasma, into the AH/POA of rabbits, did not differ significantly from that observed after injection of plasma taken from a non-febrile sheep.

3) Cross-sensitization

Two lambs received SAE pyrogen at 4 h age and remained non-febrile. When these lambs received typhoid-paratyphoid vaccine at age 60 h they both developed fevers. Similarly 2 lambs first exposed to typhoid-paratyphoid vaccine, then challenged at 60 h age with SAE also developed fever at this time (Fig. 12).

In another series of experiments, lambs were injected at 4 h age with either SAE pyrogen or the Y.enterocolitica killed cell suspension. None of these lambs became febrile. When these lambs were challenged at 60 h age with the substance to which they had not been previously exposed, 14 of 17 lambs developed fever.

4) Plasma transfer

In this series of experiments, lambs at 4 h age were given maternal or foetal plasma and then tested for their ability to develop fever at 60 h age. Four lambs which were given maternal plasma at 4 h age developed fevers averaging 1.57 ± 0.18^{0}C at 60 h age in response to 0.3 µgSAE pyrogen. Nine lambs were not given any infusions, but were given 0.3 µg SAE pyrogen at 60 h age. These lambs, which served as the control group, developed fevers averaging 1.03 ± 0.27^{0}C.

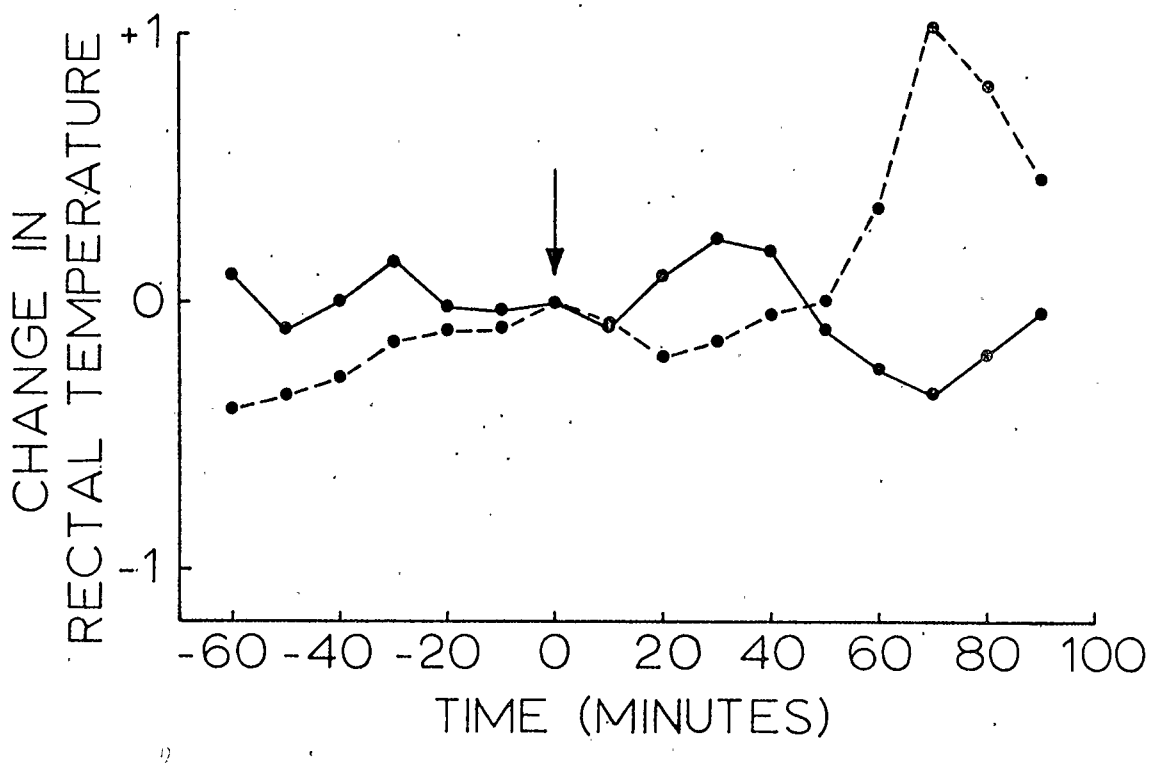

Figure 12. Temperature records of a lamb given intravenous typhoid-paratyphoid vaccine (solid line) at 4 h age and SAE pyrogen (broken lines) when 60 h old.

An additional 4 lambs were given infusions of foetal plasma at 4 h age, and when challenged with iv bacterial pyrogen when 60 h old, developed fevers averaging 1.38 $\pm$ 0.26^{0}C.

The temperature increases observed in each group were not significantly different from each other (Student t-test). However, when the temperature records of matched controls were examined, in many cases the lambs that had received maternal plasma developed greater fevers than did their siblings which had not been infused at 4 h age. For example, in a group of triplets, two lambs that had received plasma developed fevers of 1.4^{0}C and 1.3^{0}C, whereas the third lamb of the group which served as the control showed a temperature rise at 60 h of only 0.5^{0}C. In another case, the experimental lamb developed a fever of 1.45^{0}C, whereas its twin showed an increase of 0.6^{0}C. Similar differences were seen in yet another set of twins. Due to a scarcity of twins during the remainder of the study, in which further injections were given to lambs receiving foetal plasma, it was not possible to have data using paired controls. Consequently the observations are made on single lambs born at different times.

5) Route of sensitization - gastrointestinal tract

When 4 lambs which had received intragastric SAE pyrogen (15-30 µg) at 4 h age were given 0.3 µg iv at 60 h age, none of them developed fever in the 90 min period following the injection. When an additional 4 lambs which had been given SAE pyrogen into the colon were tested for their ability to develop a fever following intravenous pyrogen at 60 h age, one of the four developed a fever while the others

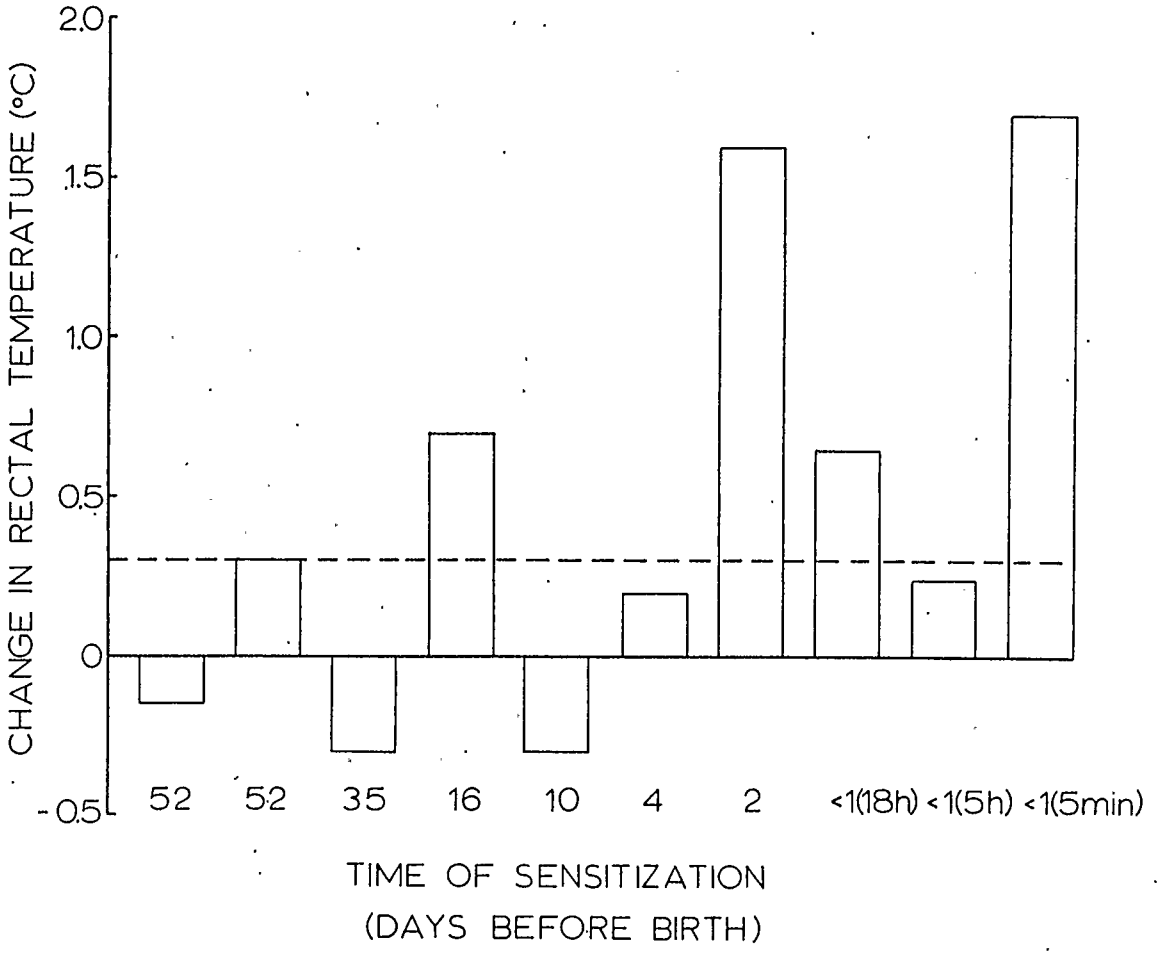

Figure 13. Temperature responses of ten lambs given 0.3 µg SAE pyrogen at 60 h age. Each of these lambs had previously been given an intravenous injection of 0.3 µg SAE pyrogen in utero at the times indicated along the lower axis. The broken line shows the upper limit of random temperature fluctuations.

remained non-febrile.

6) Sensitization in utero

The temperature responses of ten 60 h old lambs following iv endotoxin are shown in Fig. 13. These lambs had each received an injection of pyrogen at intervals of 52 days to 5 min before birth. It can be seen that four lambs which had been exposed to bacterial pyrogens at either 16 days, two days, 18 hours or 5 min before birth developed fever after the second injection. Six lambs that had been sensitized at intervals of 52 days to 5 hours before birth did not develop fever after the second injection. Thus 3 out of 4 lambs sensitized within the last 2 days of gestation were able to develop a fever at 60 h age, whereas only one out of six challenged before this age became febrile at 60 h age.

Discussion

Some newborn lambs have been observed which can develop fever to endogenous pyrogen, but remain non-febrile after bacterial pyrogen injection. Since the action of endogenous pyrogen is believed to be within the central nervous system (Cooper et al., 1965; Cooper, 1972) this would suggest that the sensitization process occurs in the peripheral tissues of the body. It is possible, therefore, that "sensitization" is involved in the development of the ability of the leucocytes and other body tissues (Atkins and Snell, 1964; Bodel and Atkins, 1967; Dinarello et al., 1968) to synthesize and release endogenous pyrogen. Indeed, when blood from 4 h old lambs was incubated, in vitro with bacterial pyrogen, the leucocytes did not produce any detectable endogenous pyrogen. This contrasts with the results of Blatteis (1976)

and Dinarello (1975) who showed with newborn guinea pig and human blood, respectively, that neonates of these species have the ability to generate endogenous pyrogen. However, the inability of the neonatal lamb leucocytes to produce endogenous pyrogen as demonstrated in the present experiments, and the possible requirement of previous exposure for this process, is open to question. The uncertainty arises from the problems that were encountered in producing endogenous pyrogen even from adult sheep leucocytes. With one exception, attempts to produce endogenous pyrogen from adult leucocytes were unsuccessful.

The reason for this inability to produce endogenous pyrogen is not known. It is possible that the leucocytes were, in fact, producing endogenous pyrogen, but that the test dose of supernatant was not adequate to produce fever in the recipient animal. However, supernatant from as many as 2.5×10^8 leucocytes constituted a single assay dose, in some instances, and no fever was observed in the recipient sheep. Other investigators have found that 10^6 or 10^7 rabbit white cells provide detectable endogenous pyrogen in rabbits (Atkins et al., 1967; Bodel, 1970, 1974). Even if the sheep is less sensitive to the endogenous pyrogen, it would seem that the 10-fold or more increase in dosage would be sufficient to produce fever in the present experiments.

It could be that the conditions under which the incubations of the white cells with the endotoxin were carried out were not ideal for pyrogen production. Even though there are no published procedures for the production of sheep endogenous pyrogen, similar techniques to those employed in the present experiments have been used successfully to

produce endogenous pyrogen from a number of species including rabbits (Murphy, 1967), humans (Cranston et al., 1956) and cats (Clark and Moyer, 1972). The variety of approaches utilized in the present studies incorporated many of those used successfully in other species and it would seem unlikely that conditions for synthesis and release of endogenous pyrogen should differ markedly in the sheep.

It could be that, in the sheep, the majority of the endogenous pyrogen comes from body tissues other than the leucocytes. Muscle tissue, heart, lung, spleen and kidney (Atkins and Snell, 1964) and liver Kupffer cells (Dinarello et al., 1968) have been shown to be capable of producing endogenous pyrogen in rabbits, and they could have an important role in pyrogen production in sheep. If so, the association of leucocytes with pyrogen, as demonstrated in vivo by neutropenia after endotoxin injection, is unexplained.

It is unlikely that the sheep develops fever without the involvement of an endogenous intermediate pyrogen. The long (45-60 min) latency seen after bacterial pyrogen injection into sheep is also seen in other species and is associated with the synthesis and release of endogenous pyrogen.

Based on the fact that a successful batch of endogenous pyrogen was made, one could tentatively suggest that sheep leucocytes do synthesize and release the molecule. The subsequent lack of success in producing endogenous pyrogen is still unexplained. It may be due to contamination from glassware or foodstuffs but several different procedures were used for washing glassware, without any success in pyrogen production.

The feed was analyzed (Canadian Department of Agriculture) for trace metal and steroid content, and no irregular constituents were reported. Whatever the case, the difficulties encountered in producing endogenous pyrogen from adult sheep leucocytes make it impossible to draw conclusions concerning the ability of neonatal leucocytes to reliably elaborate endogenous pyrogen.

