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# Modern views on the pathogenesis of fever and the mode of action of antipyretic drugs

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## INTRODUCTION

In 1763 the Reverend Edward Stone of Chipping Norton in Oxfordshire presented to the Royal Society in London the results of his observations on the use of the bark of the Willow tree in the treatment of ague. This is the first scientific account published in Great Britain on the use of an antipyretic drug. Just over 200 years later we are beginning to understand the mechanisms of action of antipyretic drugs.

In order to understand the action of these drugs we must have some knowledge of the pathogenesis of fever.

Fever is the rise in deep body temperature which occurs following infection and inflammation, and may be produced by a wide variety of organisms including both Gram-negative and Gram-positive bacteria, viruses, fungi, yeasts and protozoa, and by many inflammatory and related reactions such as tissue damage and necrosis, malignancy, antigen-antibody reactions and tissue graft rejection.

Fever occurs as a result of changes in the central control of deep body temperature produced by pyrogenic substances released following infection and inflammation, and differs from the hyperthermia associated for example with exposure to a hot environment or following heavy exercise. These latter hyperthermias are brought about as a result of the inability of the body to balance heat loss with heat gain, in fever in contrast the balance is maintained at a higher temperature than normal.

The causative agents of fever are referred to as pyrogens, many different pyrogens are known and unfortunately the nomenclature which has been used during the past 100 years is confusing and needs to be clarified. Pyrogens may be differentiated into two basic categories, firstly those pyrogenic substances which are external to the body such as those produced by infectious agents, *exogenous pyrogens*, and secondly those pyrogenic substances which are produced by the body, *endogenous pyrogens*.

*Endotoxins*, often referred to as bacterial pyrogens, form part of the cell wall of Gram-negative bacteria, and are thought to be present in all such bacteria. There is no evidence for the presence of endotoxins in Gram-positive bacteria (Westphal, 1957). Endotoxins are lipolysaccharides composed of three separate regions (Lüderitz, Westphal & others, 1971). Region I comprises the O-specific-chain which is specific for each organism and is responsible for its immunological properties. The O-side chain is composed of repeating groups of oligosaccharides which differ from species to species. Region II comprises the basal core which is also polysaccharide in nature and includes 2-$\beta$-3-deoxyoctonic acid (KDO) as well as attached phosphate groups and ethanolamine. Region III is known as Lipid A and is attached to the basal core by a link to KDO. It is this Lipid A fraction which is responsible for the pyrogenic activity of the endotoxins. Little is known about the pyrogenic components of any of the other exogenous pyrogens.

The existence of endogenous pyrogen was first demonstrated by Beeson in 1948 who found that saline extracts of rabbit neutrophils were pyrogenic when injected intravenously into rabbits, and in 1953 Bennett & Beeson showed that the pyrogenic material obtained from neutrophils was different from endotoxin, it was heat labile, the onset of the fever response was more rapid, it did not produce tolerance following repeated administration and it was able to produce fever of equal magnitude in both normal and in endotoxin-tolerant animals.

Since this pyrogenic material was first obtained from neutrophils (loosely described as polymorphonuclear leucocytes) it is often referred to as leucocytic pyrogen, though since it is now recognized that not only neutrophils but also monocytes, Kupffer cells, and other fixed cells of the reticular endothelial systems can produce the same or at least very similar substances, it is more correctly referred to as endogenous pyrogen.

It is now generally accepted (see Atkins & Bodel, 1974) that the cells capable of producing endogenous pyrogen are activated either by exogenous pyrogens or by endogenous factors such as inflammation, tissue damage etc. to synthesize and subsequently release endogenous pyrogen, and it is this circulating material which is the common mediator of fever.

The characterization studies of Murphy, Chesney & Wood (1971) and Gander & Goodale (1962), Rafter, Collins & Wood (1960) and Bodel, Wechsler & Atkins (1969) indicate that endogenous pyrogen is a protein with a molecular weight in the 10 000–20 000 range and with an isoelectric point of approximately pH 7·0. It is a heat-labile substance and is readily inactivated by alkali. Activity lost during purification can often be restored by the addition of mercaptoethanol suggesting that free sulphydryl groups are necessary for pyrogenicity.

