PYROGENS and FEVER

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Preface

For some 15 years Sir George Pickering, Dr T. D. Whittet and Dr K. E. Cooper had been urging us to hold a Ciba Foundation symposium to review the problems of pyrogens and fever. However, it always seemed that more progress in research was desirable to make such a review profitable. But by 1969 there was evidence, in particular from Dr D. R. Bangham, of an urgent need for new, standardized and generally acceptable methods for the assay of pyrogens. This, together with the persisting pressure from the original quarters, led to this symposium being held in July 1970.

The meeting revealed the need for still further research, and it is encouraging that the discussions gave rise to new lines of work within a matter of weeks. It is hoped that more investigations in this vital area, which affects both clinical treatment and the production of therapeutic agents, will be stimulated by thoughtful study of these papers and discussions by readers in various parts of the world.

The editors are most grateful to all who contributed to the meeting and to the preparation of this volume, particularly those already named above.

The Ciba Foundation

The Ciba Foundation was opened in 1949 to promote international cooperation in medical and chemical research. It owes its existence to the generosity of CIBA Ltd (now CIBA-GEIGY Ltd), Basle, who, recognizing the obstacles to scientific communication created by war, man's natural secretiveness, disciplinary divisions, academic prejudices, distance, and differences of language, decided to set up a philanthropic institution whose aim would be to overcome such barriers. London was chosen as its site for reasons dictated by the special advantages of English charitable trust law (ensuring the independence of its actions), as well as those of language and geography.

The Foundation's house at 41 Portland Place, London, has become well known to workers in many fields of science. Every year the Foundation organizes six to ten three-day symposia and three to four shorter study groups, all of which are published in book form. Many other scientific meetings are held, organized either by the Foundation or by other groups in need of a meeting place. Accommodation is also provided for scientists visiting London, whether or not they are attending a meeting in the house.

The Foundation's many activities are controlled by a small group of distinguished trustees. Within the general framework of biological science, interpreted in its broadest sense, these activities are well summed up by the motto of the Ciba Foundation: *Consocient Gentes*—let the peoples come together.

NOTE ON TERMINOLOGY

E. Atkins

Throughout the discussions, the terms "bacterial pyrogen" and "endotoxin" are often used interchangeably. This usage, though honoured by tradition, is unfortunately misleading and stems from the discovery that the so-called "injection fevers" of the 19th century were due to the inadvertent contamination of biological materials with pyrogens of bacterial origin present in the air and water. These agents, which are now known to be derived from the cell walls of gram-negative bacteria, have been given the more specific name "endotoxins" and have been identified biochemically as lipopolysaccharides of high molecular weight. Bacteria of other classes, e.g. gram-positive bacteria and mycobacteria, do not appear to contain substances with the same biochemical and physiological properties as endotoxins, although these bacteria may contain or produce a number of agents that will cause fever when given intravenously and therefore should properly be included in the older term "bacterial pyrogens". In a number of instances, these substances are proteins and are presumably pyrogenic by virtue of antigen-antibody reactions occurring in naturally or specifically sensitized hosts.

It is apparent, therefore, that "bacterial pyrogen", as opposed to "gram-negative bacterial endotoxin", is a general descriptive term for many different substances. Since other microbial agents may also be pyrogenic (e.g. certain viruses and fungi) it would seem preferable to retain the term "bacterial pyrogen" only in this generic sense and not as a substitute for endotoxin.

These microbial substances as a group may be referred to as examples of "exogenous pyrogens" to contrast them with pyrogens derived from the tissues of the animal host which are now known collectively as "endogenous pyrogens" or "leucocyte pyrogens".

"Leucocyte pyrogen" is a term usually reserved for the pyrogenic agent isolated from either blood or exudate leucocytes. The major cell type in these instances is the granulocyte although, as will be apparent from the papers and discussions in this symposium, other cell types, e.g. monocytes, macrophages and Kupffer cells, also produce a pyrogen, the biochemical nature of which has not yet been worked out in any detail.

