STUDIES ON THE PATHOGENESIS OF FEVER

V. THE RELATION OF CIRCULATING ENDOGENOUS PYROGEN TO THE FEVER OF ACUTE BACTERIAL INFECTIONS*

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A circulating endogenous pyrogen, which acts directly on the thermoregulatory centers of the brain (1), has been demonstrated in the blood of rabbits with fever produced by the intravenous injection of typhoid vaccine (2, 3). Bennett, Petersdorf, and Keene have recently reported that bacterial endotoxins may also, under special circumstances, exert a direct pyrogenic action on the central nervous system (4-7). Their detailed observations clearly indicate that the endotoxin model, which has been used so extensively in the past for studying fever (8), is relatively complex and possesses certain features which pertain only to the action of exogenous pyrogens (1).

It has been suggested elsewhere (3) that the endogenous pyrogen referred to above may represent a common factor in the pathogenesis of fevers caused by a wide variety of inflammatory states, in many of which bacterial endotoxins are obviously not involved. To test further this hypothesis, we have employed in the present study two experimental models designed to simulate the conditions that obtain in naturally occurring bacterial infections. In neither model is a highly injurious agent injected into the blood stream, as in the case of endotoxin-induced fever. The results obtained with both models reveal that the fevers of acute pneumococcal and streptococcal infections are caused by a circulating endogenous factor which is similar to, if not identical with, leucocytic pyrogen (9, 10) and the blood-borne endogenous pyrogen previously described (3). Evidence is also presented that bacterial (exogenous) pyrogen is not involved in the fevers produced by either of these experimental infections.

Methods

The methods of handling the rabbits and recording their temperatures,† and the precautions taken to avoid contamination of solutions and glassware with extraneous bacterial pyrogens have been described in a previous paper (10).

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† Fever indices were calculated as in the preceding study (1). A cut-off point of 240 minutes.
Experimental Infections:

1. Pneumococcal Peritonitis.—The method of producing pneumococcal peritonitis in donor rabbits was similar to that used by Bennett (11). A stock culture of Diplococcus pneumoniae, Type I (strain A-5), which was passed through mice every 2 to 3 weeks, was stored in defibrinated rabbit blood under vaseline at 4°C. Subcultures were made by transferring 0.05 ml. of the stock culture to 5 ml. of beef infusion broth containing 10 per cent sheep serum and 0.2 per cent dextrose. After incubation at 37°C. for 16 hours, 0.5 ml. of the broth culture was transferred to 4.5 ml. of fresh broth. The new culture was incubated at 37°C. for 4 hours and was then appropriately diluted with tryptose phosphate broth. The dose of organisms injected intraperitoneally was that contained in 0.1 ml. of either a 1:10,000 or 1:100,000 dilution of the 4 hour culture.

Before intraperitoneal inoculation the skin of the rabbit's abdomen was shaved with electric clippers. Following inoculation each rabbit was returned to its cage for a period of 14 to 16 hours before the recording of its temperature was begun.

2. Streptococcal Cellulitis.—A stock culture of strain AD-504 of Streptococcus pyogenes (Group A, Type 30) was kindly provided by Dr. Armine T. Wilson of the Alfred I. duPont Institute. This organism was also stored in defibrinated rabbit blood under vaseline, at 4°C. Subcultures were prepared as described above except that the final culture was incubated for only 2 hours at 37°C. before being diluted with tryptose phosphate broth. The dose of organisms injected intradermally was that contained in 0.1 ml. of either a 1:10 or 1:100 dilution.

After the skin of the rabbit's abdomen and flanks had been shaved with electric clippers, a total of 16 to 24 intradermal sites were inoculated in four rows. Within 24 to 48 hours an erythematous, indurated, raised lesion, 3 to 4 cm. in diameter, regularly appeared at each site (see Fig. 1).

Selection of Donors:

In all experiments blood was used only from donor rabbits with fevers of at least 41°C. Rabbits with lower temperatures were excluded on the assumption that their sera would be less likely to contain detectable amounts of transferable pyrogen.

All of the animals with pneumococcal peritonitis died or were sacrificed within 36 hours. Rectal temperatures of 41°C. or higher usually occurred between 16 and 24 hours after inoculation. However, 21 of the 51 pneumococcal rabbits either died without having had any detectable fever or succumbed before their body temperatures had reached a level of 41°C. All such animals were discarded.

The donor rabbits with streptococcal cellulitis, on the other hand, rarely died of the infection. With only a few exceptions they regularly developed fevers of 41°C. or higher on the 1st or 2nd day after inoculation.