Recently, Gander & Goodale (1975) have investigated the release of endogenous pyrogen from various cells of the body. They found that intravenously administered bacteria and also influenza virus stimulated the spleen and liver to synthesize and release endogenous pyrogen. In contrast, intravenous endotoxin was more active in stimulating the blood neutrophils and monocytes to produce endogenous pyrogen. Gander & Goodale interpret their results by saying that though Kupffer cells, spleen and blood monocytes and neutrophils are all able to synthesize and release endogenous pyrogen following stimulation either as a result of phagocytosis or by bacterial endotoxin, only the cells which are most capable of removing exogenous pyrogen from the blood normally release endogenous pyrogen. Consequently since the fixed cells of the reticular endothelial system (RES) are most efficient in removing foreign particulate matter from the circulation these cells will be mainly responsible for the release of endogenous pyrogen. However, as was shown by Atkins (1960) endotoxin binds most readily with the circulating neutrophils and this explains why endotoxin is most effective in activating neutrophils to produce endogenous pyrogen.

Gander & Goodale conclude that in many naturally occurring fevers it is the fixed cells of the RES which are responsible for the circulating endogenous pyrogen causing the rise in temperature, and the neutrophils may be of very slight importance in such fevers. However, they suggest that in acute inflammatory reactions the neutrophils may be activated to produce endogenous pyrogen, and also when circulating endotoxin is present.

It is now generally accepted that endogenous pyrogens are the circulating mediators of fever and that they induce changes in the central nervous system, presumably in the region of the anterior hypothalamus to decrease heat loss and increase heat gain resulting in an increase in deep body temperature. The sequence of events in the pathogenesis of fever is summarized in Fig. 1.

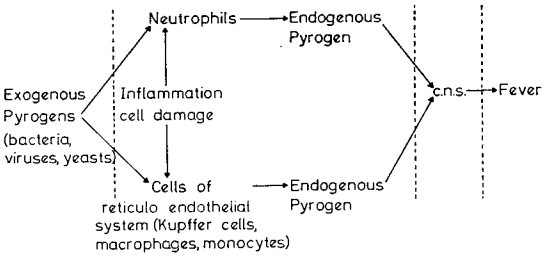


FIG. 1. Schematic diagram of the pathogenesis of fever.

*Actions of pyrogens in the central nervous system*

There is considerable evidence that both exogenous and endogenous pyrogens when injected directly into the central nervous system (cns) produce fever in the majority of species which have been studied, and that the fevers produced are very similar to those resulting from the peripheral administration of these pyrogens.

The evidence to suggest that pyrogens can enter the cns from the periphery is, however, sadly lacking. At the present time the evidence is against endotoxin crossing the blood brain barrier. In 1956, Rowley, Howard & Jenkin were unable to detect any radioactivity in the brains of either mice or guinea-pigs following the peripheral administration of $^{32}P$ labelled bacterial lipopolysaccharide, similarly Braude, Carey & Zalesky (1955), using very large (lethal) doses of endotoxin prepared from *E. coli* and labelled with $^{51}Cr$, found no radioactivity in the brains of rabbits following parenteral administration, and Cooper & Cranston (1963) using $^{131}I$ labelled endotoxin also failed to find any activity in the brain. The only claim put forward to suggest that endotoxin can in fact pass into the cns was made by Bennett, Petersdorf & Keene (1957) who found that animals made tolerant to endotoxin by repeated administration would still respond to a large dose of endotoxin by developing a fever, and they suggested that this was due to leakage of small amounts of endotoxin into the cns, where tolerance does not develop. However, their evidence is not convincing, since it is doubtful that complete tolerance ever develops, and their effects may be explained by a residual effect due to the large dose of endotoxin administered. There are virtually no studies on the passage of other exogenous pyrogens into the cns.

