STUDIES ON EXPERIMENTAL FEVER WITH PARTICULAR REFERENCE TO THE PATHOGENETIC ROLE AND CHEMICAL PROPERTIES OF LEUCOCYTIC PYROGEN*

BY W. BARRY WOOD, JR., DONALD L. BORNSTEIN, AND GALE W. RAFTER

DEPARTMENT OF MICROBIOLOGY, JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE AND SCHOOL OF HYGIENE AND PUBLIC HEALTH

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Fever is a very common manifestation of disease. Its value as a diagnostic sign has been recognized for more than 20 centuries,1 and yet the precise mechanisms which cause it are still unknown.

About a hundred years ago, a group of German clinicians and pathologists2-4 advanced the theory that fever is in some way related to inflammation. This hypothesis was supported by the demonstration that purulent exudates contain an extractable component which, if injected intravenously, causes fever.3-5

The temperature chart in Figure 1 depicts the response of a cat given an intravenous injection of autogenous pus. The experiment was performed in 1866 by a predoctoral medical student at the University of Dorpat.5 Although there is no doubt that he produced fever by this method, his results are difficult to interpret in the light of present-day knowledge concerning the ubiquity of bacterial pyrogens.6

These substances, now usually referred to as bacterial endotoxins, are lipopolysaccharides of high molecular weight,7 which are extremely toxic to animal tissues and give rise to a characteristic febrile response when injected intravenously6 (Fig. 2). The fever which they produce is preceded by an appreciable latent period and is usually biphasic and relatively prolonged. It is regularly accompanied by an initial fall in the peripheral white cell count, followed by a leucocytosis. When injected repeatedly, bacterial endotoxins give rise to a refractory state, known as tolerance,6 in which the latent period is prolonged, the fever is depressed,

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*Fig. 1.—Fever and tachycardia produced in a cat by intravenous injection of 1.5 ml of a gauze filtrate of autogenous pus.5 (Reprinted with permission of the Editor of the New England Journal of Medicine.)
Fig. 2.—Febrile and leucocytic responses of a normal rabbit injected intravenously with 1.5 ml of a 1:10 dilution of typhoid vaccine. (Reprinted with permission of the Editor of the New England Journal of Medicine.)

Fig. 3.—Temperature and leucocytic response of tolerant rabbit to same dose of vaccine. (Reprinted with permission of the Editor of the New England Journal of Medicine.)
and the leucopenia is lessened (Fig. 3). The occurrence of tolerance has been shown to be unrelated to antibody formation. Because of their extraordinary stability to heat, bacterial endotoxins are not destroyed by autoclaving and are often encountered in hospital wards as harmful contaminants of glassware and rubber tubing.

In 1948, a very different kind of pyrogen was isolated from polymorphonuclear leucocytes. Unlike the bacterial pyrogens, it was relatively heat-labile, it caused no leucopenia (when injected in moderate doses), and it produced a monophasic febrile response, preceded by a relatively short latent period and terminating in a prompt defervescence. In addition, it failed to produce tolerance when repeatedly injected into the same animal. Furthermore, its pyrogenicity in normal recipients was found to be the same as in recipients previously made tolerant to bacterial endotoxin.

![Graph](Image)

Fig. 4.—Upper curves show comparative responses of normal and tolerant rabbits to bacterial endotoxin. Note that response to endotoxin is suppressed in tolerant animals (dotted line). In contrast, lower curves indicate that fever-producing effect of leucocytic pyrogen is uninfluenced by tolerance. (Reprinted from *Scientific American*, 196, 62 (1957), with permission of the Editors.)
This important difference in the responses of tolerant recipients to the two kinds of pyrogens (bacterial and leucocytic) is illustrated in Figure 4. In the upper graph are shown the fevers produced by a given dose of bacterial endotoxin in a normal rabbit (solid line) as compared to that produced in a tolerant rabbit (broken line). The depressive effect of the tolerance is evident from the contrasting temperature curves. The lower chart, on the other hand, depicts the responses of normal and endotoxin-tolerant recipients to leucocytic pyrogen. It will be noted that the pyrogenicity of the leucocytic factor is completely unaffected by the tolerance. The striking difference in the comparative behaviors of the two pyrogens in normal and tolerant recipients provides a useful biological method of distinguishing the one from the other.13