In view of the difficulty encountered in producing endogenous pyrogen *in vitro*, it was decided to try and develop a method for detection of the substance *in vivo*. Endogenous pyrogen has been detected in rabbits (Atkins, 1960), but attempts to demonstrate it in other animals or man have not been particularly successful (Greisman and Hornick, 1972). In preliminary experiments involving plasma transfer between adult sheep, no transferrable pyrogen was demonstrated. Consequently, the sensitive assay involving microinjection of plasma into the AH/POA of rabbits was used. This method has been used successfully to detect endogenous pyrogen in febrile human patients (Cooper *et al.*, 1976c) because of its high sensitivity (Cooper and Roark, 1972). This procedure also is advantageous from the point of view that the rabbits do not appear to develop hypersensitivity reactions to the human plasma like they do after intravenous administration (Cooper, 1971a). However, when plasma from febrile sheep was microinjected into rabbits, no fever developed. It may be that the amount of circulating endogenous pyrogen in sheep is less than in humans, but the amount of endotoxin administered to the sheep was 100 times the threshold dose. This would seem to be sufficient stimulus to produce a high titer of circulating endogenous pyrogen. One difference between the human studies and those

reported here is that in the former, plasma was taken from patients suffering febrile illnesses, in which there may have been a continuous release of endogenous pyrogen. In the present experiments, the endotoxin stimulus was given as a single injection, and the consequent endogenous pyrogen that was formed may have reached its highest level at a time other than when the bleedings were undertaken. Since no circulating endogenous pyrogen could be detected after administration of high levels of endotoxin, it would appear that this assay method is inadequate for determining if newborn lambs release endogenous pyrogen when first exposed to iv endotoxin.

It has been shown that the part of the lipopolysaccharide molecule which contains the pyrogenic moiety is different from the side chains which induce immunological specificity (Work, 1972). When lambs were initially exposed to a particular pyrogen and then exposed to a different type, the animals became febrile after the second injection. Thus, cross-sensitization occurred between pyrogens that are either antigenically similar (for example, typhoid-paratyphoid & SAE pyrogen) or are unrelated, as in the case of SAE pyrogen and Y.enterocolitica. It is likely, therefore, that in order for an animal to develop fever, it must recognize the core or lipid A component of the lipopolysaccharide molecule. A similar type of recognition appears to exist in which animals made tolerant to a particular pyrogen can extend this tolerance to endotoxins from other bacterial species through recognition of common core antigens (Greisman & Hornick, 1975).

Three of the 17 lambs which had been sensitized at 4 h did

not develop fever at 60 h age when challenged with a different pyrogen. This has not been seen when the sensitizing pyrogen and the subsequent challenge are the same, unless, as seen in an earlier study, the lambs were tested at 48 h age instead of 60 h age. Possibly these lambs, and the three observed in the present study, still had an immaturity in their febrile process such that fever does not occur, even after sensitization. Also, the time required for sensitization between antigenically unrelated molecules may be longer than that required for related or identical pyrogens. Some evidence for this suggestion is that one lamb which did not respond with fever at 60 h to a pyrogen it had not been previously exposed to, did develop fever when challenged shortly thereafter with the original sensitizing pyrogen.

The results from the plasma transfer studies are difficult to interpret. Indeed, the infusion of maternal plasma into newborn lambs appeared to induce sensitization, in that the lambs developed marked fevers after iv endotoxin, at 60 h age. However, many of the lambs that had not been sensitized in this manner also proved capable of developing moderate fevers at 60 h age such that the difference between the two groups was not significantly different. It is not known why such a high percentage of unsensitized lambs developed fever at 60 h age. This phenomenon is unique to this portion of the work reported in this thesis. On occasion, during other parts of the study an unsensitized lamb would develop fever at 60 h age, but this was definitely the exception to what was generally observed. It may have been that, at the time of year the present studies were conducted, the lambs were exposed naturally to

greater than usual numbers of gram-negative bacteria. As a result, the lambs were naturally sensitized and could develop fever at 60 h age.

The fact that lambs which had received maternal plasma developed higher fevers than did their twins would suggest that the maternal plasma contained some factor which enhanced the febrile process. Alternatively, the infusion of approximately 50 ml of a foreign protein may have had a non-specific activating effect on the reticulo-endothelial system. It was difficult to obtain evidence in support of this hypothesis due to the problems encountered in obtaining a pyrogen-free protein preparation. However, a similar type of effect was produced by infusing plasma obtained from foetal lambs. If maternal plasma did indeed contain a "sensitization factor", the effects produced by infusing maternal and foetal plasma should differ, as the foetal plasma should not contain this substance. However, such a difference was not seen, with lambs that had received foetal plasma developing fevers that were not significantly different in magnitude from those seen in lambs that had been given maternal plasma. Thus, infusions of both foetal and maternal plasma may have produced a non-specific activation of the mechanisms involved in the febrile process. Alternatively, these lambs may also have become naturally sensitized by environmental pyrogens. It would appear that resolution of this question would require experiments in a larger number of lambs, and secondly, the administration of the challenging pyrogen at a younger age, before the lambs had become naturally sensitized.

Since it would appear that cross-sensitization does exist

between pyrogens, it is possible that the lambs may become sensitized naturally to a wide variety of pyrogens by exposure to pyrogens absorbed from the gut. Microbial activity has been found in the alimentary tract of lambs as young as one day old (Smith, 1965) and it has been suggested that the presence of a microbial population in the gut would provide a constant endotoxin stimulus to an animal (Landy and Weidanz, 1968; Miler et al., 1964). However, when endotoxin was administered orally or into the rectum to 4 h old lambs, 7 of 8 lambs did not become sensitized so as to permit them to develop fever when given intravenous pyrogen at 60 h age. This would suggest that, under natural conditions, animals do not become sensitized as a result of absorption of pyrogen from the gut.

It is possible, however, that the amount of endotoxin administered was not adequate to cross the intestinal wall in sufficient quantities to sensitize the lamb. Nolan et al. (1974) placed milligram quantities of endotoxin in an isolated rat gut preparation and found that about 0.5% of the endotoxin crossed the gut wall. If similar amounts of endotoxin would cross in the present studies, the amount of SAE pyrogen entering the portal circulation would be about 0.075 - 0.5 μg SAE pyrogen, which, at the higher dose, is adequate for sensitization when administered intravenously. The amount of pyrogen which may cross the intestine in lambs may be considerably less, however. In fact, although there is evidence for absorption of endotoxin from the intestinal tract of piglets (Miler et al.,1964), no evidence for this could be found in dogs (Sanford and Noyes, 1958) or rabbits (Ravin et al.,1960)

unless the animals were subjected to hemorrhagic shock. Even if pyrogen is absorbed from the alimentary tract under natural conditions, a bolus injection of endotoxin probably bears little resemblance to a slow, sustained release of pyrogens as may occur to a certain extent via the alimentary tract.

The results from in utero sensitization studies would suggest that sensitization does not necessarily occur following exposure to bacterial pyrogen in utero. However, the sensitization process appears to mature, in some foetal lambs, within the last few days before birth. It is unlikely that the lack of sensitization in younger foetuses is due to an inability of the white cells to recognize the injected lipopolysaccharide. In foetuses as young as 101 days gestational age, the typical neutropenia was seen after pyrogen injection.

It is possible that the injected pyrogen is inactivated more quickly in the younger foetuses. Furthermore, the unique pattern of foetal blood flow includes the placenta, an active drug-metabolizing organ (Juchau and Dyer, 1972) but largely omits the lungs (Rudolph, 1970). However, injections were made into both the saphenous vein, draining the lower limbs, and the jugular vein, draining the head and the different distribution of the injectate did not affect the subsequent ability to develop fever.

The ability to become "sensitized" to a pyrogen may depend, in part, on the immunological competence of the foetus which may in turn be highly dependent on the nature of the stimulus. The foetal lamb is capable of forming antibodies to a specific bacteriophage as early

as the 66th to 70th day of gestation, yet does not form antibodies to S. typhosa at anytime in foetal or early neonatal life (Silverstein et al.,1963). Whether or not antibody formation is a component of the "sensitization" process is unknown. If it is, it surely involves the presence of only minute amounts of antibodies since van Miert and Atmakusuma (1971) showed that goat kids would develop fever in the absence of detectable antibodies.

IV. CENTRAL NERVOUS SYTEM STUDIES

A. Temperature Responses of Lambs after Intraventricular Injection of Prostaglandins and Pyrogens

Febrile responses in the sheep have been reported (Bligh and Milton, 1973) after infusion of PGE_1 into a lateral ventricle at different ambient temperatures. In addition, other workers (Hales et al., 1973) have observed febrile responses in sheep following intraventricular injections of as little as 0.5 μg PGE_1.

Since the febrile response to iv pyrogen in the newborn lamb appears to depend upon both a maturation and a sensitization process, it was of interest to see if the newborn lamb was able to respond consistently with a fever following direct injection of PGE_1 or pyrogens into the cerebral ventricles.

Methods

Lambs of mixed breeds were born in the vivarium of the Medical School. At various times after birth, rectal temperature was measured in each lamb as described previously and recorded continuously. In some experiments, in addition to rectal temperature, respiratory rate was measured, general activity was assessed by observation, and, by feeling the ears, an estimate of gross changes in ear temperature was obtained. During the experimental period, lambs and their mothers were kept in a small pen at an ambient temperature of $21 \pm 1^{\circ}C$.

Body temperature was recorded for a minimum of 1 h before an injection was made. In order to inject into a lateral ventricle, lambs were gently restrained, the scalp wool was clipped and iodine was app-

lied to the skin. Under local anesthesia (4% Xylocaine Hydrochloride, Astra Limited), a small incision was made in the scalp to expose the skull and a hole was drilled through the skull 7 mm lateral to the midline and 5 mm posterior to the coronal suture. To make the injections, a 23 gauge needle was lowered into the brain to a depth of 15-16 mm below the outer surface of the skull. Attached to the needle was a graduated length of polyethylene tubing (P.E. 50, Clay Adams, Parsippany, N.J.) containing the drug solution, and from which a volume of 200 µl was delivered by gravity. To establish that the solution flowed into the ventricle, the free end of the tubing was held approximately 10 cm above the estimated level of the tip of the needle. Flow did not occur if the tip of the injection needle was in the substance of the brain instead of in the ventricular space. Following the injection, the needle was withdrawn and the hole in the skull was filled with sterile bone wax (Ethicon Sutures Ltd., Peterborough, Ontario). Antibiotic powder (Polybactrin, Calmic Ltd., Toronto), was sprayed in the wound and the incision was closed. The entire injection procedure took less than 5 minutes, after which the lamb was returned to the pen with its mother. This injection procedure did not appear to cause any discomfort to the lamb, nor, apart from the effects of the injected solution, was there any observable difference in behaviour other than a slight increase in activity when the lamb was handled. A similar increase in activity was observed when lambs were handled but no injection was made.

In order to determine if the injected solution was passing from the lateral ventricle into the third ventricle near the AH/POA, two

lambs were injected with 200 µl of 0.5% bromophenol blue utilizing the procedure described above. Immediately following the injection, the lambs were killed with an overdose of iv sodium pentobarbital, the skull was opened and the brain was removed. A mid-sagittal cut was made to bisect the brain and the cerebral ventricles were examined for the presence of dye.

Some lambs were given iv bacterial pyrogen and their body temperatures were recorded. The bacterial pyrogen used in this study was SAE pyrogen. This pyrogen was injected aseptically into the external jugular vein of the recipient animal in a dose of 0.3 µg dissolved in 3 ml of pyrogen-free sterile saline.

PGE_1 and PGE_2 (Upjohn, Kalamazoo, Michigan) were dissolved in ethanol in a concentration of 1 mg/ml and then diluted to the appropriate concentration (0.01 - 1.0 mg/ml) in sterile, non-pyrogenic 0.9% sodium chloride. At periodic intervals the vehicle was tested for pyrogenicity using the rabbit assay method, and it was found to be pyrogen-free. All glassware was made pyrogen-free by baking at 180°C for 3 h and tubing used for injections was stored in 70% ethanol when not in use. Disposable syringes were sterile and pyrogen free.

Results

Forty intraventricular injections of PGE_1 were made into 28 lambs, varying in age between 4 and 168 h. Because of temperature fluctuations observed in the lambs due to handling and general activity, only changes of 0.3°C or more in the 100 min following the injection were considered of physiological importance. Following injection

of PGE_1 in dosages of 2 - 200 µg into lateral ventricles of newborn lambs, 15 of 40 injections were followed by rises in deep body temperature. In the remaining experiments, rectal temperatures either remained unchanged or fell. Temperature responses did not appear to be related to age since lambs of identical ages often showed variable responses to the same dosage of PGE_1. In addition, lambs which were capable of developing a fever in response to intravenously injected bacterial pyrogen often did not respond with a rise in body temperature following administration of PGE_1 into their lateral cerebral ventricles.

Table 2 shows the responses observed after 40 injections of PGE_1 in dosages of 2 - 200 µg, and it can be seen that PGE_1 in higher concentrations caused falls in body temperature. A fall in rectal temperature of 1.3°C was observed in one lamb injected with 200 µg PGE_1. This fall was accompanied by detectable warming of the ears but not by panting or adoption of a relaxed, spread-out posture, such as had been observed in other lambs during defervescence or during exposure to high ambient temperatures. There appeared to be no relationship between body temperature changes and the dosage of prostaglandins administered when the dosage was either 2 or 20 µg. Five lambs at ages 4 to 168 h, each received 2 injections of 2 µg PGE_1, 60 h apart, and only 4 of the injections were followed by rises in temperature, which ranged in magnitude from 0.3 to 0.8°C. The remaining six injections produced no change in body temperature. In the animals that became febrile, the pattern of response varied, with 2 animals showing rapid rises in temperature and the other 2 developing fevers over a longer time period. In only one instance was a lamb observed to shiver and get cold ears

TABLE 2. Effects on body temperature observed when prostaglandin E_1 (PGE_1) is injected into lateral cerebral ventricles of conscious newborn lambs. The arrows indicate the direction of temperature change (↑ increase; ↓ decrease; → no change).