The evidence for endogenous pyrogen passing the blood brain barrier is also lacking. Allen (1965) prepared serum from afebrile and febrile rabbits and labelled the total serum proteins with $^{131}I$. The

labelled serum was injected into the carotid arteries of recipient rabbits, the animals were subsequently killed and autoradiographs prepared from brain slices. In rabbits receiving labelled serum from afebrile donors no radioactivity could be detected in any of the brain slices, in contrast in animals receiving serum from febrile animals radioactivity was detected in the posterior hypothalamus in 4 out of 5 animals used. Radioactivity was not found in any other area of the brain, including the pre-optic anterior hypothalamic area. More recently Gander & Milton (unpublished results) have labelled endogenous pyrogen prepared from rabbit neutrophils (peritoneal exudate cells) with $^{125}I$. When this material was injected intravenously into conscious rabbits no significant amounts of radioactivity could be detected in any areas of the brain.

It still remains then for the answer to this question to be found, for if endogenous pyrogen does not enter the cns, and if it is the mediator of pyrogen fever then it must be initiating events outside the brain tissue perhaps at the level of the brain capillaries.

How do pyrogens affect the anterior hypothalamus to produce a rise in deep body temperature and how do antipyretic drugs bring down fever? A possible answer to these two questions has come from studies on the role of the prostaglandins.

*The role of prostaglandins in fever*

The first evidence that prostaglandins might be involved in fever was provided by Milton & Wendlandt working in the Pharmacology Department at The School of Pharmacy when they reported in 1970 that prostaglandin $E_1$ ($PGE_1$) when injected directly into the third cerebral ventricle of the conscious cat produced vigorous shivering, ear skin vasoconstriction and a rapid rise in deep body temperature. The threshold dose needed to produce a rise in deep body temperature was extremely small, in the order of $3 \times 10^{-11}$ mol, and the duration of the response was short, particularly when compared with the long lasting fever produced by the intraventricular injection of bacterial pyrogens. In all other respects the hyperthermia produced by $PGE_1$ resembled that produced by bacterial pyrogens, both behaviourally and autonomically. Milton & Wendlandt found that prostaglandins $A_1$, $F_{1\alpha}$ and $F_{2\alpha}$ were inactive at the same dose levels as $PGE_1$ with respect to thermoregulatory effects. In a more detailed investigation published in 1971 Milton & Wendlandt showed that prostaglandin $E_2$ ($PGE_2$) had almost identical actions to $PGE_1$, and also that $PGE_1$ was hyperthermic in the rabbit. In another publication in 1971, Milton & Wendlandt reported on the hyperthermic effects of $PGE_1$ in the rat.

In their original publication in 1970, Milton & Wendlandt reported that though the fever produced by the intraventricular injection of pyrogen was abolished by the antipyretic drug 4-acetamidophenol (first reported in 1968 by Milton & Wendlandt) the hyperthermia produced by $PGE_1$ was not affected, and they put forward the theory that $PGE_1$ might be a modulator of body temperature and more important that bacterial pyrogens might produce fever by causing the release of prostaglandin and that antipyretic drugs might act by preventing that release.

By 1971, Feldberg & Saxena had confirmed the original observations of Milton & Wendlandt on the hyperthermic effects of $PGE_1$ in the cat and had also shown that this substance was hyperthermic in the rabbit and rat. In addition they had made two important discoveries, firstly, that when $PGE_1$ was infused into the cerebral ventricular system of the cat the hyperthermia produced was sustained for only as long as the infusion lasted, thereafter deep body temperature soon returned to the pre-infusion level, and secondly, they located the site of action of $PGE_1$ to the preoptic area of the anterior hypothalamus (PO/AH).

These observations then, of Milton & Wendlandt, and of Feldberg & Saxena, indicated that a prostaglandin of the E series would be an ideal substance for modulating increases in body temperature, including fever, since it was active in very small amounts, its duration of action was short, it acted in the area of the brain considered to be the centre for thermoregulation and it was hyperthermic in all the species in which it had at that time been administered.

