The physiological mechanisms by which fever is produced in man and experimental animals have, for many years, intrigued medical practitioners and basic physiologists. Body tissue do not normally contain transferable fever-producing substances. If, however, phagocytic cells are activated by certain stimuli, they may synthesize pyrogenic factors of endogenous origin. First recognized about three decades ago, these endogenous substances were found to initiate the onset of fever if injected into a suitable assay animal (1-5). A variety of factors are produced and released from circulating leukocytes or tissue macrophages when these cells are stimulated either by pyrogenic substances or by engaging in phagocytic activity. Although initially called granulocytic or neutrophilic pyrogen, the fever-producing factors of cellular origin are now generally known as endogenous pyrogen, or EP.

The entire field of body temperature regulation has been stimulated by the recent discovery that many cold-blooded animal species increase their internal body temperatures as a response to an experimental inoculation of pathogenic bacteria (3-5). Cold-blooded animals (and some infant mammals) increase their internal temperature by moving to a warmer environment. This for of "behavioral" fever is analogous to the development of clinical fever during the course of infectious processes in man.

These exciting discoveries and the additional demonstrations that fever may be of benefit in terms of host survival have led to a renewed interest in fever and its underlying mechanisms. This interest is evidenced, in part, by the fact that the American Physiological Society has sponsored two recent symposia on this topic (4-5).

Hormone-Like Role of EP

It is now widely accepted that a variety of infectious microorganisms or inert substances are able to "turn on" or activate certain pyrogen-producing body cells, stimulating them to release EP. This mediating substance then moves via the blood stream to initiate the onset of fever through its ability to stimulate hypothalamic temperature-regulating centers. As depicted in Figure 1, this entire system meets the concept of a hormone-like control mechanism. The individual pyrogen-producing cells are analogous to the cells in endocrine glands which release well known hormones. EP in turn produces its effects on a distant target tissue, i.e., certain neurons within the central nervous system. EP is believed to alter the "set point" of a thermostat-like control mechanism located in temperature-regulating areas of the anterior hypothalamus. This stimulus promptly initiates complex physiological responses mediated via efferent neural pathways to peripheral skeletal muscles and blood vessels. Dermal vasoconstriction and muscular shivering occur as a consequence, leading to an increase in the production and retention of heat within the body. Hormonal responses may also be involved.

The Molecular Nature of EP

Despite much effort by many laboratories during the past three decades, no one is yet able to provide an exact structural formula for this important mediator. Rather, much of the available information about EP has been derived by exclusion. EP is generally thought to be a small protein with a molecular weight of 13,000 to 15,000 daltons. Pyrogenic EP species with larger molecular weights have been identified and it is postulated that some EP may circulate as a dimer or a trimer. Various attempts to concentrate and purify EP have shown that as little as 30-50 ng of purified EP can produce 1.0°C fever in a test rabbit. However, pyrogenic fractions have been purified to the point that they no longer exhibit a chemical reaction for protein, and it is therefore possible that EP may contain carbohydrate or lipid components.

EP is thought to have three sulfhydryl groups and it can inactivate by heat or high pH. The febrile effects of EP are not species-specific. EP does not produce tolerance in the manner of bacterial endotoxin and the molecule is poorly immunogenic.

Assay of EP

All studies of EP are complicated by the need to quantitate the activity of this substance with relatively crude bioassay procedures. Traditionally, this has been done using rabbits carefully selected for their propensity to develop a reproducible febrile response pattern when they are tested. This form of bioassay requires relatively large amounts of pyrogen and the assay alone can consume major portions of any product emerging from fractionation and purification procedures.

Mice may be employed as bioassay animals (4) and a procedure for cannulating the lateral ventricle of the brain of rats for bioassay purposes has been described (5). Both the mouse and the chronically cannulated rat require far smaller quantities of EP.
than rabbits to produce a satisfactory assay. Bioassay systems are difficult to use, must be carefully standardized, and require sufficient numbers of test animals to be statistically valid. Bioassays are also susceptible to the contamination of any test sample by ubiquitous bacterial endotoxins. Endotoxin must be excluded with great care from any crude or purified preparation of EP that requires testing.

An alternative radioimmunoassay procedure has been described (7) but is of limited availability because of the great difficulty in producing highly purified EP and specific high-titer anti-EP immunoglobulin.

**Cellular Production of EP**

Only certain types of body cells are capable of producing and secreting EP. The EP-producing cells of healthy normal subjects or experimental animals have the capacity for phagocytizing particulate matter and all are thought to be of bone marrow origin. None of these cells are of the lymphoid series.