N	Age (Hours)	Dosage (µg)	Temperature Response
5	4 - 60	2	2 ↑ 3 →
5*	60 - 168	2	2 ↑ 3 →
10	4 - 60	20	3 ↑ 4 ↓ 3 →
17†	60 - 120	20	8 ↑ 3 ↓ 6 →
2	120	100	2 ↓
1	60	200	↓

* Lambs injected a second time with PGE_1.

† Lambs have been injected with either PGE_1, norepinephrine or 5-hydroxytryptamine 60 h before this injection.

in response to 2 μg intraventricular PGE_1.

Of 10 lambs first injected with 20 μg of PGE_1, at age 4-60 h, only 3 showed body temperature rises and in 3 lambs there was no change. In the remaining 4 animals, rectal temperature fell by more than 0.3°C from the temperature at the time of injection. In these animals the temperature had risen due to the injection procedure and the falls could be attributed largely to a return to normal body temperature.

Figure 14 illustrates the range of responses of 17 lambs injected at age 120 h with 20 μg PGE_1. The shaded area encloses the maximum and minimum changes in rectal temperature of the 17 lambs injected with 20 μg PGE_1 at this age. Altogether, 6 lambs showed no change in rectal temperature, 8 developed temperature increases with a mean of 0.5 ± 0.06°C (S.E.M.) and 3 showed drops in rectal temperature. There appeared to be no consistent pattern in the mechanisms used to raise body temperature, or in the latency of onset of fever. For instance, one lamb developed a fever of 0.88°C, yet its ears remained warm to touch and its respiratory rate did not slow noticeably. Its fever had peaked within 30 min of the injection, after which its respiratory rate increased slightly and body temperature decreased. Another lamb of the same age also received 20 μg of PGE_1, yet its body temperature did not begin to rise until 25 min after the time of injection. Though its ears appeared cool to touch, its body temperature only increased by 0.43°C. An additional 120 h old lamb was injected with 20 μg PGE_2 and rectal temperature rose by 0.5°C.

Some of the lambs injected at age 120 h had been previously injected intraventricularly with either PGE_1, 5-hydroxytryptamine or

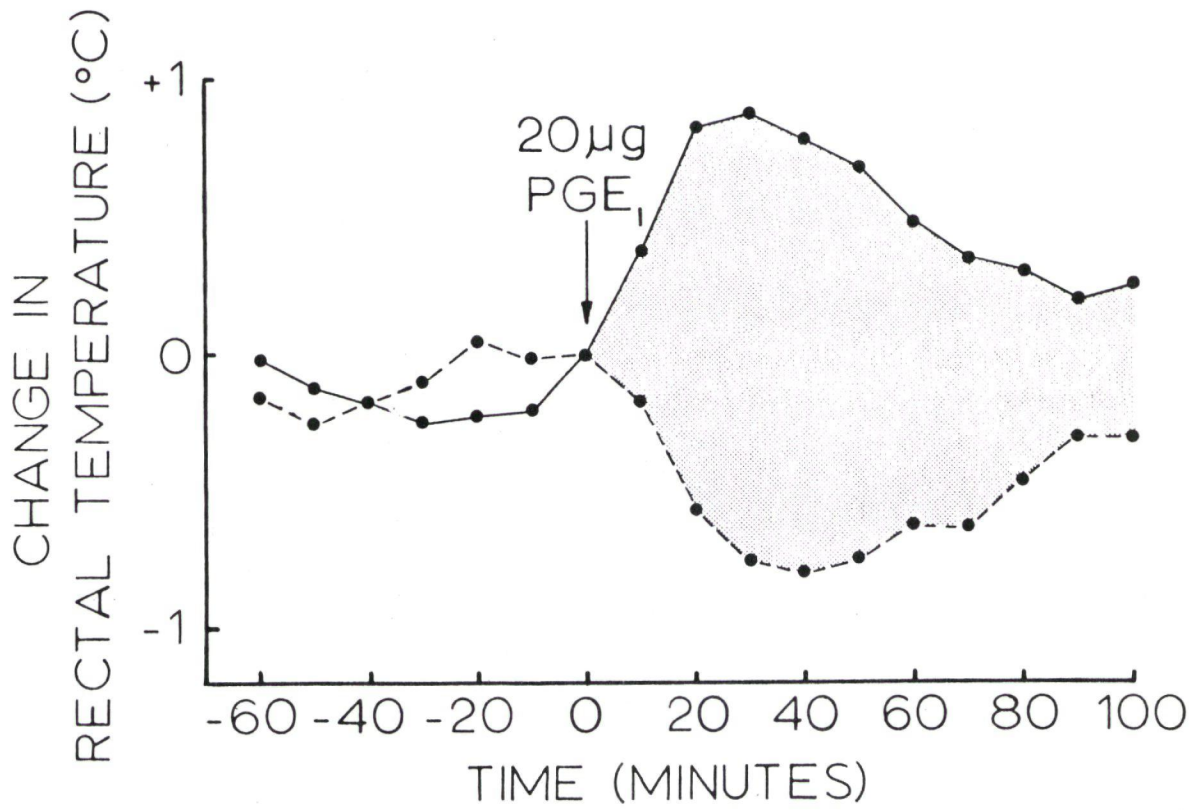

Figure 14. The shaded area encloses the temperature records of 17 lambs after injection of 20 µg prostaglandin E_1 (PGE_1) into their lateral cerebral ventricles. The two traces are the temperature records of two lambs showing the greatest changes from baseline temperature following the PGE_1 injection.

norepinephrine 60 h earlier. However, the response at age 120 h did not appear to be related to the response observed following the earlier injection.

Ten of the lambs which had received PGE_1 intraventricularly had been sensitized earlier to bacterial pyrogens, and, following a subsequent iv challenge with 0.3 µg bacterial pyrogen, developed a fever. However, 8 of these animals, even though they had been sensitized, did not increase their body temperatures in response to PGE_1 injected into the lateral ventricles. In another series of experiments, 3 other lambs, which had developed fever in response to intraventricularly injected PGE_1, did not respond with fevers when challenged at this time with intravenous bacterial pyrogen.

Bacterial pyrogen in a dosage of 3 ng was injected intraventricularly into 3 lambs first at 60 h, and then again at 120 h. None of these lambs developed fevers in response to either injection. Figure 15 is the temperature record of one of these lambs, illustrating the lack of response to two intraventricular injections of bacterial pyrogen at 60 and 120 h age. Other lambs received an amount of pyrogen, intraventricularly, equivalent to an effective intravenous dose (300 ng) and three of the four injections were associated with temperature rises of up to 0.75°C after latency periods of from 30 - 60 min.

In the lambs which had been injected with bromophenol blue, the brains were carefully removed and the cerebral ventricles were examined for distribution of dye. The ependyma of both lateral ventricles, the third ventricle and fourth ventricle was stained. No dye was seen in the subarachnoid space.

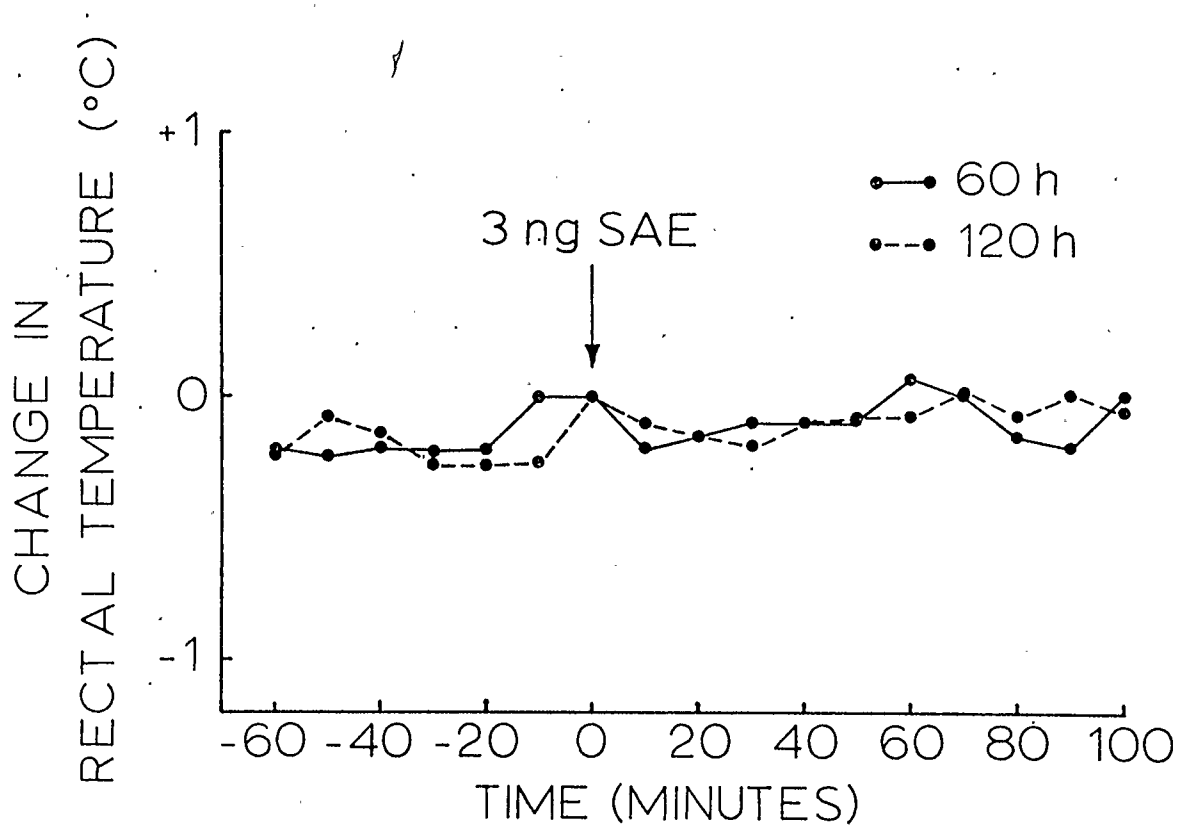

Figure 15. Records of body temperatures of one lamb following injection of 3 ng SAE pyrogen into a lateral cerebral ventricle at ages 60 and 120 h.

Discussion

The use of the intraventricular approach to identify neurochemical systems subserving central nervous system (CNS) function has been questioned (Myers, 1974). However, in order to study brain function in newborns, it would appear that the acute ventricular injection method described herein may have some advantages, because microinjection techniques necessitate anesthesia, surgery, and a recovery period of several days. The response of the newborn lamb to pyrogens undergoes a change within the first few days after birth, thus the time period over which thermoregulation studies are carried out may be important. The intraventricular injection method can be used to study CNS control of temperature regulation, and possibly other physiological systems at a very young age.

Since there were no consistent responses in temperature following the injection of PGE_1 into the ventricular system of newborn lambs, several questions are raised. The region of the brain into which local injections of PGE result in an increase in temperature appears to be the AH/POA (Stitt, 1973; Veale and Cooper, 1974, 1975), but the amount of PGE_1 reaching this site from the ventricular system may not always have been adequate to produce fever. In the adult sheep, a relatively high dose of PGE_1 (2.5 μg/min infused into a lateral cerebral ventricle for 45 min) has been shown to produce a febrile response (Bligh and Milton, 1973). The ability of PGE_1 to produce an increase in temperature in the sheep may depend on its prolonged presence at its site of action. In the newborn lamb the rate of flow of cerebrospinal fluid (CSF) could be so high that it might wash a bolus of PGE_1 out of the

ventricular system before the necessary concentration could be achieved in the AH/POA. It would appear, though, that the turnover of CSF in the newborn lamb is not more than twice that of the adult sheep and is probably similar (Evans et al., 1974). However, in other species such as the rabbit and cat (Feldberg and Saxena, 1971b; Veale and Cooper, 1973), single intraventricular injections of PGE_1 in doses much lower than those used in these experiments, were sufficient to produce sharp increases in temperature. In fact, 100 ng PGE_1 given into a lateral ventricle of the rabbit produces a fever of more than $1.0^{o}C$ (Veale and Cooper, 1974). Other investigators (Hales et al., 1973) have observed febrile responses in the adult sheep following single intraventricular injections of PGE_1 in doses ranging from 0.5 to 100 µg, and these observations have been verified by the author. Therefore, the doses of PGE_1 of 2-200 µg administered to the newborn lamb in these experiments would seem adequate. It is unlikely that the prostaglandin had lost its activity, because aliquots of the PGE_1 solution were used routinely in the laboratory to produce fever in cats, rabbits and rats throughout the time period of this study.

It is possible that the PGE was not always injected into the ventricular fluid, but this is unlikely. For each ventricular injection, the cannula was lowered slowly through the tissue of the brain whilst maintaining a slight pressure head, and, upon entering the ventricular space, the level of the solution within the tubing dropped abruptly. The flow rate into the ventricle could be changed by moving the free end of the tubing. Once flow was established, as described above, it could be stopped by slightly raising or lowering the injection

needle which caused the tip of the needle to enter the tissue of the brain. Further, in the two lambs into which bromophenol blue was injected in a manner identical to the method used for drug injections, it was evident that the walls of the entire ventricular system were stained with the dye. Of particular interest is the observation that the ventral portion of the third ventricle, anterior to the massa intermedia, was deeply stained. If injected PGE_1 were to diffuse throughout the ventricular space in a manner similar to the dye, this would suggest that substances thus injected are capable of reaching the ependyma adjacent to the AH/POA. The possibility still remains that, because of the dynamics of CSF flow, or permeability of the ependyma in the newborn lamb, the injected drug was often not able to reach an effective concentration in the tissue of the AH/POA. It is also possible that the flow of blood through the tissue of the AH/POA may have been sufficient in some lambs to transport the PGE_1 away from this area before it could exert its effect.