In 1971, Vane showed that the synthesis of $PGE_2$ and $PGF_{2\alpha}$ from arachidonic acid by guinea-pig lung homogenate was inhibited by aspirin-like drugs. Vane suggested that not only the analgesic and anti-inflammatory action of these drugs but also their antipyretic action could be explained by an inhibition of prostaglandin synthesis, a view that is now widely accepted. In 1971, Piper & Vane had indicated that since there is little preformed prostaglandin in body tissues PG synthesis could be equated with PG release, consequently Vane's observations on the inhibition of PG synthesis by aspirin-like drugs provided the explanation to the theory previously put forward by Milton & Wendlandt (1970) that antipyretic drugs acted by preventing prostaglandin release in the cns.

*Prostaglandin E release during fever and the action of antipyretic drugs*

In 1970, Milton & Wendlandt reported that a prostaglandin-like substance had been found in cat cerebrospinal fluid (csf) during pyrogen fever, and in 1973 Feldberg and Gupta obtained csf from the third ventricle of the conscious cat and assayed it for contractile activity using the rat fundus strip preparation of Vane (1957). They found that in afebrile animals the activity was very low or absent, in contrast, during fever produced by injecting pyrogen directly into the third ventricle, the activity was considerably greater. Following the administration of the antipyretic drug 4-acetamidophenol, the fever abated and the contractile activity of the csf was again low. From their results Feldberg & Gupta concluded that the contractile substance present in the csf was a prostaglandin.

In 1973, Feldberg, Gupta & others collected csf from the cisterna magna of the conscious cat and assayed it for PGE-like activity. They found that the O-somatic antigen of *Shigella dysenteriae* produced a fever when administered both into the third ventricle and into the cisterna magna and also when given intravenously. In all cases during the febrile response the PGE-like activity of the csf increased and the three antipyretic drugs acetysalicylic acid (aspirin), 4-acetamidophenol (paracetamol, acetaminophen) and indomethacin all abolished fever and at the same time the PGE content of the csf fell.

Thin-layer chromatography of the csf samples followed by bioassay and radioimmunoassay indicated that the prostaglandin present in the csf of the cat during fever was prostaglandin $E_2$.

Similar results were obtained by Harvey, Milton & Straughan (1975) in the rabbit in which they produced fever both with the O-somatic antigen of *S. dysenteriae* and also with a purified pyrogen prepared from *Pr. vulgaris* ('E' Pyrogen, Organon).

In addition, they found that if rabbits were made tolerant to the fever producing effect of the 'E' Pyrogen by injecting it intravenously every day for ten days, then on the tenth day when the animals were refractory to the pyrogenic action no increase in the PGE content of the csf was found.

In 1974, Dey, Feldberg & Wendlandt injected purified lipid A prepared from a mutant strain of *Salmonella* into the conscious cat and showed that this substance also produced fever accompanied by an increase in the PGE content of cisternal csf. In addition they showed that antipyretic drugs both inhibited the fever and the release of PGE.

Since bacterial pyrogens activate neutrophils and possibly certain monocytes to synthesize and release endogenous pyrogen, Harvey & Milton (1975) prepared endogenous pyrogen from cat peritoneal exudate cells (neutrophils) and found that when this material was infused intravenously into a conscious cat it produced fever which was associated with an increase in PGE of the cisterna csf (see Fig. 2), and again this fever and the increase in PGE were inhibited by antipyretic agents. In contrast to these results, Cranston, Hellon & Mitchell (1975), carrying out similar experiments in the rabbit, showed that though endogenous pyrogen produced fever and a rise in the PGE content of the csf, when they infused aspirin at the same time as the endogenous pyrogen no increase in the PGE levels of the csf were observed though the dose of aspirin which they used had no antipyretic action and the fever still occurred. These authors maintained that since it was possible to dissociate the effect of aspirin on PGE release from its effect on fever, the direct relation between PGE and fever was in question. However, their experiments did not show whether, though the PGE content of the csf collected from the cisterna magna was reduced, sufficient PGE was still being synthesized and released in the temperature regulating region of the anterior hypothalamus. The fact that the dose of aspirin which they infused was not antipyretic could be used as an argument to support the view that sufficient PGE was still being synthesized to produce fever.

