

# OBSERVATIONS ON THE FEVER CAUSED BY BACTERIAL PYROGENS

## II. A STUDY OF THE RELATIONSHIP BETWEEN THE FEVERS CAUSED BY BACTERIAL PYROGENS AND BY THE INTRAVENOUS INJECTION OF THE STERILE EXUDATES OF ACUTE INFLAMMATION\*

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(Received for publication, May 3, 1948)

### INTRODUCTION

Menkin (1) has isolated from sterile exudates in dogs a material closely associated with the euglobulin fraction which causes a febrile response when injected intravenously in rabbits. This substance he described as a product of cell injury and termed *pyrexin*. He attributed the fever accompanying acute inflammatory states to the action of pyrexin produced at the site of inflammation. Menkin's work is open to the criticism that he failed to mention precautions against the ever present danger of contamination with bacterial pyrogens during the process of fractionating the exudate. The sterility of the whole exudate and the fractions derived from it is only one indication of freedom from such contamination, since pyrogens are heat-stable and can cause fever in extremely small quantities (2, 3).

If the fever-promoting effect of sterile exudates is due to the presence of bacterial pyrogen, animals receiving a course of daily injections of an exudate should develop tolerance for this effect and should also show comparative insensitivity to the effects of other pyrogens (4, 5). The present study was undertaken in an attempt to define the relationship between the fever following injection of exudates in rabbits and that caused by bacterial pyrogens.

### *Materials and Methods*

Sterile exudates were produced in dogs by the intrapleural injection of 1.5 to 2.0 ml. of turpentine as described by Menkin (6). Following the injection of this irritant, daily thoracenteses were performed and about 15 ml. of chest fluid was withdrawn for examination. Clotting was prevented by the addition of heparin. Cultures were made immediately in thioglycollate broth, the pH was tested, and smears were examined for bacteria and differential cell count. The cultures were examined after 24 and 48 hours of incubation and any exudate

\* The opinions expressed in this report are to be construed as those of the author alone and do not reflect those of the Naval Medical Corps or the naval service at large.

showing evidence of bacterial contamination was discarded. The pyrogenicity of the whole exudate was tested each day by intravenous injection in rabbits.

As Menkin described (1, 6), with progression of the inflammatory process, the reaction of the exudate, in most cases, changed from alkaline to acid within 4 to 6 days. The cellular content consisted almost entirely of polymorphonuclear leucocytes at first but this gradually gave way to a predominance of mononuclear cells by the time that the fluid had become acid.

Within 24 to 48 hours after the exudates became acid, they usually caused definite febrile responses in rabbits. However, three of nine acid exudates failed to produce fever and, on one occasion, an exudate with an alkaline reaction proved to be pyrogenic.

Rectal temperatures and circulating leucocyte counts were recorded twice daily in dogs injected with turpentine. These followed a fairly consistent pattern (Fig. 1). Fever generally

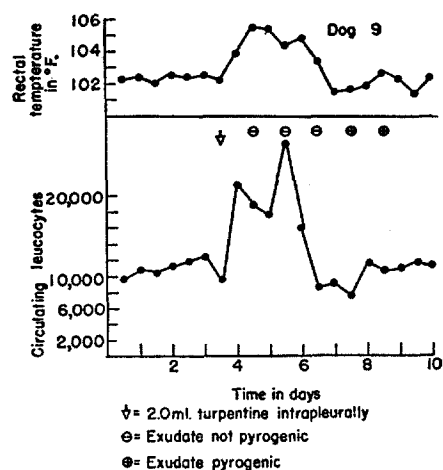


FIG. 1. Rectal temperature and circulating leucocytes in a dog given turpentine intrapleurally on the 4th day. Note that the dog's temperature had returned to normal by the time that the exudate had become pyrogenic. The sudden drop in leucocytes on the 7th day, 24 hours before the appearance of the fever-promoting factor in the chest fluid, is characteristic.

lasted about 3 days. By the time the fever-promoting factor could be demonstrated in the chest fluid, the dog's temperature had returned to normal. The circulating leucocytes increased in number after administration of turpentine and this increase persisted for 3 or 4 days, after which there was a sudden sharp drop about 24 hours before the exudate became pyrogenic. This sudden fall in leucocyte count almost invariably preceded the appearance of the fever-promoting factor in the chest fluid and came to be recognized as a reliable sign of its presence.