In lambs, the site responsive to PGE_1 to produce fever may lie too remote from the ventricular wall to allow the injected drug to reach it from the ventricular system. In rat brain, Fuxe and Ungerstedt (1968) showed that amines injected in relatively high doses into the cerebral ventricular system could be detected in a limited zone of approximately 300 μ within the tissue surrounding the ventricles. Whether a similar situation exists in the newborn lamb is not known. In fact, the locus of action of PGE_1 to produce a temperature increase in the adult sheep has not been established; although alterations in feeding have been observed in this species following the injection of

PGE_1 into the anterior hypothalamus, no significant changes in deep body temperature have been observed (Martin and Baile , 1973).

These observations, along with the finding that injection of PGE_1 into a lateral ventricle of the newborn lamb frequently does not result in fever, emphasize the need for an extensive study of the effects, both in the adult sheep and newborn lamb, of PGE_1 injected directly into the tissue of the brain.

Another possibility is that PGE_1 reached the AH/POA but the cells of this area did not respond to the prostaglandin. Hales et al. (1973) observed that, following intraventricular injection into the adult sheep, PGE_1 and PGE_2 were approximately equally effective in producing fever, with $PGF_{2\alpha}$ being slightly less effective and $PGF_{1\alpha}$ much less effective than the PGE series. It may well be that fever in the newborn lamb could be mediated by a different prostaglandin, possibly PGE_2 or prostaglandins of other series.

As had been observed earlier, the newborn lamb appears to require a "sensitization" to bacterial pyrogen before being able to develop a fever in response to iv pyrogen. It is of particular interest, however, that when lambs became capable of responding to iv pyrogen with a fever, they often failed to show a temperature rise after intraventricular prostaglandin. If, in fact, the injected prostaglandin reached the cells of the AH/POA, these results would then suggest that the newborn lamb may be able to develop a fever without the central involvement of PGE_1.

Recently, evidence has been provided which suggests that, in the rabbit, fever, in response to iv leucocyte pyrogen, can develop

independently of PGE_1. Following destruction of the AH/POA to a degree sufficient to impair the animals' ability to thermoregulate, rabbits respond to leucocyte pyrogen given intraventricularly or iv, but not to PGE_1 given intraventricularly. It may be of interest that the echidna, a monotreme, which becomes febrile after iv pyrogen also fails to develop a fever in response to intraventricular PGE_1 or PGE_2 (Baird et al., 1974). The possibility exists that the newborn mammal has a thermoregulatory system similar in some respects to this phylogenetically more primitive mammal, and only develops a PGE mediated fever response later in life.

One mechanism by which pyrogens could elevate body temperature independently of PGE release is suggested by the finding of Feldberg et al.,(1970) that perfusion of the cerebral ventricles of cats with a calcium-free solution which contained sodium caused hyperthermia. These workers suggested that the hyperthermia was due to an action of sodium along with an absence of calcium on the cells in the hypothalamus, and not on the monoaminergic neurons which innervate these cells. They further postulated that one of the actions of pyrogens may be to lower the level of calcium or prevent its action within the hypothalamus. In an extension of this work, Myers and Veale (1970, 1971) showed that the body temperature of unanesthetized cats could be raised or lowered by varying the ratio of sodium to calcium in the tissue of the posterior hypothalamus. There is some evidence to suggest that an alteration of this ratio may be produced by pyrogens (Myers, 1974). Recently, it has been shown in cats that the rise in rectal temperature occurring during perfusion of the ventricular system with a calcium-

free fluid is unlikely to be attributed to the increased synthesis of prostaglandins in the hypothalamus (Dey et al., 1974). Since sheep have been shown to respond to ionic changes in the CSF with changes in body temperature (Seone and Baile , 1973), the possibility exists that the effect of pyrogens given iv in the newborn lamb may be through this postulated ionic mechanism and not involve PGE_1.

The experimental results indicate that a febrile response is not consistently observed following the intraventricular injection of PGE_1 in the newborn, yet, on occasion, either increases or decreases in body temperature were observed following PGE_1 injection. One possible explanation for these changes in body temperature, when they occur, is that the drug may have an influence on the synapses lying within the pathways mediating thermoregulatory responses. Avanzino et al. (1966) observed that prostaglandins E_1, E_2 and $F_{2\alpha}$ produced largely excitatory effects on the firing rates of spontaneously firing neurons in the brainstems of cats, and Siggens et al. (1971) have shown that PGE_1 can antagonize responses of Purkinje cells to norepinephrine. In addition, prostaglandins could possibly modulate synaptic function through influences on the release of neurotransmitter substances (Coceani, 1974). Therefore, the flooding of the ventricular system with relatively large quantities of PGE_1, as in these experimental conditions, may have non-specific effects on the normal thermoregulatory patterns of the animal. Whatever the response, these varied temperature changes do provide evidence that the injected PGE_1 entered the tissue of the brain, possibly the AH/POA, and exerted an effect.

The lack of response of lambs to 3 ng of SAE pyrogen, which is

1/100 an iv dose which produces approximately 0.7°C fever, would suggest that the large pyrogen molecules did not reach the AH/POA in sufficient quantities to cause fever. In support of this, is the finding that, following the intraventricular injection of 300 ng SAE, fevers were observed in 3 of 4 experiments following latencies of 30-60 min. These responses may have resulted from the pyrogen having escaped into the circulation, and the time course of the fever would support this. On the other hand, a 30-60 min latency may be required for the synthesis, within the brain, of an endogenous pyrogen which then activates heat production and conservation pathways within the AH/POA.

In conclusion, the results would suggest that the newborn lamb may be able to develop a fever independently of the central involvement of PGE_1, since lambs that are capable of responding to iv bacterial pyrogen often fail to develop fever after intraventricular PGE_1. Similarly, small amounts of intraventricular bacterial pyrogen were ineffective in causing fever though larger doses of pyrogen were followed by fevers after a 30-60 min latency. Therefore, the intraventricular approach may not be a useful method for the study of central involvement of prostaglandins and pyrogens in the control of body temperature in the newborn lamb.

B. The Effect of Noradrenaline and 5-Hydroxytryptamine Injected into a Lateral Cerebral Ventricle, on Thermoregulation in the Newborn Lamb

The response of the adult sheep to intraventricular injections of monoamines has been studied (Bligh, 1966b; Bligh et al., 1971). Since the newborn lamb does not develop fever after intravenous pyrogen or to prostaglandins injected into the cerebral ventricles, it was of interest to compare the response of the newborn lamb to that of the adult sheep following injections of 5-HT and NA into the lateral ventricles.

Methods

For the experimental period, the lambs and their mothers were placed in pens in a large environmental chamber where the temperature was regulated to within 1°C at either 4°, 21° or 30°C. Body temperature was measured and a continuous written record of body temperature was obtained for a minimum of 60 min before and 90 min following the injection. Throughout the experimental period the lambs were kept under continuous observation, and at frequent intervals respiratory frequency was noted by visual count, and vasomotor tone of the ears was crudely assessed by feeling them to detect whether they were hot or cold. Except for the brief interval during the time of injection, the lambs were unrestrained and remained with their mothers for the duration of the experiment. Injections were made into conscious newborn lambs under local anesthesia as previously described.

Materials

All amine solutions were freshly prepared immediately before use.

5-HT and NA were dissolved in sterile, pyrogen-free 0.9% NaCl, then sterilized by passage through a sterile, 0.22 μ bacterial filter (Millipore). Solutions prepared in this manner were tested in both rabbit and Limulus assay (Mears, Cooper and Veale, 1975) and were found to be pyrogen-free. Amines were administered in quantities of 100 or 200 μg dissolved in 200 μl of vehicle. Weights refer to the creatinine sulfate complex, in the case of 5-HT, and to the chloride salt for NA. Both amines were supplied by Sigma (St. Louis, U.S.A.).

All glassware was made pyrogen-free by baking it at 180°C for 3 h, and injection tubing was stored in benzalkonium chloride (Zephiran chloride, Winthrop Laboratories) when not in use. Before injection, the tubing was flushed repeatedly with sterile, non-pyrogenic saline. All syringes were disposable and pyrogen-free.

Results

A total of 79 injections were made into 57 lambs. Several lambs received 2 injections, one at 4 h age and another at 60 h age, but the responses did not appear to differ as a result of a previous injection. The results from injections of NA, 5-HT and NaCl at 4°, 21° and 30°C ambient temperature are presented in Tables 3, 4, and 5. Since responses appeared to be similar in lambs at 4 h and 60 h age, the temperature responses have been pooled; however, the responses to the two different drug dosages have been presented separately.

Noradrenaline

The effect of 200 μg of NA upon thermoregulation at various ambient temperatures is illustrated in Fig.16. At an ambient temperature of 4°C, lambs were observed to shiver slightly, respiratory fre-

TABLE 3. Responses of newborn lambs to intraventricular injection of noradrenaline, 5-hydroxytryptamine or NaCl at an ambient temperature of 4°C. Arrows indicate direction of change after injection (↑ increase; ↓ decrease; → no change). Change in rectal temperature (°C) computed as maximum change (mean ± S.E.) within 90 minutes from the temperature at the time of injection.

DRUG	FUNCTION	DOSAGE (µg)	
		100	200
Noradrenaline	resp. freq.	→	→
	shivering	↓	↓
	ear temp.	→	→
	rectal temp.	-.068±0.16	-1.60±0.31
	n	9	10
5-Hydroxytryptamine	resp. freq.	→	→
	shivering	↓	↓
	ear temp.	↑	↑
	rectal temp.	-0.59±0.07	-1.27±0.28
	n	6	6
NaCl (200 µl)	resp. freq.	→	
	shivering	→	
	ear temp.	→	
	rectal temp.	-0.20±0.09	
	n	4	

TABLE 4. Responses of newborn lambs to intraventricular injection of noradrenaline, 5-hydroxytryptamine or NaCl at an ambient temperature of 21°C. See Table 3 legend for further details.

DRUG	FUNCTION	DOSAGE (µg) 100	200
Noradrenaline	resp. freq.	→	→
	shivering	→	→
	ear temp.	↑	↑
	rectal temp.	-1.04±0.43	-0.84±0.42
	n	4	4
5-Hydroxytryptamine	resp. freq.	→	→
	shivering	→	→
	ear temp.	↑↓	↑
	rectal temp.	-0.23±0.18	-0.29±0.18
	n	4	4
NaCl (200 µl)	resp. freq.	→	
	shivering	→	
	ear temp.	→	
	rectal temp.	-0.19±0.09	
	n	4	

TABLE 5. Responses of newborn lambs to intraventricular injection of noradrenaline, 5-hydroxytryptamine or NaCl at an ambient temperature of 30°C. See Table 3 legend for further details.

DRUG	FUNCTION	DOSAGE (µg)	
		100	200
Noradrenaline	resp. freq.	↓	↓
	shivering	→	→
	ear temp.	→	→
	rectal temp.	+0.74±0.22	+1.13±0.18
	n	5	5
5-Hydroxytryptamine	resp. freq.	↑	↑
	shivering	→	→
	ear temp.	→	→
	rectal temp.	-0.27±0.13	+0.09±0.12
	n	5	5
NaCl (200 µl)	resp. freq.	→	
	shivering	→	
	ear temp.	→	
	rectal temp.	-0.05±0.17	
	n	4	

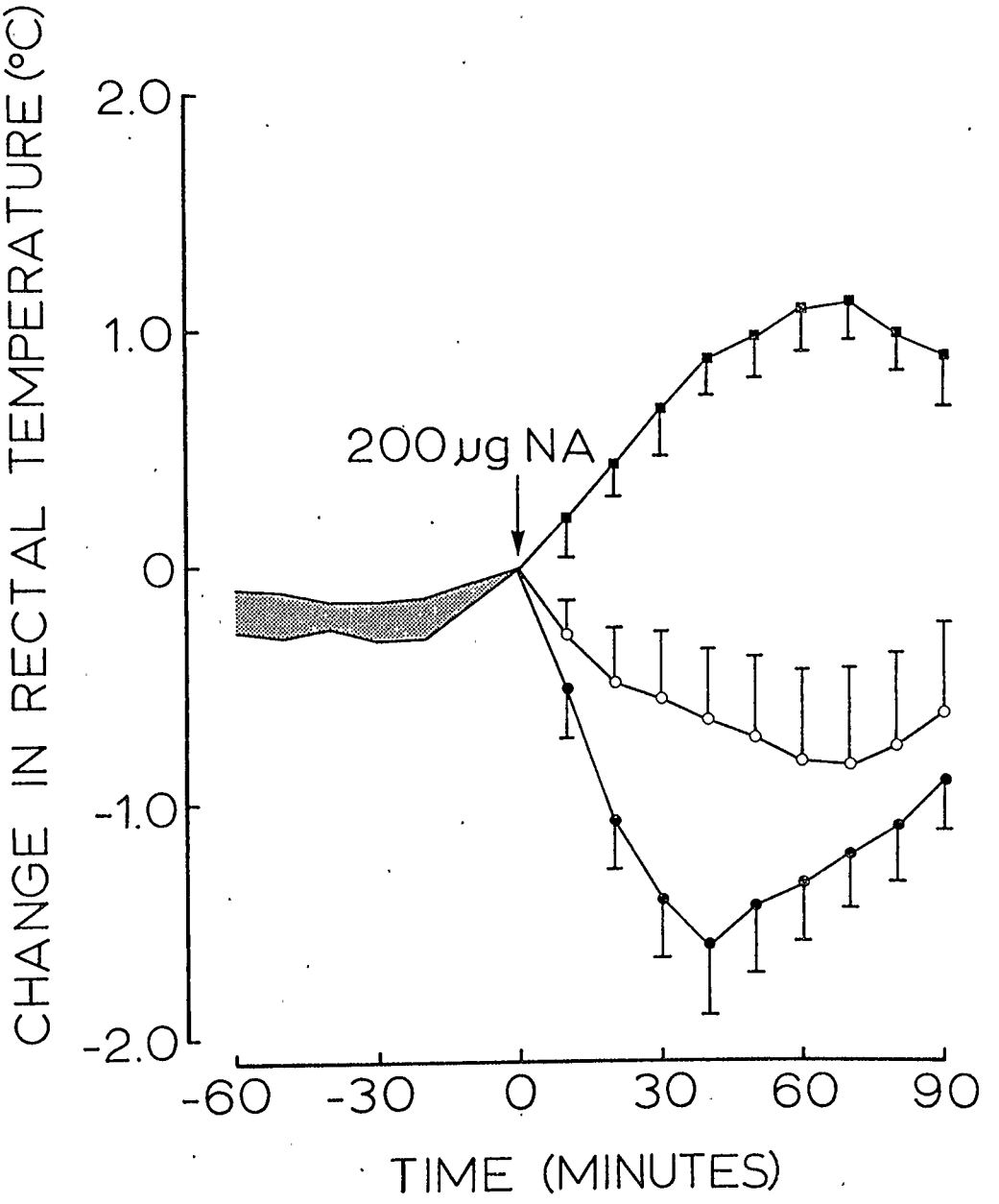

Figure 16. Mean changes in body temperature from the temperature at the time of injection of 200 µg NA into lateral cerebral ventricles of lambs at 4°C (closed circles, n = 10), 21°C (open circles, n = 4) and 30°C (squares n = 5) ambient temperature. The shaded area encloses the mean changes in body temperature from the time of injection. Vertical bars equal the SEM.

quency was about 40/min and the ears were cold to touch. At this ambient temperature, intraventricular injection of NA caused a fall in rectal temperature. The drop in temperature was accompanied by an observable cessation of shivering, but no change in respiratory frequency was noted, and the ears remained cold. Following the NA injection, the lambs usually abandoned the curled up, huddled position that they had adopted in the cold, and would walk about, frequently vocalizing. This behaviour was observed only following NA injections and not after intraventricular 5-HT or NaCl. Approximately 30-50 min after the injection the lambs began to shiver violently and their rectal temperatures rose. They again adopted a huddled up position until the body temperature had returned to normal.