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CHAIRMAN'S OPENING REMARKS

Sir George Pickering

It is nearly forty years since I did my first piece of entirely original research. Lewis and I worked on peripheral vascular disease and one of the problems was to identify and measure obstruction of the main arteries. Lewis thought that if we heated the body the extremities would get hot by vasodilatation which might be measured through skin temperature. We made a chamber to enclose the trunk. When the chamber was heated the skin temperature of the extremities rose, but it did not rise as high or as quickly when there was arterial obstruction (Lewis, Pickering and Rothschild, 1931). The method is useful and still persists.

What interested me more was the mechanism by which the effect was produced. Was it a reflex from the skin, as was currently thought, or was it the effect of warm blood on a central mechanism? I showed that when one limb with its circulation arrested is plunged into cold water there is almost immediate vasoconstriction in the other limb. This wears off. When the circulation is released the vasoconstriction returns after a latent period and persists as long as immersion is continued. When the arm with arrested circulation is plunged into warm water there is no change in blood flow in the opposite limb. When the circulation is released there is no change for several minutes and then vasodilatation begins, increases, and continues as long as the limb is immersed. It seemed quite clear that the application of cold to the skin evoked a vasoconstrictor reflex, but the application of warmth did not evoke a vasodilatation reflex, though both heat and cold had an effect on inducing vasodilatation and vasoconstriction respectively (Pickering, 1932).

This was further investigated by Snell in my department. He showed that infusing warm or cold saline into the antecubital vein would produce vasodilatation or vasoconstriction in the opposite hand. The size of the response was directly related to the amount of the heat transfer. When the responses to raising and reducing the arterial blood temperature were plotted the points lay on a single line. The central receptor was very sensitive, responding to changes of less than 0·1°C (Snell, 1954).

To locate the central receptor, Downey, Mottram and I (1964) cooled single arteries and veins in the conscious rabbit and measured the increase

in oxygen consumption. By far the most responsive area was that served by the internal carotid artery. It seems likely, therefore, that the receptor lies in that territory, in agreement with the observations on direct heating and cooling made by Ström (1950).

When man exercises vigorously the body temperature rises, sometimes by as much as 4°C. This is not a fever. The rise is accompanied by vasodilatation and sweating which, when exercise is discontinued, reduce the temperature to more or less its previous level. Fever is not a simple alteration in heat gain or heat loss. It represents a disturbance of the central mechanism regulating body temperature so that the temperature is raised. Von Liebermeister (1875) thought that fever was due to a change in setting of the central mechanism. Evidence of this was produced by Stern (1892) by rather crude methods. Much better evidence was found by Cooper, Cranston and Snell (1964), who showed that a given heat transfer produced much the same vasodilatation in a given patient when his temperature was normal or raised to a set level by injection of a pyrogen.

Gerbrandy, Cranston and Snell (1954) showed that when bacterial pyrogen was injected intravenously, cutaneous vasoconstriction and increase in mouth temperature did not begin until about fifty minutes after the injection. If, however, the same dose of pyrogen was incubated with 200 ml of blood for two hours and then injected, the latent period was reduced to about twenty minutes. This suggested that bacterial pyrogen first exerts its effect in the blood. Analysis showed that this was due to white cells, not red cells, platelets or plasma. Earlier Bennett and Beeson (1953) had shown that the leucocytes are the only tissue in the body from which it is easy to recover a pyrogenic agent. This agent, endogenous or leucocyte pyrogen, was quite different from bacterial pyrogen in that it was destroyed by heat. Thus it seemed likely that fever was produced by bacterial pyrogen causing leucocytes to produce leucocyte pyrogen, and leucocyte pyrogen then acted on some other structure.

Just before I left my department, Cooper and his colleagues showed that minute doses of leucocyte pyrogen injected into the pre-optic region of the anterior hypothalamus, near the midline, produced fever within a few minutes. When injected elsewhere much larger doses were needed and the latent period was longer. Bacterial pyrogen also acts in larger doses and after a longer latent period. It seems, therefore, that the structure on which leucocyte pyrogen acts is in the pre-optic region of the anterior hypothalamus (Cooper, Cranston and Honour, 1966).