When an exudate had been shown to be pyrogenic and cultures were negative, thoracentesis was performed and as much of the fluid as could be withdrawn was collected in sterile, pyrogen-free glass containers and stored at 4°C. Over periods as long as 2 months, no diminution was observed in the fever-promoting property of fluids so stored. No attempt was made to separate various components chemically.

Temperatures of rabbits following injection of exudates or bacterial pyrogens were recorded every 30 minutes for 7 hours and a "fever index" (5) computed to facilitate comparison of responses. The bacterial pyrogens employed were typhoid and *Escherichia coli* vaccines.

## RESULTS

*Comparison of Fever Curves Produced by Injection of Exudate and by Injection of Bacterial Pyrogen.*—The febrile response in rabbits following the injection of chest fluid differed from that following the injection of a bacterial pyrogen in that after a brisk rise to a peak at  $1\frac{1}{2}$  to 2 hours, there was an abrupt fall of the temperature to the initial level within  $3\frac{1}{2}$  hours. Though larger doses of the exudate could raise the peak of the fever, the duration of the elevation remained the same. With bacterial pyrogen, the response is prolonged, the

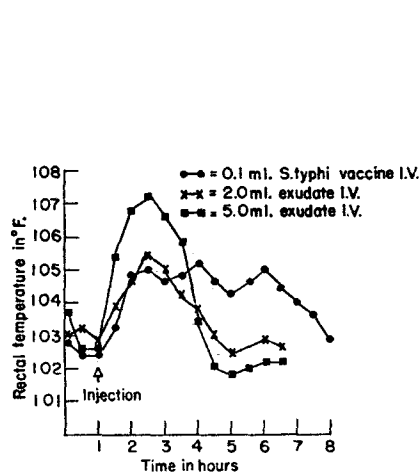


FIG. 2

FIG. 2. Mean fever curves obtained in a group of four rabbits after administration on successive days of 0.1 ml. of *S. typhi* vaccine, 2.0 ml. of exudate, and 5.0 ml. of exudate. Note the rapid rise and fall after injections of exudate as compared to the prolonged response after the bacterial pyrogen.

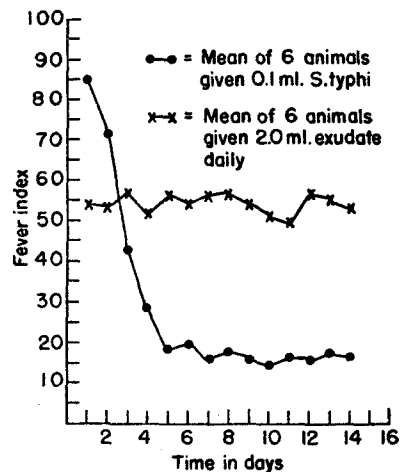


FIG. 3

FIG. 3. Comparison of the response of six animals to daily injections of exudate with the progressive diminution in response of six animals given daily injections of bacterial pyrogen.

temperature sometimes failing to return to the base line within the observation period of 7 hours (Fig. 2).

*Effects of Repeated Injections of Exudates in Normal Animals.*—Three groups of rabbits received daily injections of 2.0 ml. of whole exudate for from 10 to 21 days. In sharp contrast to the diminishing response to daily injections of bacterial pyrogens, there was no evidence that tolerance to the fever-promoting property of the exudates developed in these animals (Fig. 3).

*Effect of Exudates in Animals Tolerant to Bacterial Pyrogens.*—Six animals rendered tolerant by daily injections of 0.1 ml. of typhoid vaccine for 14 days were given 3.0 ml. of exudate intravenously on the 15th day. In normal animals, the fever index following injection of this amount of exudate ap-

proximated that resulting from 0.1 ml. of this vaccine. There was no evidence of tolerance to the exudate, since all animals responded with brisk fevers.

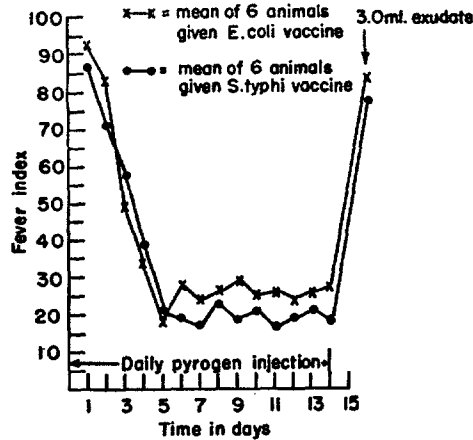


FIG. 4. Failure of two groups of animals rendered tolerant to bacterial pyrogens by daily injections for 14 days to show tolerance for the fever-promoting action of exudate given on the 15th day.