At an ambient temperature of 21°C, intraventricular NA caused no change in respiratory frequency, which was low to begin with, but the ears became hot and noticeably vasodilated. This caused rectal temperature to fall in some lambs but when responses of the 4 lambs were averaged the change in rectal temperatures was not significantly different ($p > 0.05$) from that observed following control injections at this temperature. In general, it appeared that the smaller lambs showed a greater degree of hypothermia than did the larger lambs.

Following injection of NA when the ambient temperature was 30°C, body temperature quickly began to increase. In fact, within an hour after the injection some of the lambs were removed from the heat as rectal temperature increased to over 42°C and the possibility of a deleterious effect of the high temperature became apparent. The rise in body temperature was associated with a marked reduction in respir-

atory frequency. In a 30°C environment, respiratory rate was usually in the range of 250-300 breaths/min and following NA injection this decreased to about 75/min. As rectal temperature began to return to pre-injection levels, lambs were observed to pant and often they lay out prostrate on the floor of the pen.

The changes in body temperature observed following injection of both NA and 5-HT were related to the amount of drug delivered to the brain. That is, the relative hypo- or hyperthermia was usually greater after the 200 µg dose than after 100 µg. This dose-responsiveness is illustrated for NA in Fig. 17. It can be seen that, at an ambient temperature of 4°C, 200 µg of NA injected intraventricularly caused significantly greater hypothermia than did 100 µg.

5-Hydroxytryptamine. Fig. 18 shows the response of newborn lambs following intraventricular injection of 200 µg of 5-HT at 3 different ambient temperatures. As was observed in the previous experiments with NA, the responses varied according to the environmental temperature. At 30°C, there was no significant change in rectal temperature following either 100 µg or 200 µg 5-HT given intraventricularly (Table 5). Respiratory rate increased slightly from levels of 250-300/min to over 300/min. At this temperature the ears were hot to touch and appeared to be dilated throughout the total duration of the experiment.

At a room temperature of 21°C, neither 100 nor 200 µg of 5-HT caused a statistically significant change in body temperature, though 5 of 8 lambs did develop hypothermia ranging from 0.4 to 0.8°C. In all of the lambs that were given 200 µg of 5-HT the ears became flushed

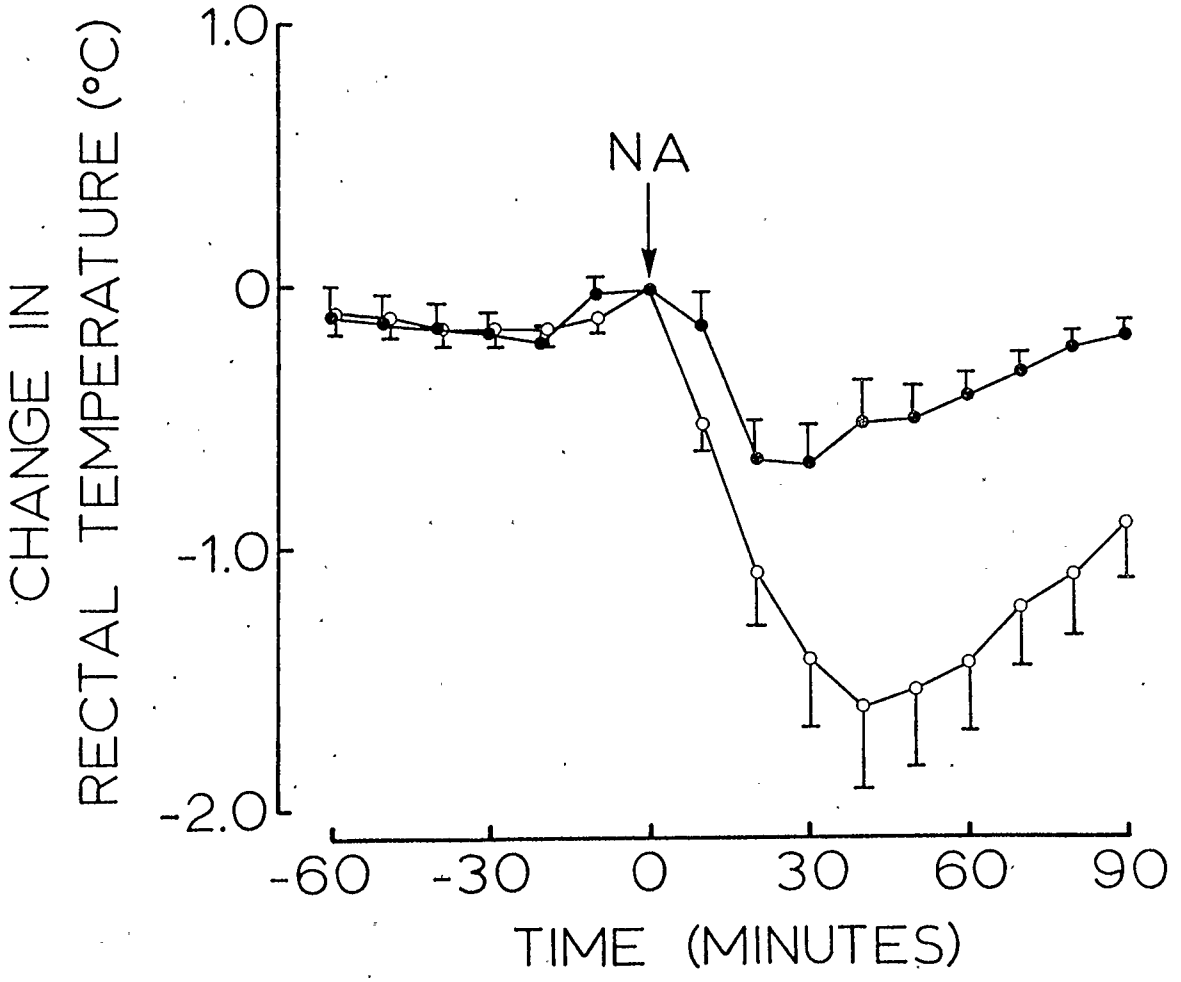

Figure 17. Mean changes in body temperature from the temperature at the time of injection of 100 µg NA (closed circles, $\underline{n}$ = 9) or 200 µg NA (open circles, $\underline{n}$ = 10) into lateral cerebral ventricles of lambs at 4°C. Vertical bars equal the SEM.

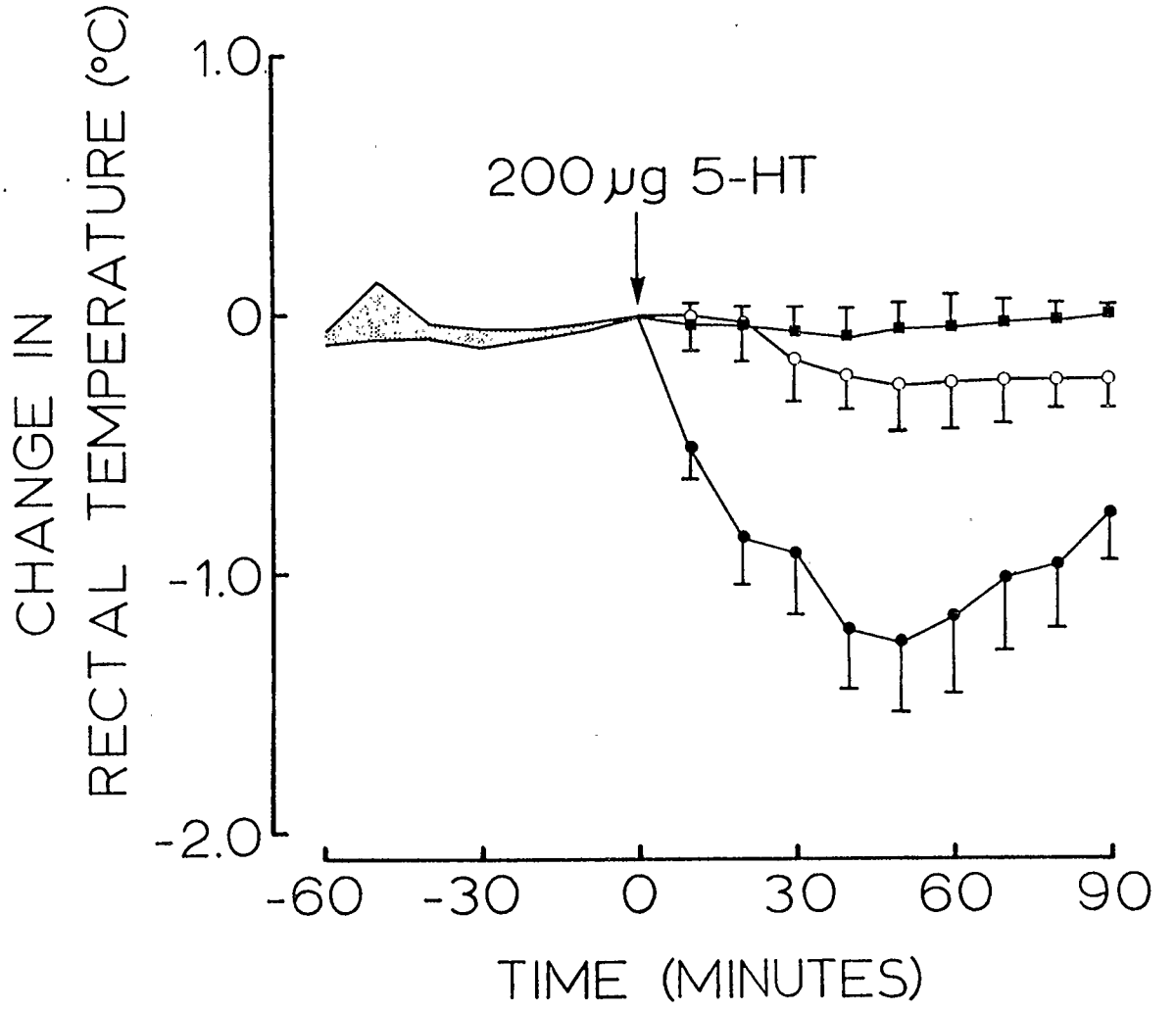

Figure 18. Mean changes in body temperature from the temperature at the time of injection of 200 µg 5-HT into lateral cerebral ventricles of lambs at 4°C (closed circles, n = 6), 21°C (open circles, n = 4) and 30°C (squares, n=5) ambient temperature. The shaded area encloses the mean changes in body temperature from the time of injection. Vertical bars equal the SEM.

with blood, but this effect was only noticeable in 1 of 4 lambs receiving 100 µg 5-HT. In none of these animals did respiratory rates differ from those seen before the injection.

When 5-HT was injected into the cerebral ventricles at an ambient temperature of 4°C, rectal temperature fell with a pattern similar to that observed following NA injection at this environmental temperature. Respiratory rate remained unchanged and shivering activity ceased. In these respects, the responses were similar to those observed after NA administration. However, unlike the response after NA, 5-HT caused ear skin temperature to increase.

NaCl. A number of lambs were also given control injections of 0.9% NaCl at the three environmental temperatures. The maximum change in body temperatures averaged -0.20 ± 0.09°C and this is within the normal variation of body temperature seen over a 90 min period in freely moving, unrestrained lambs that had not been injected.

Discussion

It was previously shown that the dye, injected in a manner identical to the method utilized in the present study, diffuses throughout the entire ventricular system and, of particular interest, stains the ependyma of the antero-ventral part of the third ventricle. This is the region of the AH/POA which has been shown in a number of species to be sensitive to the direct application of agents which cause temperature change. If the amines injected in the present experiment were to diffuse throughout the ventricular system in a manner similar to the dye, it is likely that they would affect synapses in the AH/POA. Further evidence for this is the fact that consistent, integrated thermoregula-

tory responses have been observed which would suggest that activation and recruitment of neurons involved in the control of body temperature.