By making use of this somewhat cumbersome, but nonetheless reliable biological test, it has been possible, through passive transfer experiments, to define the

![Diagram](image_url)

**Fig. 5.—Relation of mean fever curve of seven donor rabbits receiving intravenous typhoid vaccine (upper chart) to concentration of circulating endotoxin and endogenous pyrogen respectively, as measured by passive transfer to normal and tolerant recipients (lower chart).** Fever index refers to area under mean fever curve of recipients and is measure of both height and duration of fever produced. Whereas endotoxin (stippled area) is rapidly cleared, endogenous pyrogen (cross-hatching) persists throughout febrile response, its concentration being approximately proportional to height of fever.11 (Reprinted with permission of the Editors of the *Journal of Experimental Medicine.*)
principle mechanisms involved in the production of fever by bacterial endotoxins.\textsuperscript{13-14}

When an appropriate dose of endotoxin is injected intravenously, it causes the kind of temperature response illustrated by the donor fever curve in the upper chart of Figure 5. If samples of serum, obtained from the donor animal at the intervals indicated by the small arrows, are injected into recipient rabbits, the fever responses of the recipients (measured in arbitrary fever index units) indicate the amount of transferable pyrogen in the donor rabbit's circulation at the time of the various bleedings. If, in addition, each serum is tested in both normal and tolerant recipients, it is possible to determine what proportion of the pyrogen being transferred is of the endotoxin type and what proportion is of the leucocytic type. It will be seen from the lower chart that the injected endotoxin (indicated by the stippled area) is rapidly cleared from the blood stream and is eventually replaced by an endogenous pyrogen (cross-hatching) which behaves like that derived from polymorphonuclear leucocytes. Furthermore, it will be noted that the height of the donor rabbit's fever is directly proportional to the concentration of endogenous pyrogen in its circulation. Conversely, there appears to be no direct relationship between the height of the fever and the concentration of circulating endotoxin. Therefore, it has been concluded that endotoxin-induced fever is, in reality, caused by the action of the circulating endogenous pyrogen on the thermoregulatory centers of the brain, rather than by the direct effect of the injected endotoxin:

\begin{center}
\begin{tabular}{llll}
Injection & Injury of cells & Release of & Stimulation of \\
endotoxin & in circulation & endogenous & thermoregula- \\
& (leucocytes) & pyrogen & tory centers \\
& & & of brain
\end{tabular}
\end{center}

The following facts add further support to this conclusion:

(1) When radioactively tagged endotoxin is injected intravenously, it exhibits a marked affinity for the white cells of the blood.\textsuperscript{16}

(2) Endotoxin is injurious to white cells\textsuperscript{17,18} and causes circulating polymorphonuclear leucocytes to stick to the endothelium of small blood vessels.\textsuperscript{19} This phenomenon can be directly observed in the rabbit ear chamber, and appears to account for the granulocytopenia which characteristically follows the introduction of endotoxin into the circulation.

(3) The \textit{in vitro} interaction of leucocytes and endotoxin results not only in the eventual inactivation of the endotoxin, but also in the release of endogenous pyrogen from the leucocytes.\textsuperscript{20}

(4) Injected leucocytic pyrogen acts more promptly and more directly upon the thermoregulatory centers of the brain than does injected endotoxin.\textsuperscript{21}

From all of these findings, it appears reasonably well established that leucocytic pyrogen is directly involved in the genesis of endotoxin fever.\textsuperscript{15,22} Other experimental models have been similarly studied. They include fevers caused by the intravenous injection of influenzal viruses,\textsuperscript{23-25} by the production of hypersensitivity reactions to tuberculin,\textsuperscript{26} and by the induction of acute bacterial infections due to Type I pneumococci\textsuperscript{27,28} and to Group A hemolytic streptococci.\textsuperscript{28} In all cases, the fevers produced have been shown to be directly related to the presence in the circulation of a pyrogen of the leucocytic type. The evidence obtained from the study of pneumococcal peritonitis has been particularly conclusive.\textsuperscript{27,28} For
in this model, the endogenous pyrogen has been detected in the peritoneal exudate, in the thoracic duct lymph, and in the blood stream. Its presence at all three sites has been found to correlate directly with the pyrexia.