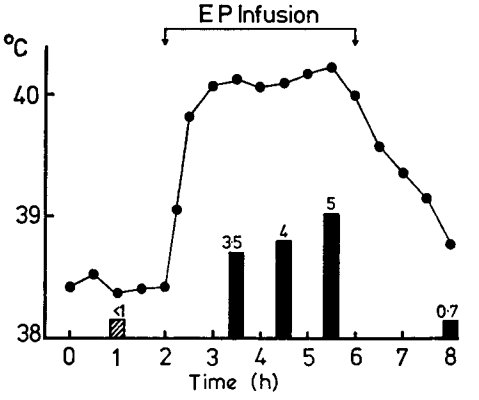


FIG. 2. Record of rectal temperature of an unanaesthetized cat. The height of the columns and the values above the columns refer to the $PGE_2$-like activity in ng $ml^{-1}$ of cisternal csf. The position of the columns refers to the time but not to the duration of the csf collection. Between the arrows endogenous pyrogen (EP) ($2 \times 10^6$ cell equivalents $min^{-1}$), for 5 min, then $2 \times 10^5$ cell equivalents $min^{-1}$ was infused into a saphenous vein (Harvey & Milton, unpublished results).

In 1975, Veale & Cooper reported that when they made extensive lesions in the preoptic area of the anterior hypothalamus (PO/AH) of the rabbit and then applied $PGE_1$ locally it no longer produced a rise in deep body temperature. This observation provided further evidence that the site of action of the $PGE_1$ is the PO/AH, they also observed that in lesioned animals locally applied leucocytic pyrogen (LP) was also ineffective in producing a fever. In contrast to these observations, when they injected $PGE_1$ and LP into a lateral cerebral ventricle the hyperthermic effect of the $PGE_1$ was abolished by the lesion, but the fever produced by the LP was not, although the onset of fever was more gradual. Similarly LP given intravenously also produced fever in a lesioned animal.

The authors conclude from their results that LP also acts at a site other than the PO/AH to produce fever and that this action is independent of the release of PGE, though in their investigations they were unable to find such a site. Unfortunately the authors did not investigate whether the fever produced by LP following lesioning of the PO/AH was inhibited by antipyretic agents. If this secondary site does exist, it is possible that LP whether administered intravenously or intraventricularly could reach the site and release a prostaglandin whereas $PGE_1$ when injected into the cerebral ventricles would be unable to reach the site.

These experiments of Veale & Cooper, and of Cranston & others obviously need further investigation for if it were shown that the action of pyrogens and of prostaglandins in producing hyperthermia were unrelated then the present theory concerning pyrogens, prostaglandins and the mode of action of antipyretic drugs becomes untenable.

Harvey & Milton (1975) found that if plasma obtained from a donor cat, in which fever had been produced by intravenous *S. dysenteriae*, was injected into a recipient cat which had been made refractory to the pyrogen then this recipient cat developed a fever which was accompanied by an increase in cisternal csf PGE levels (Fig. 3). These experiments showed that during bacterial pyrogen fever there was a circulating pyrogenic material in the plasma which differed from the bacterial pyrogen and which was itself capable of producing PGE release. It is concluded that this circulating pyrogen is endogenous pyrogen. In contrast, when they injected bacterial pyrogen directly into the cerebral ventricular system to produce fever no circulating pyrogenic material could be detected in the plasma. These results show, therefore, that centrally administered bacterial pyrogen does not activate the synthesis and release of endogenous pyrogen peripherally and must produce fever by acting on cells within the cns.