The temperature changes observed following intraventricular injections of 5-HT and NA into newborn lambs are similar to those observed by Bligh et al. (1971) in adult sheep. The concept that NA acts as an inhibitory transmitter on a heat production pathway is supported by the observations that shivering ceased following NA injection in the 4°C environment, with a consequent decline in body temperature. It is interesting, however, that the NA did not appear to affect heat conservation mechanisms such as reduced respiratory rate and ear vasomotor tone. The lack of effect of NA on heat conservation in the cold had been previously observed in adult sheep (Bligh et al., 1971). There is a possibility, however, that the intraventricular injection of NA did decrease the neural drive for vasomotor tone, but was of insufficient magnitude to reduce vasoconstriction enough for the effect to be manifest as an increase in ear temperature. Another possibility is that vasomotor control may rest, in part, in a structure outside of the hypothalamus and not under noradrenergic control. It is unlikely that such a structure is the spinal cord since spinal cooling in the sheep has minimal or no effect on ear skin blood flow (Hales and Iriki, 1975) or ear skin temperature (Bacon and Bligh, 1976).

The inhibitory role of NA on heat loss pathways was made evident by the substantial increase in rectal temperature following its intraventricular injection at an ambient temperature of 30°C. In the lamb, it is likely that the major mechanism for loss of body heat is by thermal tachypnea (Alexander and Brook, 1960) and the four to five-fold

reduction in respiratory rate that occurs after NA injection probably is largely responsible for the temperature increase. It is unlikely that heat production was activated by the NA, since there is evidence that heat production pathways within the CNS of the adult sheep are cholinergically mediated (Bligh and Maskrey, 1969; Bligh et al., 1971). Furthermore, the lambs did not shiver, which is an important source of heat in newborn lambs exposed to cold (Alexander and Williams, 1968).

Though an ambient temperature of 21° would be expected to be below the thermoneutral zone of the newborn lamb (Alexander and Williams, 1968), there was only a small drop in rectal temperature following administration of NA at this temperature. Perhaps the ambient temperature was high enough, and the metabolic rate adequate to maintain body temperature even though heat conservation mechanisms were inhibited by the NA. In this environmental temperature, the NA did cause vasodilatation in the ears as evidenced by an increase in ear skin temperature. It would appear that the more moderate environment of 21°C did not provide the stimulus for vasoconstriction that had been appareent at 4°C.

The responses in rectal temperature, respiratory rate, ear skin temperature and shivering activity following intraventricular 5-HT are in general agreement with the predictions of the model of Bligh et al. (1971). That is, the neural pathways involved in heat loss in the lamb appear to be mediated by 5-HT as do those in the adult sheep. Thus, at an environment of 30°C, in which heat loss mechanisms were already activated, intraventricular 5-HT elevated respiratory rate but this was insufficient to change rectal temperature significantly. In the 21°C

environment one would have expected a similar increase in respiratory rate, but the only change in any of the variables measured was an increase in ear skin temperature. Such a loss of vasomotor tone is predicted by the Bligh model if activation of heat loss mechanisms is accompanied by an inhibition of heat conservation. A similar situation was seen at 4°C, with intraventricular 5-HT increasing ear skin temperature but having no effect on respiratory rate. Perhaps in the cold, the peripheral drive for a decreased respiratory rate may have overcome the effects of the 5-HT. However, the very noticeable decrease in shivering indicates a decrease in heat production. The increase in ear skin temperature would indicate an inhibition of heat production and is in agreement with the predictions of the model. The evidence for 5-HT having a role in heat loss is not as strong as had been seen in the adult sheep by Bligh et al. (1971). However, it did cause an inhibition of heat production, and the means by which rectal temperature was lowered often differed from the mechanisms seen after NA.

The available evidence would indicate that lambs can thermoregulate at birth (Alexander, 1961) and the results of the present study suggest that the neural pathways involved in thermoregulation in the newborn lamb are organized in a manner similar to that in the adult sheep. Thus, the inability of the newborn lamb to develop fever after intravenous pyrogen is probably not due to the absence of a functional monoaminergic system or to the lack of development of any of the CNS pathways involved in normal thermoregulation. Also of interest is the fact that reliable, repeatable responses were obtained following injection of monoamines into the cerebral ventricular system of lambs, yet

in a previous study utilizing identical techniques, lambs often did not respond with fever following intraventricular prostaglandin E_1. This would provide further evidence that prostaglandins are not involved in the febrile response in newborn lambs. The evidence would further indicate that pathways utilizing the monoamines NA and 5-HT as transmitter agents are not sensitive to the pyrogenic action of prostaglandins.

C. Microinjections of Prostaglandin, Pyrogen and Noradrenaline into the Hypothalamus of the Newborn Lamb

The previous work would suggest that newborn lambs can develop fever without the central involvement of prostaglandins. However, the site of action of prostaglandin in the lamb may be at a locus in the hypothalamus not easily accessible from the ventricles. Since the responses of the lambs to intraventricular injections of pyrogens, prostaglandins and monoamines did not appear to differ over the first few days of life, guide tubes were implanted so that, following recovery from surgery, microinjections could be made directly into the hypothalamus.

Methods

Approximately 2-4 h after birth, each of 13 lambs of either sex was anesthetized with halothane and an endotracheal tube was inserted. The lamb's head was placed in a stereotaxic head-holder (Kopf Instruments) with the level of the infraorbital bars 7 mm below that of the ear bars. In preliminary investigations with the head in this position, the anterior commissure and the optic chiasm were found to lie in a vertical plane. While under anesthesia the temperature of the lamb was maintained above 35^{o}C with the use of a heating pad.

Surgery was carried out under aseptic conditions using the protocol of Myers and Veale (1971) for stereotaxic surgery on cats. The scalp was sterilized with tincture of Zephiran and a medial incision, approximately 50 mm long was made. The periosteum was cleared from the skull and holes were drilled to accommodate an array of four 17 gauge stainless steel tubes. These were implanted stereotaxically to serve

as guides for microinjection cannulae (Myers, 1970b). The guide tubes were bilaterally placed at 2.5 mm on each side of midline with the lower ends located 3-4 mm above the anterior or posterior hypothalamus (23 mm and 19 mm anterior to the ear bars). Guide tubes were 40 mm long, and each were fitted with an indwelling 22 gauge wire stylet. The guide taubes were cemented to the skull with cranioplast cement, (L.D. Caulk, Co.) and a polystyrene pedestal, surrounding the array was secured to the skull with 4 screws. The preparation was capped for the duration of the experiment to maintain sterility. The incision was sprayed with an antibiotic (Polybactrin, Calmic Ltd.) and the skin was drawn together around the pedestal. Each lamb was given 60,000 IU of penicillin (Derapen, Ayerst, Montreal) intramuscularly.

Following recovery from anesthesia, the lamb was returned to its mother. At a postnatal age of 50 h the lamb and its mother were brought into a walk-in environmental chamber in which the temperature was controlled to within $\pm$ 1°C at either 10°, 21° or 30°C. Body temperature was measured and recorded as previously described. Respiratory frequency was counted visually and ear temperature was periodically assessed by feeling the ears.

Injections were made through a modified push-pull cannula (Myers, 1970b) in which the inner (27 gauge) cannula protruted 4-5 mm beyond the outer (20 gauge) guide cannula. The inner cannula was attached to a length of polyethylene tubing (PE-20) which was connected to a 10 µl Hamilton syringe mounted on a Harvard infusion-withdrawal pump. The injection assembly was stored in tincture of Zephiran and before use was repeatedly flushed with sterile saline and loaded with

the drug solution. For injection, the cannula was lowered to a depth of 4-5 mm below the guide tube and a volume of 1 µl was infused over a period of 1 min. The cannula was left in place during an additional 30 secs, then it was removed, tested for flow, then placed in the contralateral site where the procedure was repeated.

Injections were made into both anterior and posterior sites in each lamb. All lambs were given injections of PGE_1 (0.1 µg bilaterally) or PGE_2 (0.1 or 10 µg bilaterally). Some lambs received injection of SAE pyrogen (0.1 µg) into these same sites. These experiments were conducted at an environmental temperature of 21°C. At other times temperature was maintained at either 10°C or 30°C and some lambs were given bilateral injections of NA (10 µg/side). Any site which produced a change in body temperature was also infused with 1 µl of sterile, pyrogen-free saline which was the vehicle in which the drugs were dissolved. In experiments in which no temperature changes were observed, successive microinjections of various drugs were given at 1.5 h intervals. In instances where temperature was observed to change, no further injections were given until the temperature had returned to the level seen before the injection and was stable for at least 30 min. The injections were given in a random order and were carried out between the ages of 50-70 h postnatal.

At the termination of the series of experiments each lamb was deeply anesthetized with sodium pentobarbital (30 mg/kg iv) and 1 µl of 0.5% bromophenol blue was microinjected at each site. The heart was exposed and clamped at its junction with the dorsal aorta. The thoracic aorta was then cannulated with a polyethylene catheter and

the blood was washed from the cerebral vessels by allowing a 0.9% saline solution to flow through the aorta and into the head and exit via severed jugular veins in the neck. After the fluid flowing from the jugular vessels was clear of blood, 2 liters of 10% formal-saline were perfused through the vessels in order to fix the brain. The brain was sectioned in the coronal plane and cut at 25 μ on a freezing microtome. Brain sections were stained for cells and myelin following a modified method of Klüver and Barrera (1953).

Results

Microinjections were made into 48 sites throughout the diencephalon of 13 lambs at ages 50-70 h. Fig. 19 is a composite anatomical map showing 44 injection sites within the hypothalamus. The remaining 4 sites were in structures such as the septal area or midbrain, not represented in these coronal planes. When PGE_1 (0.1 μg) or PGE_2 (0.1 or 1.0 μg) was infused bilaterally into each of these sites, rectal temperature did not change by more than $0.5^{o}C$ in the 90 min after the injection. Indeed, the average temperature change observed after twelve bilateral injections into the AH/POA was -0.01 ± 0.08 (S.E.M.). Fig. 20 (upper) shows the temperature response of 1 lamb following injection of PGE_1 (0.2 μg) into the AH/POA and it can be seen that on this occasion rectal temperature fell by $0.5^{o}C$. This slight fall in rectal temperature was not accompanied by any observable change in ear temperature, respiratory rate or behaviour. Other lambs which received PGE injections into similar areas of the hypothalamus showed small ($< 0.3^{o}C$) temperature increases, or no change. On no occasion was an injection of PGE into any area of the brain followed by the typical

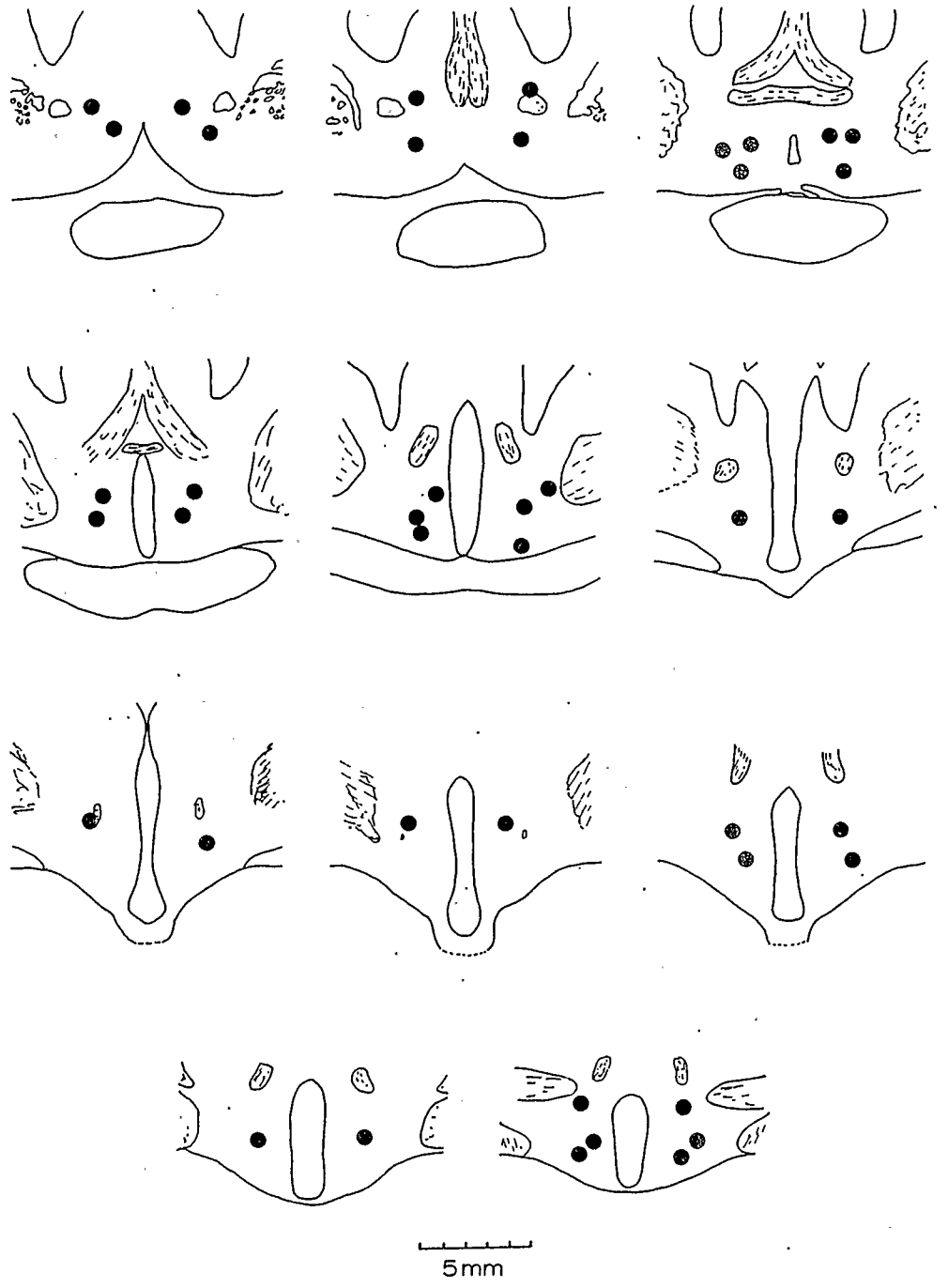

Figure 19. Representative coronal sections at 0.5 mm intervals through the lamb hypothalamus showing sites (filled circles) into which bilateral microinjections of PGE_1 or PGE_2 were made into 13 lambs.