Briefly, our current concept of the mechanism of fever is that infection releases leucocyte pyrogen from leucocytes, and perhaps from other cells, and the leucocyte pyrogen then acts on cells in the anterior hypothalamus

which regulate body temperature so that their "set-point" is raised. This is a hypothesis which can be refuted by further observation and experiment. And arising from it, I would like to ask three questions:

(1) Is endogenous pyrogen related in any way to bacterial pyrogen or is it a totally independent substance? In other words, is a single endogenous pyrogen released from leucocytes of a given species in exactly the same way regardless of the bacterial pyrogen which releases it?

(2) Is the action of bacterial pyrogen simply to release pre-formed endogenous pyrogen, or does it accelerate its manufacture by leucocytes?

(3) Approximately how many molecules of endogenous pyrogen are needed to produce a recognizable fever? If it is comparatively few, as I suspect, then presumably these molecules are preferentially taken up by the central nervous system. But are they also preferentially taken up by temperature-regulating cells? And what effect would they have on the metabolism of such cells, because presumably it is on a metabolic change that the changed setting of the temperature mechanism depends?

I hope and expect that I shall leave this conference having had my ideas expanded and sharpened and perhaps with the answers to these three questions, and others.

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SOME PHYSIOLOGICAL AND CLINICAL ASPECTS OF PYROGENS

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It would be a pity to open a symposium on fever without referring to Dr James Currie, the late 18th-century physician who was responsible for introducing the clinical thermometer into medical practice in England, and who studied many of the problems of the mechanism of fever. He investigated the epidemiology of some of the worst fevers of his time, besides doing the earliest known experimental work on accidental hypothermia. From Currie's time onwards much effort was expended in characterizing the patterns of response of body temperature to different types of infection or different phases of fever, until von Liebermeister (1875), again taking an experimental approach and studying the metabolic response to fever, produced evidence that now supports the view that fever represented a re-setting of the body's temperature regulating mechanisms at a new high level. Studies were made in 1911 by Jules Lefèvre and later by Eugéne du Bois (1936) on the metabolic responses of the whole body which occur during fever. The emphasis at this time was, quite reasonably, on attempting to cure the underlying cause of fever or to reduce the high body temperature by the empirical use of antipyretics. The real breakthrough came with the discovery by Grant and Whalen (1953) and Bennett and Beeson (1953) that a fever-producing substance could be obtained from rabbit white blood cells with or without their ever having been in contact with bacterial material, and with the work of Gerbrandy, Cranston and Snell (1954) in demonstrating that a new pyrogenic substance was liberated when human white blood cells were stimulated by bacterial pyrogen, and also with the work of Westphal and Lüderitz (1954) on the extraction and characterization of the highly pyrogenic lipopolysaccharide material obtainable from gram-negative organisms. The subsequent most important developments in Dr Barry Wood's laboratory and in the laboratories of Dr Atkins and his colleagues will become evident later in this symposium.

The story at present is that certain bacterial products, namely high molecular weight lipopolysaccharides, interact with white blood cells and stimulate them to release a substance which we now call leucocyte pyrogen.

Atkins and Snell (1964) have demonstrated that tissues other than leucocytes can have pyrogenic material extracted from them. This pyrogenic material has similar properties to leucocyte pyrogen, and this may explain the fevers that accompany aseptic tissue damage and allergic responses. Fever of this origin may, following organ transplantation, indicate tissue rejection as well as infection. Also some steroids may stimulate leucocytes to liberate pyrogen (Bodel and Dillard, 1966).

The terms "bacterial pyrogen" and "endotoxin" are both used to describe the pyrogenic material obtainable from microorganisms; however in this paper I shall stick to the term "bacterial pyrogen". Similarly the term "endogenous" pyrogen is frequently used to refer to the material derived from leucocytes, though it seems that the term "leucocyte pyrogen" is more descriptive and apt for the purpose. Pyrogens coming from other tissues could be called "tissue pyrogen", or more specifically a term could be added to denote their derivation.