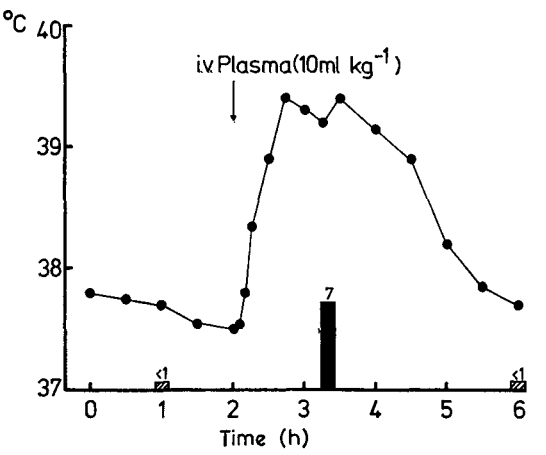


FIG. 3. Record of rectal temperature of an unanaesthetized cat tolerant to *S. dysenteriae*. At the arrow 10 ml $kg^{-1}$ of plasma, obtained from a donor cat in which fever had been induced by the O-somatic antigen of *S. dysenteriae* was infused into a jugular vein. The height of the columns and the values above refer to $PGE_2$-like activity in ng $ml^{-1}$. The position of the columns refers to the time but not to the duration of the csf collection (Harvey & Milton, unpublished results).

*Prostaglandin release in brain injury and 'non-specific' fevers*

It was first noticed by Milton & Wendlandt (1968) that the long lasting hyperthermic response following the injection of 5-hydroxytryptamine into the cerebral ventricles was inhibited by the antipyretic drug 4-acetamidophenol, and they also noticed to their surprise that following the injection of 4-acetamidophenol itself into the cerebral ventricular system there was after a period of time a hyperthermic response which was itself inhibited by an intraperitoneal injection of the antipyretic drug. In the light of our present knowledge and with subsequent experiments (Dey, Feldberg & others, 1974; Milton & Harvey, 1975), it would appear that these hyperthermic responses are not due to a direct action of the drugs themselves but are a 'non-specific' fever produced by interference with the cns and it would appear that in the absence of bacterial infection the slightest 'injury' stimulus to the brain may result in fever consequent to the release of PGE.

*Hypothermic effects of antipyretic drugs in afebrile states*

Antipyretic drugs such as 4-acetamidophenol and

indomethacin, but generally, not salicylates, may produce a fall in deep body temperature when administered to both man and animals in the absence of fever, particularly when given in large doses. In addition when given as antipyretics to reduce fever the temperature may fall from the fever level to below that found in the afebrile state.

In 1973, Milton investigated this phenomenon to determine whether the fall in body temperature in the absence of fever could be attributed to an inhibition of prostaglandin synthesis and release. Indomethacin (2–25 mg $kg^{-1}$) and 4-acetamidophenol (50–100 mg $kg^{-1}$) both produced a fall in deep body temperature when administered intraperitoneally to the conscious cat. This hypothermia was accompanied by ear skin vasodilatation and when high doses were administered by panting. When $PGE_1$ was infused into a lateral ventricle, shivering and ear vasoconstriction occurred and deep body temperature rose; when the temperature had reached a plateau, and whilst the infusion of $PGE_1$ was continued, 4-acetamidophenol (50 and 100 mg $kg^{-1}$) and indomethacin (2 mg $kg^{-1}$) were administered. Both drugs produced vasodilatation and panting but had no effect on the shivering, and deep body temperature fell slightly before reaching a new plateau level which was sustained until the infusion was stopped. From these results it was considered that the effects of the two drugs to produce ear skin vasodilatation and panting were not mediated through inhibition of prostaglandin synthesis but were due to an action of the two drugs on the heat loss mechanisms concerned. Acetylsalicylic acid, 25 mg $kg^{-1}$, did not affect deep body temperature in either the afebrile state or during $PGE_1$ infusion. These results are also regarded as further evidence that the prostaglandins are not involved in normal thermoregulation.