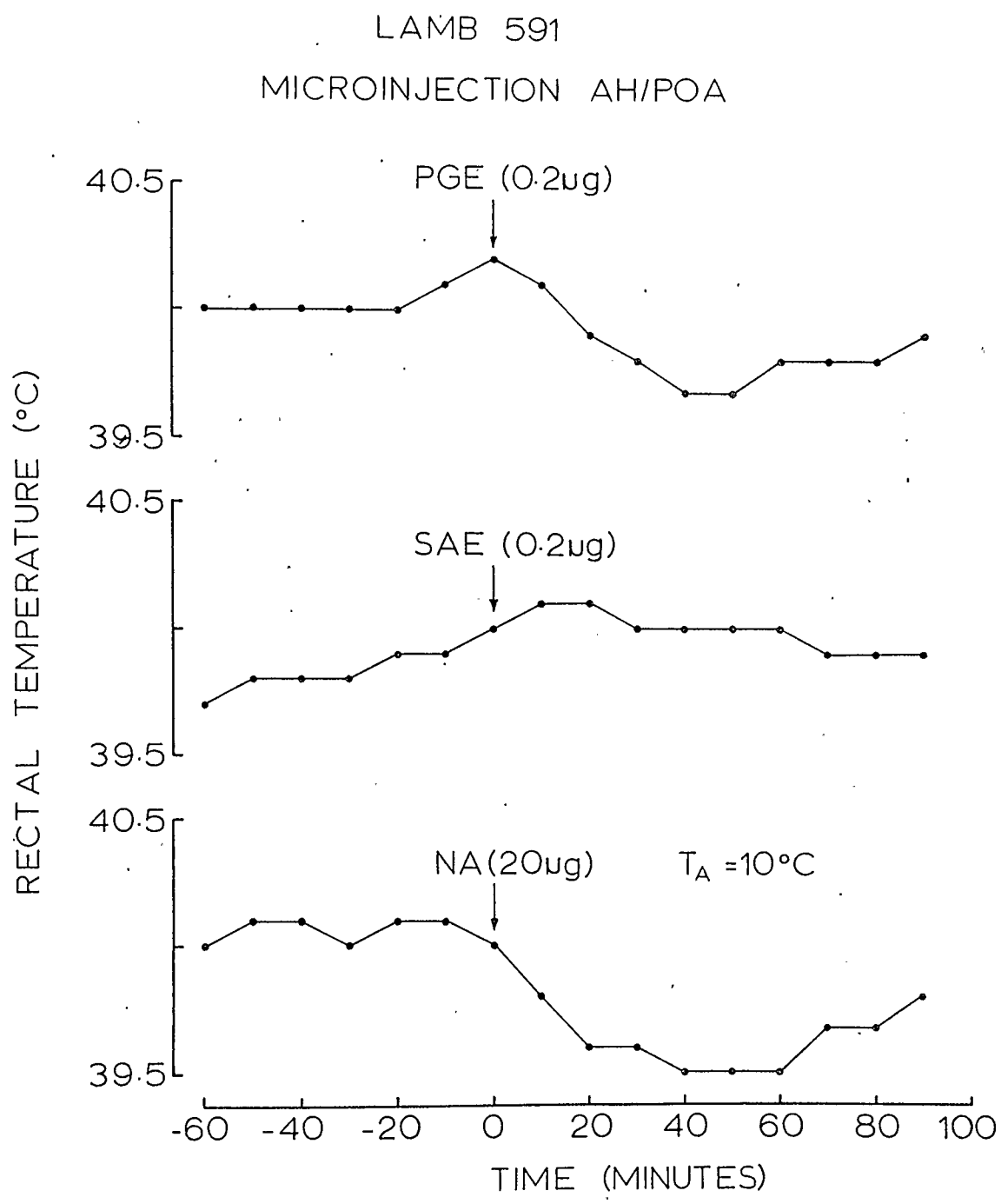

Figure 20. Temperature records of a 60 h old lamb on three separate occasions following bilateral microinjections of PGE_2 (0.1 µg/side), SAE pyrogen (0.1 µg/side) and NA (10 µg/side) into the same sites in the AH/POA.

"prostaglandin fever" that is commonly observed in other laboratory animals. It is of interest that several of the lambs had received intravenous pyrogen at 4 h age, and were capable of developing fevers to intravenous SAE pyrogen at the time of these experiments.

A number of the lambs which had been shown to be unresponsive to PGE were given microinjections of SAE pyrogen into these same loci. Fig. 21 identifies by a filled circle the loci into which bilateral injections of 0.1 µg SAE pyrogen caused a fever of more than 0.3°C. The open circles indicate sites into which similar microinjections had no effect on temperature. It can be seen that fevers were obtained, in general, after microinjections into the AH/POA, but on occasion, injections in other lambs into sites very similar to these produced no temperature changes. Fig. 20 (middle) is a temperature record of one lamb showing no response after a microinjection of SAE pyrogen (0.2 µg total dose) into the AH/POA. This lamb developed a fever to 0.3 µg SAE pyrogen given intravenously.

The fevers observed after microinjections of SAE pyrogen were of one or two patterns. The most common response was characterized by a biphasic fever of 0.85 - 1.2°C commencing after a 60 min latency (Fig. 22, upper). However, one lamb began to shiver within 15 min after the administration of pyrogen, and it developed a biphasic fever with peaks at 150 min and 310 min post-injection. The temperature record for this lamb is illustrated in Fig. 22 (lower).

Eight of the lambs which had also received PGE were placed in an environment of 10°C. At this environmental temperature, the lambs' ears were cold to touch, and the animals often adopted a huddled-up

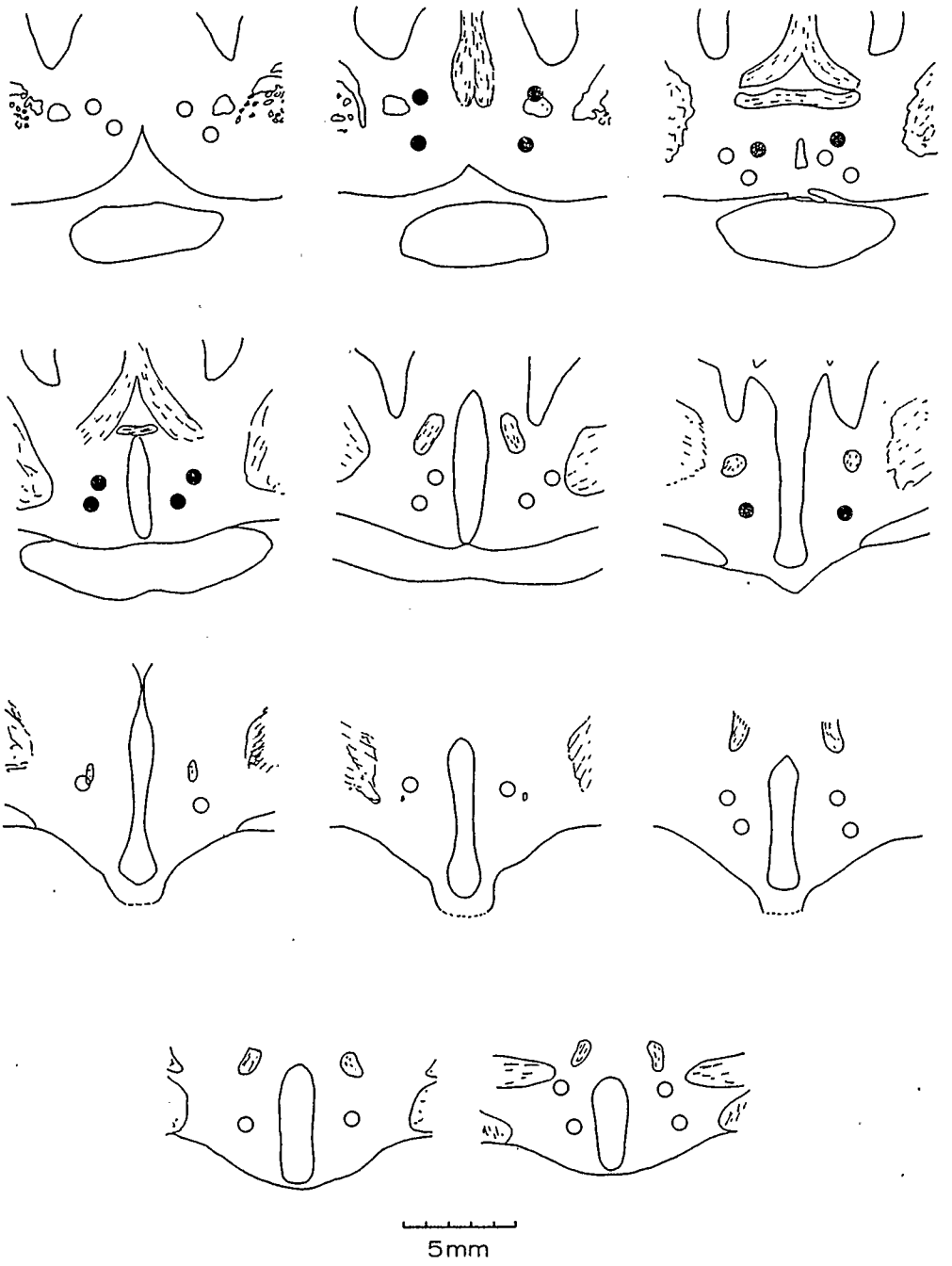

Figure 21. Representative coronal sections through the lamb hypothalamus showing sites into which injection of SAE pyrogen caused a fever (filled circles) or no temperature change (open circles).

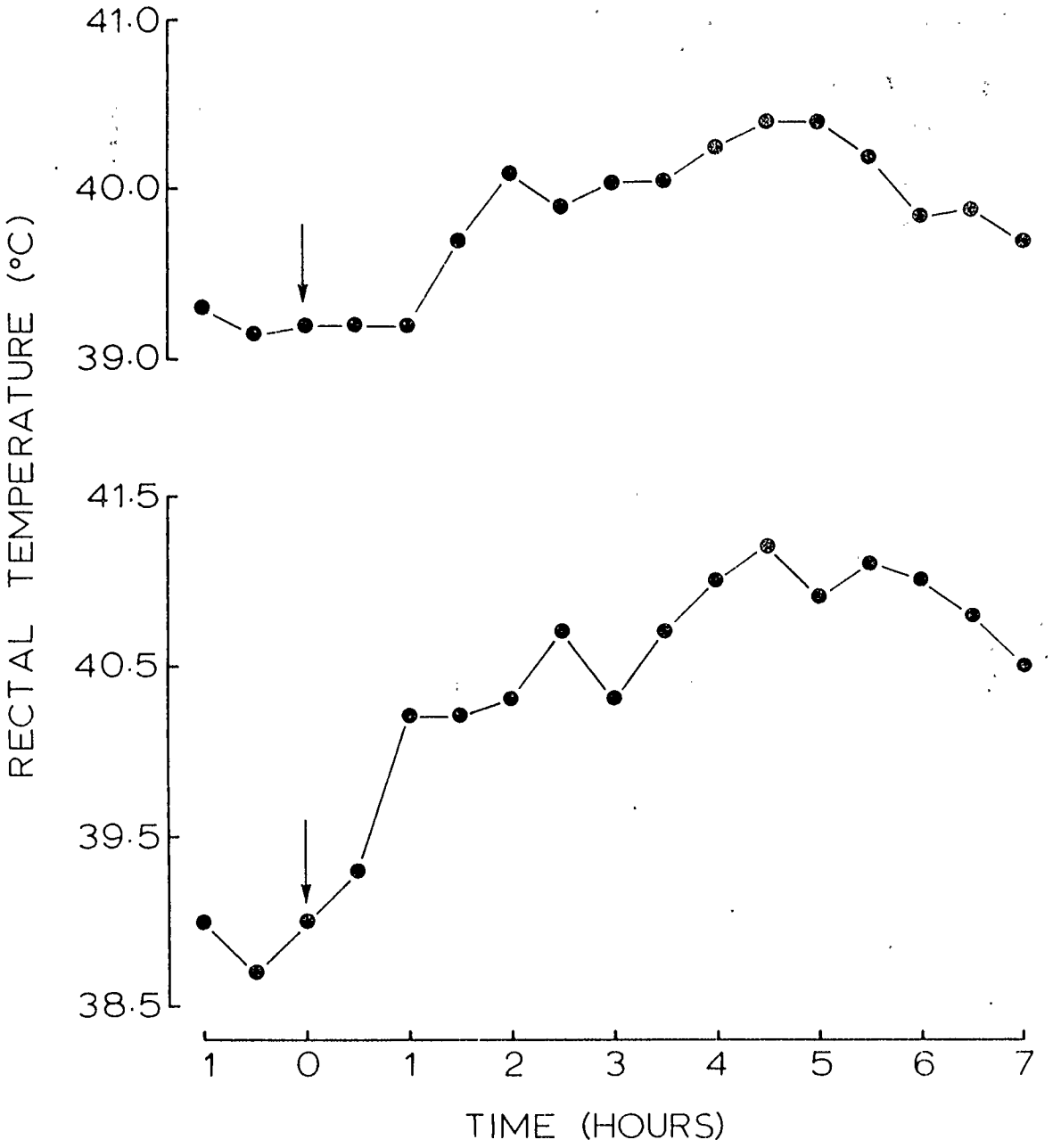

Figure 22. Temperature records of two different lambs following microinjections of SAE pyrogen (0.2 μg, total dose) into the AH/POA of time 0, as indicated by the arrows.

position. Following injections of 10 µg NA into 7 of 16 bilateral sites, rectal temperatures fell by 0.5 - 1.4°C. These sites were subsequently found to lie within the AH/POA. Fig. 20 (lower) shows the temperature response of 1 lamb after microinjection of NA into the AH/POA. The only consistent behavioural response observed during the period of hypothermia was the abandonment of the huddled position. No changes in respiratory rate or ear skin temperature were observed. However, the increase of rectal temperature after the hypothermic interval was sometimes accompanied by shivering. All lambs which showed temperature falls after NA was microinjected were given control saline injections, and on these occasions, temperatures did not change. When lambs at an environmental temperature of 10°C were given microinjections of NA and their temperature did not fall, the injection sites were subsequently found to lie within areas of the hypothalamus other than the AH/POA.