Leucocyte pyrogen has been shown (Cooper, Cranston and Honour, 1967; Jackson, 1967; Repin and Kratzkin, 1967) in both the rabbit and the cat to act in minute quantities in the pre-optic area and the anterior hypothalamus close to the wall of the third ventricle and near the floor of the brain. Recently Rosendorff, Mooney and Long (1970) found evidence that there may also be a site of pyrogen action further back in the midbrain in the rabbit. Whether or not the leucocyte action on the brain involves monoaminergic pathways, and the extent to which it involves brain cations, will be discussed later in this symposium.

In man, Cooper, Johnson and Spalding (1964) have shown that leucocyte pyrogen does not appear to act on the nervous tissue in the spinal cord below the level of the sixth cervical vertebra or on the autonomic ganglia. The typical responses to pyrogen, namely peripheral vasoconstriction, shivering over the whole muscle mass of the body, and headache, do not occur in patients with a high spinal cord transection. Shivering does occur in a few muscles innervated from above the level of the transection, and if the transection is above the level of outflow of the sympathetic nerves no vascoconstriction is detectable on the body surface and, interestingly, the patient does not get headache.

Cooper, Cranston and Fessler (1960*a*, *b*) showed that leucocyte pyrogen can be precipitated or co-precipitated with ammonium sulphate and recovered; it is destroyed by precipitation with trichloroacetic acid and acetone, and inactivated by trypsin. It appears to be destroyed rapidly on the alkaline side of neutrality, particularly at pH values above 8·1, and more gradually at pH values below 6·5. Attempts were made to purify the leucocyte pyrogen by serial elution with different buffer

molarities from DEAE columns. The best purification obtained was one in which a fever response of approximately 1°C rise in body temperature was produced by a fraction containing 4·75 μg protein. [Further purifications have been carried out (Rafter *et al.*, 1966; Gander, Mitchell and Goodale, 1970) and the leucocyte pyrogen identified as a lipid-polypeptide complex.] This seemed obviously impure and Dr Murphy will show how he has been able to purify this material more than 1000-fold. During purification we lost a large quantity of the purified material on storage in glass; comparison of the light absorption curves of the material before and after it lost its pyrogenicity showed that the material had lost an absorption peak at about 580 mμ. I mention this in the hope that it might be of use to someone attempting chemical purification, though at that wavelength it could also be the beginning of a red herring.

Most of the bacterial pyrogen is cleared from the circulation by a short rapid phase of a few minutes, and then the remainder by a longer phase (Rowley, Howard and Jenkin, 1956; Braude, Zalesky and

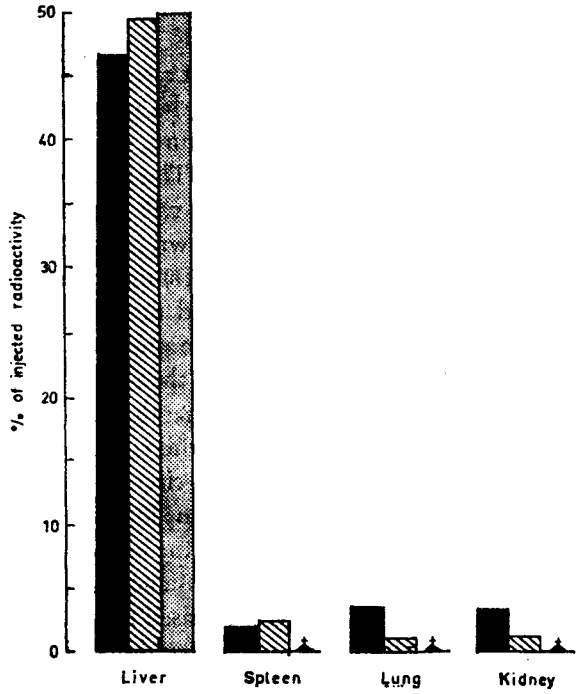

FIG. 1. Proportion of radioactive labelled bacterial pyrogen sequestered in various organs. Results of three independent observers. ■: [^{131}I] Pyrexal (Cooper and Cranston, 1963); ■ : [^{51}Cr] *E. coli* endotoxin (Braude, Zalesky and Douglas, 1958); ■: [^{32}P] *E. coli* endotoxin (Rowley, Howard and Jenkin, 1956).