## CONCLUSIONS

Since the possibility was first suggested by Milton & Wendlandt just 6 years ago, the evidence that a prostaglandin of the E series, probably $PGE_2$, is a mediator of pyrogen fever is fairly convincing. When injected directly into the thermoregulatory area of the anterior hypothalamus both $PGE_1$ and $PGE_2$ activate heat gain and inhibit heat loss mechanisms in a manner very similar to that produced by bacterial and endogenous pyrogens. $PGE_1$ and $PGE_2$ are among the most potent substances known which increase deep body temperature when applied directly by injection into the cns, the threshold dose for $PGE_1$ to produce a significant rise in deep body temperature is in the order of $3 \times 10^{-11}$ mol (1 ng). They produce a rise in deep body temperature in all the placental mammals in which they have so far been studied. In this respect they differ from the monoamines which appear to have different effects on deep body temperature in different species and under different ambient conditions.

Bacterial pyrogens, lipid A and endogenous pyrogen all of which produce fever increase the level of PGE found in the cerebrospinal fluid. The concentration of PGE in the csf found during fever would be sufficient to produce a rise in deep body temperature if it were applied to the region of the anterior hypothalamus.

During bacterial pyrogen fever a circulating pyrogenic material, which is not the administered bacterial pyrogen but is of endogenous origin, is found in the plasma. When this is transferred to a recipient animal, it produces both fever and a rise in the PGE levels of the csf. In animals which have been made refractory to the pyrogenic action of bacterial pyrogen by its chronic administration, not only does fever not develop but no increase in csf PGE levels is seen.

The antipyretic drugs such as acetylsalicylic acid, 4-acetamidophenol and indomethacin which have been shown to inhibit the enzyme systems concerned with prostaglandin synthesis all inhibit the rise in PGE levels of the csf when administered during pyrogen fever at the same time as they produce antipyresis. This is true whether this fever is produced by bacterial pyrogens, lipid A, or endogenous pyrogen. It is therefore reasonable to assume that the antipyretic drugs produce their action by inhibiting prostaglandin synthesis and release.

In contrast to their postulated role in fever, there is no evidence as yet that prostaglandins are involved in normal thermoregulation (Cammock, Dascombe & Milton, 1976).

In their paper in 1973, Feldberg & others posed the intriguing question as to whether the general malaise seen in fever was due not to the increase in body temperature as has been suggested but was due to the action of prostaglandins released by the bacterial pyrogens acting on other areas of the brain.

At present we do not know how bacterial or endogenous pyrogens activate the synthetic mechanisms responsible for prostaglandin synthesis and in addition we have almost no information on the role of prostaglandins in fever in man.

REFERENCES


ALLEN, I. V. (1965). *Br. J. exp. Path.*, **46**, 25–34.

ATKINS, E. (1960). *Physiol. Rev.*, **40**, 580–646.

ATKINS, E. & BODEL, P. (1974). Chapter II, Fever. In: *The Inflammatory Process*, vol. III, 2nd edn. Editors: Grant, L. & McClusky, R. T., pp. 467–514, New York and London: Academic Press.

BEESON, P. B. (1948). *J. clin. Invest.*, **27**, 425.

BENNETT, I. L. Jr. & BEESON, P. B. (1953). *J. exp. Med.*, **98**, 477–492.

BENNETT, I. L. Jr., PETERSDORF, R. G. & KEENE, W. R. (1957). *Tr. Ass. Am. Physicians*, **70**, 64–73.

BODEL, P. T., WECHSLER, A. & ATKINS, E. (1969). *Yale J. Biol. Med.*, **41**, 376–387.

BRAUDE, A. I., CAREY, F. J. & ZALESKY, M. (1955). *J. clin. Invest.*, **34**, 858–866.

CAMMOCK, S., DASCOMBE, M. J. & MILTON, A. S. (1976). In: *Advances in Prostaglandin and Thromboxane Research.* vol. 1, pp 375–380. Editors: Sam B. and Paoletti, R. New York: Raven Press.

COOPER, K. E. & CRANSTON, W. I. (1963). *J. Physiol. (Lond.)*, **166**, 41–42P.