Six lambs were placed in a 30°C environment, and at this temperature, their ears were hot to touch and respiratory rates were high (100-300/min.) Each lamb received bilateral injections of 10 µg NA into both the anterior and the posterior sites. Twelve of the injection sites were found to lie within the AH/POA, yet no consistent temperature change was observed following microinjections into this area or other parts of the hypothalamus. In fact, during the 90 min period after the injection, rectal temperature remained within +0.4°C and -0.45°C of the temperature at the time of injection.

Discussion

The present results show that newborn lambs do not develop fever after intrahypothalamic injection of PGE_1 or PGE_2. It is unlikely that this lack of response is due to inadequate dosage, for other laboratory animals develop prompt fevers after much smaller amounts of prostaglandin. For example, cats will develop fever after as little as 5 ng of PGE_1 injected bilaterally. The present experiments were carried out with up to 1 µg injected bilaterally, and even accounting for the fact that the hypothalamus of the newborn lamb is slightly larger than that of the cat, this should be more than adequate dosage.

These results support the previous findings that, on many occasions, intraventricular injections of PGE_1 did not produce fever in newborn lambs. They would suggest that the lack of fever observed on these instances was not due to an inability of the drug to diffuse into the hypothalamus. It is of interest that fever develops in adult sheep after intraventricular injection of prostaglandin (Bligh and Milton, 1973; Hales et al., 1974; personal observations), yet Martin and Baile (1973) did not observe fever after intrahypothalamic injection of microgram quantities of PGE_1. It may be therefore that the fevers observed in adult sheep, and, on occasion, in newborn lambs after intraventricular injection of prostaglandin, are affecting neurons involved in temperature regulation, but in structures outside of the hypothalamus. There is evidence that fever can occur in rabbits in which the hypothalamus is lesioned (Cooper et al., 1976c)but, at the present time, there is no evidence for a locus of action outside of

the AH/POA for the febrile action of prostaglandins (Veale and Cooper, 1975). Rosendorff and Mooney (1971) were able to obtain small fevers after microinjection of a purified leucocyte pyrogen into the medulla, and this area could also be sensitive to prostaglandins. However, in the present experiments, this locus was not examined for its sensitivity to the febrile action of prostaglandins.

The present experiments show that there are discrete areas of the hypothalamus that are sensitive to NA, but are not sensitive to PGE_1 or PGE_2. Though a dissociation between sites sensitive to NA and PGE_1 has been previously observed in rabbits (Preston and Cooper, 1976), areas which are responsive to PGE_1 are also sensitive to NA in cats (Feldberg and Saxena, 1971; Cooper et al., 1976a)and rats (Veale and Whishaw, 1976).

Newborn lamb brain has prostaglandin-synthesizing and catabolizing activity that approximates that of the adult sheep brain (Pace-Asciak, 1976). Nevertheless, the data presented here provides additional support for the hypothesis that fever can occur in newborn lambs without the central involvement of prostaglandins. If this is true, it follows that fever in newborn lambs should not be antagonized by salicylates, which are believed to exert their antipyretic affects by inhibiting prostaglandin synthesis (Vane, 1971; Milton and Wendlandt, 1971). However, when two lambs were made febrile by intravenous pyrogen, intravenous salicylate (300 mg) was effective in lowering their fever. Consequently, one must conclude either that fever in newborn lambs does involve prostaglandins, but at an undefined locus at present, or that the current thinking with respect to the mechanism of action

of salicylates is open to question.

The use of bacterial pyrogen, injected directly into the hypothalamus, to identify areas of the brain involved in fever can be criticized because it is thought to be an endogenous pyrogen that actually enters the hypothalamus. However, the inability to produce endogenous pyrogen for this purpose necessitated the use of bacterial pyrogen. In addition, the AH/POA has been shown in other species to be very sensitive to direct application of minute quantities of endotoxin (Villablanca and Myers, 1965; Cooper et al., 1967; Repin and Kratskin, 1967; Myers et al., 1974).

Despite the high sensitivity of the AH/POA region to directly applied endotoxin in other species, this area in the newborn lamb appears to be relatively unresponsive to endotoxin. In several instances microinjections of an effective intravenous dose of SAE pyrogen directly into the AH/POA did not cause fever. The dosage would seem more than adequate, since it is possible in other animals to obtain fevers after intrahypothalamic injection of less than 1/1000 the effective intravenous dose (Villablanca and Myers, 1965; Myers et al., 1974). Since the lambs had received previous intravenous injections of bacterial pyrogen and were capable of developing fever to intravenous endotoxin, the lack of fever after intrahypothalamic injection is not due to lack of sensitization. It is also very unlikely that the absence of fever was due to an incomplete delivery of the drug to the hypothalamic tissue. Safeguards that were utilized to ensure that the injection was completed included a) tracking a bubble along the PE tubing during the time of the injection, b) allowing time for the injectate to diffuse

from the tip of the needle after completion of the injection, and c) testing the needle for unimpeded flow immediately after removal from the brain.

When fevers were observed after central injection of pyrogen it was observed that the sites from which febrile responses were elicited lay in, or close to, the AH/POA. Because such large quantities of endotoxin were required to elicit this response, the effect could have been due to the leakage of the injected pyrogen into the general circulation and a subsequent action elsewhere. If this were the case, however, it would be expected that injections into sites more caudal or anterior to the AH/POA which did not produce fever would have had equal access to the cerebral circulation. Such large quantities of pyrogen were used because it had been found that smaller amounts injected intraventricularly did not cause fever.

Another possibility is that the fevers observed after intrahypothalamic pyrogen injection were caused, not by the pyrogen, but by physical disruption of the tissue by the cannula. However, this would appear unlikely since neither control injections, prostaglandin, or noradrenaline injections produced similar temperature increases.

The latency of the febrile response after intrahypothalamic injection of pyrogen varied. Most of the fevers had a latency of about 1 h, which is similar to that observed in the monkey (Myers et al., 1974) but longer than that seen in the cat (Villablanca and Myers, 1965) or rabbit (Cooper et al., 1967) after intrahypothalamic pyrogen injection. One of the lambs, however, developed a fever after a latency of 15 min. This lamb also developed a biphasic fever. Both monophasic and biphasic

fevers have also been observed after intrahypothalamic injection of endotoxin in monkeys (Myers et al., 1974).

The mechanism of action of endotoxin injected directly into the hypothalamus is unknown. Although it may act through prostaglandin release, the evidence in the lamb does not even support a role for prostaglandin in fever. Alternatively, the endotoxin may have a direct effect on neuronal function within the hypothalamus. Indeed, there is evidence that endotoxin can increase transmitter release at the neuromuscular junction in the crayfish (Parnas et al., 1971). However, it is unlikely that the mode of action within the hypothalamus resembles that in the invertebrate peripheral nervous system, as it is highly likely that the transmitter in the latter case is glutamate (Prosser, 1973). In the sheep, it is most likely that fever occurs through activation of a cholinergic heat production pathway (Bligh et al., 1971). Furthermore, if the endotoxin was acting directly on neurons within the hypothalamus, an effect would likely have been seen within a few minutes, rather than after a latency of one hour. Cooper et al. (1967) observed that the injection site in the hypothalamus became infiltrated with polymorphonuclear leucocytes after a microinjection of bacterial or leucocyte pyrogen. Myers et al. (1974) have suggested that these invading leucocytes could produce an endogenous pyrogen which would in turn have an effect on the neurons involved in elevating body temperature.

It is of interest that no temperature change was observed following microinjection of SAE pyrogen into the posterior hypothalamus, where the set-point for body temperature is postulated to exist (Myers

and Veale, 1970, 1971). The insensitivity of this region to locally applied pyrogens is well known (Villablanca and Myers, 1965; Cooper et al., 1967; Repin and Kratskin, 1967; Myers et al., 1974). If fever due to pyrogens does indeed involve a shift in the ratio of the Na^{+} and Ca^{++} ions within the posterior hypothalamus (Myers and Tytell, 1972), it is apparent that pyrogens must exert an additional effect in the anterior hypothalamus or elsewhere in order to be effective in causing fever (Myers et al., 1974).

The results obtained from the injection of NA into the hypothalamus of newborn lambs represent the first data obtained in lambs from this experimental approach. The temperature falls observed following microinjection of NA into the AH/POA of newborn lambs are in agreement with the responses seen after intraventricular injection of this substance into lambs and adult sheep (Bligh et al., 1971). Furthermore, these results show that the locus of action of these substances that are injected intraventricularly is most likely within the AH/POA. These data support the hypothesis that NA acts in an inhibitory manner on heat production. As had been observed in the intraventricular injection studies, NA in the cold environment did not appear to antagonize such heat conservation mechanisms as ear vasoconstriction.

The responses observed after microinjection of NA into the AH/POA during exposure to a hot environment do not provide support for the hypothesis that NA also acts in an inhibitory manner on heat loss (Bligh et al., 1971). It could be that the discrete loci into which the few injections were made may not have been appropriate for affecting synapses where NA was an inhibitory transmitter on a heat loss

pathway. Since responses were observed after intraventricular injection, but not after injection directly into the tissue of the hypothalamus, it is possible that the neuronal pathways subserving heat loss lie very close to the ventricular wall.

With the exception of the responses observed after NA injection in the hot environment, it would appear that the results between the intraventricular approach and the microinjection method agree for prostaglandin, pyrogen and NA injections. Both the intraventricular and the microinjection data would indicate that the newborn lamb is relatively insensitive to central injections of pyrogens and prostaglandins but probably responds well to monoamines.

V. GENERAL DISCUSSION AND CONCLUSIONS

The ability of a newborn mammal to respond to injected pyrogens has been questioned (Stetson, 1961; Bondy, 1971; Landy, 1971). The work reported in this thesis was undertaken to investigate the febrile response to pyrogens in the newborn lamb.

With the use of the cannulated, unanesthetized foetus, it was determined that the number of circulating white cells was reduced after endotoxin administration directly to the foetus. This would suggest that the white cells were capable of recognizing the injected lipopolysaccharide and transporting it to the cells of the reticuloendothelial system. Even though the injected pyrogen had an effect on the foetus, it did not cause an increase in foetal temperature. An increase in foetal temperature, however, was seen when pyrogen was administered to the ewe, and this increase paralleled that seen in the mother. Available evidence would suggest that the foetal fever seen under these circumstances is entirely passive and merely a result of increased build-up of heat in the foetus due to the higher maternal temperature. The epitheliochoreal placenta of the foetus (Wynn, 1965) is likely to exclude passage of the lipopolysaccharide to the foetus and, indeed, administration of pyrogen to the mother did not cause any change in foetal white cell count. Finally, it is unlikely that maternally produced endogenous pyrogen crossed the placenta and elevated foetal temperature, because administration of salicylate to the foetus during a maternal fever did not lower foetal temperature.

When newborn lambs were given bacterial pyrogen or endogenous

pyrogen shortly after birth, they did not become febrile, even though they could increase heat production and conservation in response to a cold stimulus. Over the first few days of life an increasing number of lambs responded with fever to endogenous pyrogen, suggesting that there was a maturation in the central mechanisms involved in fever. However, lambs that could develop fever to endogenous pyrogen often did not become febrile after bacterial pyrogen. This would suggest that there is a maturational response to endotoxin over and above that required in order to respond to endogenous pyrogen. The data would suggest that lambs require a "sensitization" to endotoxin. The sensitization process can be enhanced by iv injection of pyrogen at birth or even during the last few days of gestation. Under natural conditions lambs likely become sensitized by exposure to the ubiquitous endotoxins through the respiratory or gastrointestinal tract. The evidence would favour the former route since sensitization through the gastrointestinal tract could not be demonstrated experimentally.

The mechanism of the sensitization process is still open to study. It appears to occur between antigenically unrelated pyrogens but does not appear to involve antibody production. It would have been of interest to determine when the foetal white cells were capable of elaborating endogenous pyrogen, but technical difficulties frustrated such attempts. Nevertheless, studies in humans (Dinarello, 1975) and guinea pigs (Blatteis, 1976) show that these newborns can produce the molecule, so it is likely that the lamb also has this ability. However, it is difficult to extrapolate to other species due to different placentation (Amoroso, 1961) which may allow passage of endotoxins

to the foetus from the maternal circulation.

It is thought that the maturational process to endogenous pyrogens which occurs over the first few days of life involves some change in the hypothalamic mechanisms of fever. A possible candidate for such a change is in the ability of the lamb to respond to prostaglandins, the supposed "final mediator" of the febrile process (Feldberg and Milton, 1973). Indeed, the results showed that newborn lambs did not respond with fever after direct microinjection of PGE_1 or PGE_2 into the AH/POA, or, in most instances, after intraventricular injection of these substances. However, this unresponsiveness to PGE was shown to be independent of the ability to develop fever after iv pyrogen. Furthermore, it contrasted with the findings that lambs responded to 5-HT or NA injected intraventricularly with temperature changes similar to those seen in adult sheep (Bligh et al.,1971). Results from microinjection and ventricular injection studies suggest that the newborn lamb is capable of developing fever without the central involvement of prostaglandins. This is unlikely to be a function of age, since studies in adult sheep, not reported in this thesis, show that they too are unresponsive to PGE microinjected into the hypothalamus. This evidence may be an important challenge to the theory which suggests that endogenous pyrogen produces a febrile response through the release of prostaglandins.

The data presented in this thesis supports the concept that fever is a pathological process superimposed on normal thermoregulation (Cooper and Veale, 1974). Though the thermoregulation in the newborn lamb is functional at birth, the ability to develop fever appears to depend both on intrinsic developmental aspects of brain function and on

the exposure of the lamb to environmental pyrogens.

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