The EP-producing cells that normally circulate within the blood stream include neutrophils, eosinophils, and monocytes. The EP-producing cells usually located within tissues include macrophages, Kupffer cells, and other cells of the reticuloendothelial system. Several varieties of cultured tumor cells spontaneously produce EP in vitro. Tumor cell lines presently known to produce EP include cultured human histiocytic lymphoma cells, Hodgkin disease cells and renal carcinoma cells, and the mouse histiocytic and myelomonocytic tumor cell lines.

Table 1 lists a variety of pyrogenic substances (including both living organisms and nonviable substances) known to stimulate the release of EP from phagocytic cells. Viable or killed microorganisms can generally initiate cellular EP production and release by stimulating an initial episode of phagocytic activity. In addition, many nonviable substances can also activate cellular EP-producing and -releasing mechanisms when studied under in vitro conditions that do not require phagocytosis. The molecular mechanisms leading to the physiological activation of EP-producing cells remain unclear. However, these mechanisms may involve some interaction between a stimulating substance and a receptor on the exterior surface membrane of EP-secreting cells. As shown in Table 2, the production and release of EP is influenced by the source and type of producing cell as well as by the conditions used for in vitro production.

**Table 1. Pyrogens that stimulate EP release.**

<table>
<thead>
<tr>
<th>A. Living Organisms</th>
<th>B. Nonviable Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses</td>
<td>Bacterial endotoxins</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Bacterial exotoxins</td>
</tr>
<tr>
<td>Fungi</td>
<td>Pyrogenic steroids</td>
</tr>
<tr>
<td>Plasmodia</td>
<td>Lymphokines</td>
</tr>
<tr>
<td>Other Microorganisms</td>
<td>Poly I:Poly C. Other polynucleotides</td>
</tr>
<tr>
<td></td>
<td>Antigen-antibody complexes</td>
</tr>
<tr>
<td></td>
<td>Antigens that stimulate delayed dermal hypersensitivity</td>
</tr>
<tr>
<td></td>
<td>Particulate matter</td>
</tr>
<tr>
<td></td>
<td>Bleomycin</td>
</tr>
<tr>
<td></td>
<td>Colchicine</td>
</tr>
<tr>
<td></td>
<td>Muramyl dipeptide</td>
</tr>
<tr>
<td></td>
<td>Nonorganic substances</td>
</tr>
</tbody>
</table>

**Table 2. Cellular EP Production**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood</td>
<td>Bone marrow expansion</td>
<td>Increased neutrophil levels</td>
<td>Increased neutrophil levels</td>
</tr>
<tr>
<td>Sterile Exudate</td>
<td>Occurs in vivo</td>
<td>Prostaglandin levels are increased</td>
<td>Prostaglandin levels are increased</td>
</tr>
<tr>
<td>Collected Cells</td>
<td>Occurs in vivo</td>
<td>Prostaglandin levels are increased</td>
<td>Prostaglandin levels are increased</td>
</tr>
<tr>
<td>Tumor Cells</td>
<td>Occurs in vitro</td>
<td>Prostaglandin levels are increased</td>
<td>Prostaglandin levels are increased</td>
</tr>
</tbody>
</table>

Early studies generally dealt with EP species released in vitro from already-activated cells contained in sterile exudates. Activated human cells can be obtained from sterile exudate fluids found in some acutely inflamed joints. In laboratory animals, exudate cells can come from subcutaneous sterile abscesses previously induced by phlogistic agents or from peritoneal cavity exudates induced by an earlier intraperitoneal injection of an irritant, such as 2% shellfish glycogen solutions. The latter method has generally been used in rabbits to obtain the largest quantities of pyrogen-producing cells.

Exudate cells are activated during their accumulation in vivo and are probably releasing EP at the time they are collected. If placed in sterile physiological saline, these cells release EP spontaneously and promptly, but for a period of only several hours. Peritoneal exudate cells show no requirement for protein synthesis following their collection, although EP synthesis undoubtedly takes place within the exudate cells while they are being mobilized in vivo.

The in vitro release of EP from sterile exudate cells is maximized by holding them in physiological saline at 37°C, but it is inhibited if K⁺ or Ca²⁺ are added to the media. EP release may be a regulated phenomena involving the exterior cellular membrane. This is suggested by the reversal of K⁺ inhibition which is observed following the addition of ouabain as well as K⁺ to the incubation medium.