Collection of Blood from Donors:

Donor animals were exsanguinated by intracardiac puncture. The bleedings were performed with 18 gauge needles and 50 ml. syringes. Samples of whole blood were prevented from clotting with heparin. Serum was collected by allowing the blood to clot at 37°C. for 1 hour; after storage overnight at 4°C., the serum was removed and cleared by centrifugation. The samples of blood and serum were separately pooled.

Heparin sodium, 0.04 mg. was added per ml. of blood.
Blood Cultures:

A 1 ml. sample of blood from each donor rabbit was cultured in a pour plate containing 2 per cent nutrient agar. The plates were examined for growth after 48 hours of incubation at 37°C. All blood cultures from donor rabbits with pneumococcal peritonitis showed heavy growths. All those from the streptococcal cellulitis donors remained sterile.

Handling of Blood:

The pools of whole blood were stored at 4°C. and were tested for transferable pyrogen within 5 days. No effort was made to kill or remove the organisms contained in the whole blood pools from the donors with pneumococcal peritonitis.

The serum pools from donors with both types of infections, on the other hand, were passed through pyrogen-free Seitz filters before being used in the passive transfer tests. Postfiltration cultures of both types of serum were uniformly negative.

Assay of Pyrogen:

The method employed to stabilize the pre-injection temperatures of the recipient rabbits, and the techniques of preparing and injecting those with carotid catheters have been described in previous studies (1, 2). All other recipients were injected rapidly via the marginal ear vein, except those given 100 ml. of whole blood or serum.

The recipients injected with 100 ml. samples were prepared as follows:

On the day prior to injection each rabbit was anesthetized lightly with intravenous pentobarbital, and a small incision was made in the skin overlying the right external jugular vein. A polyethylene catheter, previously filled with heparin and tied at the distal end, was inserted toward the vena cava for a distance of 15 cm. from the point of entrance into the external jugular vein. The catheter was secured in place by ligatures which were drawn tightly around both the vein and the catheter above and below the point of insertion. The distal end of the catheter was brought out through a second small skin incision on the back of the neck in a manner similar to that previously described (1). Both skin incisions were closed with cotton sutures.

The injections of 100 ml. of whole blood or serum were made through the catheter immediately after 40 ml. of the recipient's blood had been removed via the same route. The combined maneuver was regularly accomplished in a period of less than 3 minutes.

When volumes as large as 50 or 100 ml. were used, they were prewarmed to 37°C. as routine before being injected.

Tolerant recipients were prepared by 7 daily intravenous injections of 1 ml. of undiluted typhoid vaccine. Each recipient was tested for tolerance on the last day of the series of injections.

RESULTS

1. Demonstration of Transferable Pyrogen in the Sera of Rabbits with Pneumococcal Peritonitis.—The fever responses of individual recipients injected with 10, 50, and 100 ml. of serum from donors with pneumococcal peritonitis are shown in Text-fig. 1. The responses produced by the 50 and 100 ml. doses clearly indicate the presence of a pyrogenic factor in the blood streams of rabbits with this infection. It will be noted that the transferable pyrogen:

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*The dimensions of the catheter were: internal diameter, 0.047; outside diameter, 0.057 inches. The heparin solution contained 5 mg. of heparin sodium per ml.

*From the same lot as used in previous experiments (2).
(a) causes a febrile reaction characterized by a short latent period, (b) is detectable only when relatively large volumes of serum are passively transferred, and (c) is capable of producing a relatively prolonged biphasic fever when given in sufficient quantity.

2. Evidence That the Circulating Pyrogen is Endogenous.—

(a) Comparative responses in normal and tolerant recipients: As emphasized elsewhere (1-3, 10) exogenous pyrogens, such as those produced by bacteria,
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cause less fever in tolerant than in normal recipients (8), whereas the response to endogenous pyrogen, including that in leucocytic exudates (9, 10) and in the sera of rabbits made febrile with typhoid vaccine (3), is uninfluenced by tolerance. The comparative fevers produced in normal and tolerant recipients by injections of 50 ml. of pneumococcal donor serum, are shown in Text-fig. 2.

![Text-fig. 3. Comparative febrile responses of normal recipient to slow intracarotid and intravenous injections of 50 ml. of pooled, filtered serum obtained from rabbits with fever caused by pneumococcal peritonitis. Note the differences in the latent periods and in the amounts of fever produced.](image1)

![Text-fig. 4. Same as Text-fig. 1 except that serum was obtained from rabbits with fever caused by streptococcal cellulitis.](image2)

The similarity of the responses in the two types of recipients strongly suggests that the pyrogen present in the circulation is of endogenous origin.