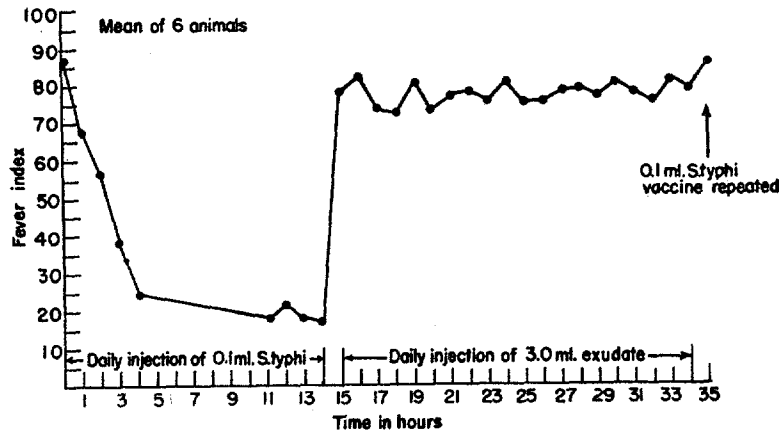


FIG. 5. Chart showing febrile responses of six animals given daily injections of bacterial pyrogen until tolerance appeared, then given daily injections of exudate for 3 weeks. At the end of this time, the high fever, after a repeated injection of bacterial pyrogen, indicated that the daily injections of exudate failed to maintain tolerance in these animals. Tolerance for bacterial pyrogens is normally lost in about 3 weeks.

This experiment was repeated with animals made tolerant to *E. coli* vaccine and again no tolerance to the temperature-raising effect of the exudate was noted (Fig. 4).

Finally, a group of animals tolerant to typhoid vaccine received intravenous injections of 3.0 ml. of exudate daily for 21 days. Tolerance to bacterial pyrogens normally lasts about 3 weeks (4, 5). At the end of this time, a repeated injection of typhoid vaccine was followed by high fever, indicating that tolerance for this bacterial pyrogen had not been maintained by daily injections of the exudate (Fig. 5).

#### DISCUSSION

These findings confirm Menkin's observation that there is present in the chest fluid of dogs given an intrapleural injection of turpentine a substance which causes definite febrile response in rabbits.

The failure of rabbits to develop tolerance to repeated injections of sterile exudates, the failure of animals tolerant to bacterial pyrogens to show tolerance to the fever-promoting effect of these exudates, and the failure of repeated injections of exudate to maintain tolerance for bacterial pyrogen, indicate that the production of fever by these exudates is not due to contamination with bacterial pyrogen. The shorter duration of the febrile response following the injection of exudate as compared with that following administration of bacterial pyrogens furnishes additional evidence that the substance in the exudate which causes fever is not a bacterial product.

The fact that the exudate itself is not pyrogenic until the dog's temperature has returned to normal makes it difficult to attribute the dog's fever to absorption of this substance.<sup>1</sup> Further studies of this apparent inconsistency are under way. The significance of the sudden drop in circulating leucocyte count shortly before the appearance of the fever-promoting factor in the chest fluid is also under investigation.

#### SUMMARY AND CONCLUSIONS

The relationship of the fever caused in rabbits by bacterial pyrogens to the fever produced by the injection of the sterile exudates of acute inflammation was investigated by recording the responses of normal and pyrogen-tolerant animals to injections of exudate. Exudates were produced by the intrapleural injection of turpentine in dogs.

The duration of the febrile response in rabbits after a single dose of exudate was found to be much shorter than the fever following an injection of bacterial pyrogen.

Animals given daily injections of exudate demonstrated no tolerance to its fever-promoting effect.

Animals tolerant to bacterial pyrogens showed no diminution in responsiveness to exudates.

<sup>1</sup> The intravenous injection of chest fluid in dogs also causes a febrile response.

Daily injections of exudate failed to establish tolerance to bacterial pyrogens in rabbits.

It is concluded that the fever-promoting property of sterile exudates is not due to the presence of bacterial pyrogen.

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