A different set of circumstances holds if white blood cells are obtained from whole blood and then studied in vitro. Circulating blood leukocytes are not activated in vivo and demonstrate no spontaneous in vitro release of EP. When whole blood neutrophils, eosinophils, or monocytes are studied, they must first be activated by inducing them to engage in phagocytic activity or by stimulating them with endotoxin. In contrast to sterile exudate cells, EP-producing cells obtained from whole blood require an initial period of protein synthesis after the cells are activated. This protein synthesis can be blocked by puromycin or actinomycin. Production of EP can also be blocked by inhibiting cellular energy expenditure with fluoride. The occurrence of in vitro protein synthesis has been confirmed by using radioactively tagged amino acids which are incorporated into EP prior to its release.

Studies with whole blood leukocytes are of special value, for the molecular processes required for EP production can be differentiated from those governing EP release. Unlike the findings in sterile exudate cells, the release of EP from whole blood leukocytes occurs over a prolonged period. Release does not begin until several hours after in vivo activation and then it continues for almost a day. In contrast to its synthesis, EP release
from whole blood leukocytes appears to be independent of energy expenditure, since it is not blocked by fluoride.

In combination, these data suggest that circulating white blood cells do not contain stored EP. This concept is in keeping with earlier studies which consistently failed to demonstrate the presence of preformed transferrable pyrogenic substances in these cells. Thus, the production of EP within circulating leukocytes requires the conventional molecular mechanisms for synthesizing new protein, including the expenditure of cellular energy, the nuclear transcription of genetic information and the de novo synthesis of protein by ribosomes.

Few details are yet known about the molecular aspects of EP production in cultured tumor cells. Since pyrogen output by the tumor cells is spontaneous, does not require activation, and occurs continuously for prolonged periods of time, it has been speculated that a genome which controls EP production remains unexpressed and operates continuously. There are no reports of specific comparisons among the pyrogens produced by the different tumor cell lines.

Since the capacity for synthesizing new protein molecules is known to differ among various EP-producing cells, it is reasonable to expect that they would exhibit different abilities to produce EP. Neutrophils and monocytes have been said to differ in their production of EP. Neutrophils are not activated by phagocytosis of latex beads although they do produce pyrogen after phagocytosis of viable or killed bacteria or after stimulation with bacterial endotoxin. The monocytes, in contrast, can be activated by these latter stimuli as well as by the phagocytosis of latex beads. In vitro pyrogen production by neutrophils involves an early burst of protein synthetic activity, while the production of EP by monocytes occurs over a longer time period. Monocytes contain many more ribosomes than neutrophils, a fact reflected by their capacity to produce 20- to 40-fold more pyrogen per cell than neutrophils. Monocytes may also release EP in a large molecular form as trimers. The EP produced by neutrophils is said to be immunologically distinct from that produced by monocytes. Despite these reported differences, it is likely that some monocytes are present in neutrophil preparations used to generate EP. Both forms of EP have an equal capacity for stimulating a febrile response in assay animals.

**Mechanism of EP Action**

EP is believed to act in some manner upon temperature-regulating centers in the hypothalamus. In addition, close relatives of EP termed leukocytic endogenous mediators (LEM or EP/LEM), and lymphocyte activating factors (LAF) are produced and released concurrently from stimulated cells in a manner similar to that of EP (8). LEM (or EP/LEM) can account for the initiation of many physiologic effects in other target tissues including the liver, bone marrow, heart, and possibly the pancreas. LAF produced by macrophages can initiate immunological responses by lymphoid cells.

It is not known how EP crosses the blood-brain barrier, or precisely where or how it interacts with neurons in the hypothalamic area. Much current thinking is based on the fact that the pyrogenic action of EP can be inhibited by antipyretic drugs, such as aspirin. Accordingly, the effects of EP on neurons are thought to be indirect, mediated locally via secondary neuroactive or neurotransmitter substances released within the brain itself. It has been postulated that EP may stimulate the formation of prostaglandin E1 from arachidonic acid within the hypothalamus. It has also been suggested that other metabolites of arachidonic acid (thromboxanes or prostacyclins) may be the key neurotransmitter substances for fever production rather than prostaglandin. These biologically active substances could lead to an increased local cellular activation of adenylate cyclase to cause cAMP formation within the neurons, or they could alter the local Ca++:Na+ ratios. Although these are plausible concepts, the molecular neurophysiologic mechanisms operative within the thermoregulatory centers are exceedingly complex and have not yet been defined with certainty.