(b) Intravenous versus intracarotid injection: Because endogenous pyrogens of the type referred to above have been shown to cause greater and more prompt febrile responses when injected via the carotid artery than when given intravenously, it has been concluded that they act directly upon the thermoregulatory centers of the brain (1). Exogenous pyrogens derived from bacteria, on
the other hand, exhibit no such difference, and the conclusion has therefore been drawn that they act by a different and less direct mechanism (1). The comparative febrile responses to intracarotid and intravenous injections of 50 ml. doses of serum from donors with pneumococcal peritonitis are shown in Text-fig. 3. It will be noted that the intracarotid response was greater and more prompt than that caused by intravenous injections. These results indicate that the circulating pyrogen, like the other forms of endogenous pyrogen previously studied, acts directly upon the central nervous system.

3. Analogous Experiments Involving Streptococcal Cellulitis.—The febrile responses of individual recipients injected with 10, 50, and 100 ml. of serum from rabbits with experimental streptococcal cellulitis are shown in Text-fig. 4. The presence of circulating pyrogen is clearly indicated by the fevers which
resulted from the transfer of 50 ml. and 100 ml. of serum. It will be noted from the results summarized in Text-figs. 4 to 6 that the properties of the circulating pyrogen in streptococcal cellulitis are identical with those of the fever-producing factor in pneumococcal peritonitis.

### TABLE I

*Demonstration of Pyrogen in Circulation during Febrile Phase of Experimental Bacterial Infections*

<table>
<thead>
<tr>
<th>Infection (Donor)</th>
<th>Sample transferred from donor</th>
<th>Type of recipient</th>
<th>Fever index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Volume (ml)</td>
<td></td>
</tr>
<tr>
<td>Pneumococcal peritonitis</td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Normal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Normal</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Tolerant</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Normal</td>
<td>24.3</td>
</tr>
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<td></td>
<td>100</td>
<td>Normal</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Tolerant</td>
<td>27.0</td>
</tr>
<tr>
<td>Whole blood</td>
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<td>Normal</td>
<td>3.0</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>Normal</td>
<td>3.7</td>
</tr>
<tr>
<td>Streptococcal cellulitis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Normal</td>
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</tr>
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<td>Normal</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>Normal</td>
<td>21.7</td>
</tr>
</tbody>
</table>

4. **Transfer of Pyrogen in Whole Blood.**—Three individual recipients were injected with 100 ml. each of heparinized whole blood, pooled from donors with pneumococcal peritonitis. A prompt, but weak, febrile response occurred in all 3 recipients (see Table I). Each recipient was found dead on the following morning. Since all recipients of filtered serum from pneumococcal donors remained healthy following injections of similar doses, it was assumed that the death of the animals receiving the unfiltered blood was due to the presence of pneumococci in the injected samples. It was further assumed that the relative weakness of the febrile response to the approximately 50 ml. dose of serum
in each 100 ml. of infected whole blood was due to a depressive effect of the immediate and heavy bacteremia induced by the transfer.

Two individual recipients were likewise injected with 100 ml. each of heparinized pooled blood from donor rabbits with streptococcal cellulitis. A prompt, and in this case, strong febrile response occurred in both animals (see Table I). Both recipients remained healthy following the injections. It was assumed, therefore, that the survival of the recipients and their ability to respond normally to the pyrogen contained in the transferred blood (compare fever indices in Table I) was due to the fact that the streptococcal donor rabbits were not bacteremic.

DISCUSSION

From the above studies two principal conclusions may be drawn: first, that a circulating pyrogen is regularly demonstrable in the blood sera of rabbits with high fevers from acute pneumococcal peritonitis and acute streptococcal cellulitis, and, secondly, that the newly detected pyrogen is biologically indistinguishable from leucocytic pyrogen, and, like it (1), acts directly upon the thermoregulatory centers of the brain. Each of these conclusions warrants separate discussion.

In previous experiments dealing with pneumococcal peritonitis, Bennett (11) was able to demonstrate, both in the peritoneal exudate and in the thoracic duct lymph, a heat-labile substance which appeared to have all of the biological properties of leucocytic pyrogen. Its presence at both sites was closely correlated with the febrile course of the infection. Despite repeated attempts, however, Bennett was unable to detect a similar pyrogenic factor in the blood stream. His failure to do so led him to conclude that a causal relationship between the fever and the pyrogen in the exudate and lymph had not been established (11). Conclusive proof of the causal relationship appears to have been provided by the present study.