CRANSTON, W. I., HELLON, R. F. & MITCHELL, D. (1975). *Ibid.*, **248**, 27–28P.

DEY, P. K., FELDBERG, W., GUPTA, K. P., MILTON, A. S. & WENDLANDT, S. (1974). *Ibid.*, **241**, 629–646.

DEY, P. K., FELDBERG, W. & WENDLANDT, S. (1974). *Ibid.*, **239**, 102P.

FELDBERG, W. & GUPTA, K. P. (1973). *Ibid.*, **228**, 41–53.

FELDBERG, W., GUPTA, K. P., MILTON, A. S. & WENDLANDT, S. (1973). *Ibid.*, **234**, 279–303.

FELDBERG, W. & SAXENA, P. N. (1971). *Ibid.*, **217**, 547–556.

FELDBERG, W. & SAXENA, P. N. (1971). *Ibid.*, **219**, 739–745.

GANDER, G. W. & GOODALE, F. (1962). *Exp. mol. Path.*, **1**, 417–426.

GANDER, G. W. & GOODALE, F. (1975). In: *Temperature Regulation and Drug Action*, pp. 51–58. Editors: Lomax, P., Schönbaum, E. & Jacob, J. Basel: Karger.

HARVEY, C. A. & MILTON, A. S. (1975). *J. Physiol. Lond.*, **250**, 18–20P.

HARVEY, C. A., MILTON, A. S. & STRAUGHAN, D. W. (1975). *Ibid.*, **248**, 26–27P.

LÜDERITZ, O., WESTPHAL, O., STAUB, A. M. & NIKAIDO, H. (1971). In: *Bacterial Endotoxins*, vol. IV, Ch. IV, pp. 145–233. Editors: Weinbaum, G., Kadis, S. & Aji, S. J. New York and London: Academic Press.

MILTON, A. S. (1973). In: *Advances in Biosciences* 9, pp. 495–500. Viewig., Oxford and Braunschweig: Pergamon.

MILTON, A. S. & HARVEY, C. A. (1975). In: *Temperature Regulation and Drug Action*, pp. 133–142. Editors: Lomax, P., Schönbaum, E. and Jacob, J. Basel: Karger.

MILTON, A. S. & WENDLANDT, S. (1968). *Br. J. Pharmac.*, **34**, 215–216P.

MILTON, A. S. & WENDLANDT, S. (1970). *J. Physiol. Lond.*, **207**, 76–77P.

MILTON, A. S. & WENDLANDT, S. (1971). *Ibid.*, **217**, 33–34P.

MILTON, A. S. & WENDLANDT, S. (1971). *Ibid.*, **218**, 325–336.

MURPHY, P. A., CHESNEY, P. J. & WOOD, W. B. Jr. (1971). In: *Pyrogens and Fever.* Editors: Wolstenholme, G. E. W. & Birch, J. Edinburgh and London: Churchill Livingstone.

PIPER, P. & VANE, J. (1971). *Ann. N.Y. Acad. Sci.*, **180**, 363–385.

RAFTER, G. W., COLLINS, R. D. & WOOD, W. B. Jr. (1960). *J. exp. Med.*, **111**, 831–840.

ROWLEY, D. HOWARD, J. G. & JENKIN, C. R. (1956). *Lancet*, **1**, 366–367.

STONE, E. (1763). *Phil. Trans.*, **53**, 195–200.

VANE, J. R. (1957). *Br. J. Pharmac.*, **12**, 344–349.

VANE, J. R. (1971). *Nature New Biol.*, **231**, 232–235.

VEALE, W. L. & COOPER, K. E. (1975). In: *Temperature Regulation and Drug Action*, pp. 218–226. Editors: Lomax, P., Schönbaum, E. & Jacob, J. Basel: Karger.

WESTPHAL, O. (1957). In: *Polysaccharides in Biology*, Editor: Springer, G. F. p. 115. New York: Josiah Macy Jr. Foundation.