Most physiologists agree that any thermoregulatory control center within the hypothalamus must possess at least three major functional components (5). These include input receptors, a central neuroregulatory mechanism and output effector pathways (Fig. 2). Input signals to the hypothalamus can come from hormone-like mediator substances as well as from a network of temperature-sensing neurons located throughout the brain and peripheral body tissues. These input signals appear to be coordinated within a thermoregulatory center in the hypothalamus which is variously termed the thermostat, comparator, integrator, or summation junction. Lastly, appropriate output responses must be formulated. The output signals may include both neuronal and endocrine mechanisms. These, in turn, would cause a peripheral increase in body heat conservation to produce a fever, or an increase in heat dissipation to cool the body.

This integrated concept may involve more than one central control mechanism within the hypothalamus. As suggested by Satinoff (9), there may be two central thermostats, one which controls all behavioral thermoregulation while the other integrates all EP and neural thermosensory input signals. Alternatively, multiple thermostats may exist, with each type of input signal having its own regulatory mechanisms to generate a single type of output response. While these possibilities should be considered, most workers attempt to explain the hypothalamic regulatory center functions by means of a complex but unified integrating mechanism which is influenced by all incoming signals. To obviate a theoretical requirement for the additional input of a constant reference signal, the system would need to possess multiple feedback loops from thermosensitive neurons.

The conceptual diagram shown in Figure 2 represents an extension of the neuronal model suggested by Bligh and Bacon (10) in which a variety of input signals and control mechanisms may be
It must also be remembered that an experimentally induced fever is associated with an increased production of nonmetabolizable amino acids and producing more glucose, which is taken up more by the liver. In addition to taking up more glucose, the liver also increases its production and output of phagocytic white blood cells.

It is possible that the key mediator for all of these widespread and concomitant metabolic changes is EP itself. However, much evidence is at hand to suggest that many of these concomitant metabolic and physiologic events are initiated by closely related LEM species which are produced and released from phagocytic cells in a manner analogous to that of EP.

Nonfebrile Actions of LEM (or EP/LEM):

A large number of nonfebrile physiologic and metabolic responses are known to begin shortly before or during fevers of infectious or inflammatory origin. These include hyperventilation with respiratory alkalosis, alterations in salt and water homeostasis, and measurable losses of nitrogen, potassium, phosphorus, and magnesium from the body. Many of these responses appear to be initiated by the hormone-like effects of LEM (or EP/LEM). The caloric energy to permit a febrile response can best be explained by the accelerated oxidation of carbohydrate and an increase in gluconeogenesis within the liver. This is made possible by the accelerated catabolism of somatic proteins, with flux of gluconeogenic amino acids from muscle to liver. The hormones that normally function to regulate body carbohydrate metabolism contribute to some degree in this response. Both insulin and glucagon are released in increased amounts from pancreatic islets. In addition to taking up more amino acids and producing more glucose, the liver also takes up and stores increased amounts of iron and zinc during febrile disease states. It rapidly begins to increase production and output of the entire group of "acute-phase resistant" plasma proteins. These include fibrinogen, haptoglobin, C-reactive protein, complement components, alpha-1 acid glycoprotein and ceruloplasmin, and in the rat, alpha-2-macroglobulin as well. The liver also accelerates its synthesis of a number of hepatocellular enzymes and hepatic metallothioneins, but it reduces the production of albumin. At the same time the bone marrow increases its production and output of phagocytic white blood cells.

Bioassay of LEM (or EP/LEM):

Although it has not yet been possible to detect the presence of circulating EP in the serum or plasma of febrile human beings, LEM in plasma can be bioassayed in rats by its ability to stimulate an abrupt decline in serum zinc and iron. LEM also stimulates an abrupt increase in the hepatic uptake of a nonmetabolizable amino acid, L-[14C]cysteine, which is injected prior to the assay for use as an additional marker. The responses induced in assay rats become maximal within 6-8 hours after an intraperitoneal injection of a sample containing LEM. The magnitude of changes in assay rats are linearly correlated with the log-dose of administered LEM; changes fail to occur if normal human or animal plasma samples are tested. However, if human plasma is collected during fever due to bacterial, parasitic, or some viral infections or during active inflammatory bowel diseases, it can be shown to contain LEM. Longitudinal studies conducted throughout the course of typhoid fever in man showed the appearance of LEM in the plasma a day or two before the onset of fever. LEM disappeared only after fever had subsided.