Despite the impressive evidence that endogenous pyrogens play a central role in the pathogenesis of fever, there is yet no proof that they are all identical, or are derived from polymorphonuclear leucocytes.\textsuperscript{15, 22} Johanovsky has recently reported that cells obtained from lymph nodes of sensitized guinea pigs, when re-exposed to the sensitizing antigen \textit{in vitro}, produce a thermogenic substance similar to leucocytic pyrogen.\textsuperscript{19} Presumably the cells involved are lymphocytes, plasma cells, and monocytes. Except for these as yet unconfirmed observations of Johanovsky, however, all attempts to detect endogenous pyrogen in mammalian cells, other than polymorphonuclear leucocytes, have been unsuccessful.\textsuperscript{30, 31}

Because of the indications that leucocytic pyrogen \textit{per se} is involved in the fever of acute bacterial infections, and because of its ready availability, a systematic study has been undertaken of its chemical properties. The conclusions which may be drawn from the work to date are the following.

The pyrogenic component released from rabbit polymorphonuclear leucocytes during incubation in normal saline is a nondialyzable protein.\textsuperscript{22} It is precipitated by perchloric acid, and removed by extraction with phenol. Soluble in 50 per cent methanol and in 33 per cent saturated ammonium sulfate, it is readily destroyed by the proteolytic action of both trypsin and pepsin.

Efforts to isolate the pyrogen in a homogeneous state have, thus far, resulted in the preparation of a partially purified product which contains less than 1 per cent carbohydrate and no readily extractable lipid. This material produces a brisk febrile response in quantities of 50 micrograms (a 200-fold increase in specific activity), and its chemical properties readily distinguish it from bacterial endotoxins,\textsuperscript{7} from Menkin’s “pyrexin,”\textsuperscript{32} and from the pyrogenic tissue polysaccharides of Landy and Shear.\textsuperscript{34}

The physico-chemical data thus far available suggest that leucocytic pyrogen is a basic protein. Since leucocytic lysozyme is also a basic protein, and since it is known to be released from injured white cells and is present in the circulation during experimentally induced fever, the question has been raised as to whether leucocytic lysozyme and leucocytic pyrogen may not be one and the same substance. The data

<table>
<thead>
<tr>
<th>Purification fraction</th>
<th>Protein (Mg)</th>
<th>Pyrogenicity*</th>
<th>Lysozyme†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original saline extract</td>
<td>12.0</td>
<td>11.8</td>
<td>600</td>
</tr>
<tr>
<td>Butanol (20%) treated extract</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Methanol (50%) supernatant</td>
<td>3.0</td>
<td>10.0</td>
<td>...</td>
</tr>
<tr>
<td>DEAE (pH 8.0) effluent</td>
<td>0.60</td>
<td>10.3</td>
<td>350</td>
</tr>
<tr>
<td>Acetone (50%) supernatant</td>
<td>0.24</td>
<td>10.9</td>
<td>120</td>
</tr>
<tr>
<td>XE-64 (pH 6.2) effluent</td>
<td>0.05</td>
<td>10.3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Fever index units.
† Microgram equivalents of egg white lysozyme.

summarized in Table 1 appear to provide a conclusive answer to this question.\textsuperscript{35} It will be noted from the figures in the right-hand column that the lysozyme activity of the initial pyrogen preparation is relatively high and that this activity is well retained through most of the steps of purification. In the final step, however,
which involves a cationic exchange column-XE-64, the lysozyme activity falls to zero, whereas the pyrogenic activity is undiminished. The evident separation of the two factors by ion exchange chromatography clearly indicates that they are not identical.

Manifestly, many questions remain unanswered concerning the behavior of leukocytic pyrogen. Although its release from leucocytes has recently been shown to be temperature-dependent and to be markedly depressed by such enzyme inhibitors as arsinite, iodoacetate, and p-chlormercuribenzoate, the precise mechanisms involved in its formation and release are still obscure. It is hoped that their nature may be revealed by the study of subcellular fractions now in progress. The manner in which the pyrogen acts upon the thermoregulatory centers of the brain is also completely unknown. Further information concerning the structure of the pyrogen molecule may conceivably suggest an experimental approach to its mode of action. And finally, it is not yet clear whether all of the endogenous pyrogens thus far implicated in the genesis of experimental fevers have a common cellular origin. The preparation of a specific neutralizing antiserum would greatly facilitate the study of this problem.

Not until these questions have been answered will the pathogenesis of fever be understandable on a molecular basis.