It is now clear that Bennett's failure to demonstrate the circulating pyrogen was due primarily to the fact that he did not use large enough volumes of serum in the passive transfer tests. In addition, the temperatures of many of his donor rabbits were not as high, at the time of bleeding, as in the present experiments. That the quantitative aspects of the passive transfer test are crucial is clearly indicated by the data summarized in Text-figs. 1 and 4. Particularly noteworthy is the fact that the endogenous pyrogen present in the serum will cause a relatively prolonged biphasic fever, when it is transferred in sufficient quantity. This observation appears to negate the previously accepted conclusion that endogenous pyrogen is capable of producing only a brief, monophasic response when injected intravenously (6, 7, 13).

As emphasized in the preceding report (1), the quantitative limitations of the passive transfer method also necessitate a cautious interpretation of such studies as those recently described by Bennett, Petersdorf, and Keene (5-7), which appear to reveal a dissociation of endotoxin-produced fever from the presence of endogenous
pyrogen in the bloodstream. The failure to demonstrate circulating endogenous
pyrogen in such experiments may be due, not to its absence, but rather to the fact
that it is not present in sufficient quantities to be detectable by the passive transfer
technique.

That leukocytic pyrogen and the circulating pyrogenic factor described in
the present experiments are closely related, if not identical, appears to be well
established. Both cause prompt febrile responses with relatively short latent
periods; the pyrogenic effects of both are uninfluenced by tolerance; and both
act directly upon the thermoregulatory centers of the brain (1, 9, 10). Furthermore,
the demonstration of a heat-labile pyrogenic factor, indistinguishable
from leukocytic pyrogen, in the peritoneal exudate and thoracic duct lymph
during the febrile phase of pneumococcal peritonitis (11), makes it appear
inescapable that the pyrogen in the circulation is derived from the same source.
Since the pyrogen present in granulocytic exudates has been shown to be
released from polymorphonuclear leukocytes (10), it is concluded that the
endogenous pyrogen which is demonstrable in the blood of rabbits with fever
from acute pneumococcal peritonitis is derived from the leukocytes of the
inflammatory exudate at the site of infection.

The evidence that the circulating endogenous factor is unrelated to bac-
terial pyrogens (8), to Menkin's pyrexin (14), and to the tissue polysaccharides
recently isolated by Landy and Shear (15) is equally convincing.

All of the latter pyrogens are heat-stable, produce fever only after relatively pro-
longed latent periods, cause tolerance when repeatedly injected, and produce less
fever in tolerant than in normal recipients (8, 9, 14, 15). Although no satisfactory
method has yet been found for testing the heat stability of pyrogens contained in
serum (13), it can be stated with assurance that none of the other properties referred
to is shared by either the circulating factor described in the present study or the
analogous endogenous substance previously discovered in the serum of rabbits with
endotoxin fever (3).

Due to their apparent sites of origin within the body, both of the latter
pyrogens have been referred to as endogenous. Unfortunately, the same term
has been used by Grant and Whalen (17) to designate the combined form in
which exogenous (bacterial) pyrogen exists in blood serum. The resulting
semantic conflict has naturally led to confusion (18). The endogenous pyrogens
described in the present studies (1-3, 10) must be clearly differentiated from
those which, in their original states, were obviously obtained from exogenous
sources (8, 17).

* Menkin's recent demonstration that large doses of pyrexin fail to cause tolerance (16)
does not detract from the important finding of Bennett and Beeson (9) that smaller doses do
so. It has been clearly established that tolerance to exogenous pyrogen may be effectively
masked if the doses used for repeated injections are sufficiently large (7).
Reference has already been made to the complexity of the endotoxin fever models, as recently revealed by the extensive studies of Bennett, Petersdorf, and Keene (4–7).

They have convincingly shown that bacterial endotoxins will cause fever when injected intrathecally in relatively small quantities (4), and will also, under special circumstances involving relatively large intravenous doses, “spill over” into the spinal fluid and thus presumably initiate a pyrogenic reaction (5–7). These findings do not necessarily indicate that a direct endotoxic mechanism operates in fever produced in normal rabbits by more moderate doses of endotoxin, in which quantitative studies have already revealed that the febrile response is directly related to the amount of endogenous pyrogen in the blood stream (3). They do, however, serve to emphasize the desirability of studying other experimental models in which conditions resemble more closely those which obtain in naturally occurring disease.