Differences Between EP and LEM:

Because identical techniques have been used to obtain EP, LEM, and LAF from stimulated rabbit peritoneal exudate cells, it remains possible that these may be identical substances. Partially purified preparations containing EP and/or LEM are able to stimulate in vitro colony formation in bone marrow cultures and fibrinogen synthesis in fetal rat hepatocyte cultures. Such findings show that a variety of body cells are targets for these mediators. Some preparations of LEM do not stimulate fever in assay rabbits. LEM does not adhere to glass as does EP, and LEM release from peritoneal exudate cells is not inhibited by the low concentrations of K+ shown to block EP release.

Arguments have been advanced suggesting that the multiplicity of fever-related metabolic and physiological responses and the differences among these various responses during different kinds of infections and inflammatory diseases can best be explained by a large family of closely related mediators rather than by a single mediator. Nevertheless the present uncertainty concerning the relationships between EP and LEM can only be solved by side-by-side comparisons of pure preparations of each putative endogenous mediator in order to define their exact molecular compositions and biological activities.

Summary:

When appropriately activated, mobile and fixed phagocytic cells can produce and release endogenous pyrogen and/or a variety of endogenous mediating substances into surrounding body fluids. The EP/LEM substances have hormone-like stimulatory effects upon distant body tissues. Although their molecular composition and nature of action at the cellular level remain undefined, these endogenous mediating substances appear to initiate or modulate fever as well as many of the generalized host metabolic and physiologic responses that accompany an infectious or inflammatory disease.
REFERENCES


BOOK REVIEWS


This book is intended as an introductory text in pharmacology for the lay public. The principles of pharmacology which are presented, such as a trivial explanation of the cholinergic nervous system, require little or no scientific background and yet may aid the consumer in understanding the effects of drugs. On the other hand, understanding some of the mechanisms of action of specific drugs would require background in animal or cell physiology. Most of the major non-prescription medications are discussed, such as histamine/antihistamines, cough remedies, sleep-aids and laxatives. The few citations of drug interactions and precautions would probably be particularly valuable to the home pharmacist. The third chapter on antibiotics gives the reader a "feel" for antibiotic therapy, which he may or may not apply appropriately under conditions where a physician's judgement is warranted. A text such as this gives the lay person a little knowledge and awareness of drug use in our community, and hopefully would stimulate a cautious outlook on indiscriminate self-administration of drugs as medication.

Suzanne G. Laychock, Ph.D.
Medical College of Virginia


This volume is part of Springer-Verlag's continuing series in sensory physiology; as in their other volumes on the vertebrate visual system this one is directed to areas of interest to researchers on invertebrate vision. The book begins with an introduction by Autrum of photosensory mechanisms from an evolutionary point of view and is followed by a discussion of photoresponses in protozoa as a model system for studying photobehavior. The emphasis in this chapter by D. Diehn is directed to the photoreceptor system for phototaxis of the protozoan algal flagellate Euglena. Two well developed chapters are devoted to discussions of extrasensory photosensitivity; that is, photosensory processes that are initiated not through the eye. One is by M. Yoshida on dorsal, nerve and brain photoreceptors as related to photobehavior. The other is a discussion by M. F. Bennett on extracellular photo reception in relation to circadian and migratory rhythmic photobehavior. These two chapters develop an area of research that is becoming of great interest to photobiologists. The remainder of the book is devoted to the eye. There are four chapters concerning the optics of invertebrate eyes; these are by W. H. Miller on intracellular filters, the physics of optical light gathering systems by A. W. Snyder, pseudo pupils by D. G. Stavenga, and a discussion by P. Kunze of the classical work going back to Exner on the optics of opposition and superposition compound eyes. The invertebrate visual cell electrophysiology is covered in some detail by M. Järvilehto. There are two interesting chapters; one on the spectral sensitivity of the eye and color vision by R. Manzel, and the other on pigments and physiology of invertebrate eyes by K. Hamdorff. The book concludes with how the techniques developed for genetics can be applied to isolate the mechanisms of the visual system of invertebrates which is demonstrated by M. Heisenberg.

In all, the text is well written and profusely illustrated with drawings, and numerous electron micrographs including scanning and transmission micrographs of the various eye structures and photoreceptors. These are very helpful in following the experiments described and the discussions that follow. There is an authors index to publications as well as to species and a subject index.

This volume does not cover all the diverse invertebrate species or their optics and photochemistry of the visual pigments, for much is yet to be discovered. Though the presentations by the various authors are directed to invertebrate visual and photoreceptor systems, comparisons are made to the visual system of vertebrates. On the whole, the book indicates the direction of the research and therefore serves as an invaluable reference source. The price is prohibitive for most students and researchers; nevertheless, it should be in the libraries for students and researchers interested in photobehavior and vision of invertebrates.