Even in such models as those employed in the present study, the question may be raised as to whether the fever observed is not due to the combined actions of the circulating endogenous pyrogen and pyrogenic components derived from the invading bacteria. Although the possible participation of pyrogens produced by infecting organisms cannot be completely excluded, there is convincing evidence that they do not play a significant role in the forms of fever studied in the present experiments. In the case of experimental pneumococcal peritonitis, the pyrogen present in the peritoneal exudate and lymph was previously found to be heat-labile, to be equally active in tolerant and normal recipients, and to be incapable of producing tolerance (11). In other words, no heat-stable component, compatible with a bacterial pyrogen, could be detected in either the exudate or the lymph. Neither could a pyrogen be obtained from the pneumococci themselves (11). Under these circumstances it is almost inconceivable that bacterial pyrogen could be present in the spinal fluid. Furthermore, the activity of the pyrogen demonstrated in the blood was not affected by tolerance (see Text-figs. 2 and 5), as would be expected of a bacterial pyrogen acting in the blood stream (3). And, finally, prolonged pneumococcal infection has been found not to induce pyrogen tolerance (19), which certainly should occur if a circulating bacterial pyrogen were involved in the pathogenesis of the fever.

Concerning the beta hemolytic streptococcal infections, the evidence is less complete. Unlike Pneumococcus, this species of organism possesses an endotoxin with properties similar to those of endotoxins extractable from Gram-negative bacilli (20). The endotoxin, however, does not appear to play an important role in streptococcal fever, for as shown in the present study, the pyrogen demonstrable in the circulation during the febrile phase of the infection is uninfluenced by tolerance, and acts directly upon the central nervous system.

Whether bacterial pyrogens are involved in the pathogenesis of fevers caused by other infections, particularly those due to Gram-negative bacilli remains
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to be determined. Bennett's demonstration that *E. coli* infections of 7 to 8 days' duration in rabbits do not cause tolerance (19) would appear to indicate that, even in this form of infection, a factor other than endotoxin is responsible for the fever. Accordingly, it seems logical to suggest that the principal common factor in most, if not all, fevers due to bacterial infections is the presence in the circulation of an endogenous pyrogen, derived from sites of inflammation and similar to the circulating factor described in the present study. Indeed, because inflammation is a prominent pathological feature of most febrile states, including those caused by viral infections, vascular lesions, hypersensitivity reactions and even neoplastic diseases, one is tempted to extend the theory still further (21). Recent observations of Atkins (22) have a direct bearing upon the general hypothesis.

Not only has he found that fevers caused by the intravenous injection of influenza viruses (23) are directly correlated with the presence of an endogenous pyrogen in the blood stream, but he has also demonstrated a similar factor in the circulations of rabbits with fever caused by tuberculin hypersensitivity (24). Both of these observations, together with those reported in the present study, are in keeping with the thesis that endogenous pyrogens derived from cells of inflammatory exudates play a central role in the pathogenesis of a wide variety of naturally occurring fevers.

Current knowledge of the chemistry of endogenous pyrogens is extremely fragmentary (9). Whether they are produced by cells other than polymorphonuclear leucocytes is at present not known. The fact that they have already been found, in at least one instance, to be species-specific (25), suggests that they represent more than a single chemical entity. Now that rabbit leucocytic pyrogen can be readily obtained in solutions presumably free of many other cellular constituents (10), it would appear feasible to undertake additional studies relating to its chemical identity, despite the difficulties which are certain to be encountered from possible contamination with extraneous pyrogens and from the necessity of using the relatively cumbersome bioassay method for identifying endogenous pyrogen. Such studies are in progress.

**SUMMARY**

An endogenous pyrogen, which is indistinguishable from leucocytic pyrogen, has been demonstrated in the blood streams of rabbits with fevers caused by experimental pneumococcal and streptococcal infections. Like the endogenous pyrogen previously detected in the serum of animals with fever produced by the intravenous injection of typhoid vaccine, the newly discovered circulating factor acts directly upon the thermoregulatory centers of the brain. Its origin from polymorphonuclear leucocytes at the site of infection appears to have been established.

The possible relationship of this circulating endogenous pyrogen to the pathogenesis of other forms of fever is discussed.
BIBLIOGRAPHY


22. Atkins, E., personal communication.


EXPLANATION OF PLATE 31

Fig. 1. Lesions produced by intradermal injections of Stræptococcus pyogenes. Photograph taken at 48 hours. (Reduced to approximately one-half size.)
Fig. 1

(King and Wood: Pathogenesis of fever. V)