STUDIES ON THE PATHOGENESIS OF FEVER

IX. CHARACTERISTICS OF ENDOGENOUS SERUM PYROGEN AND MECHANISMS GOVERNING ITS RELEASE*

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The intravenous administration of bacterial endotoxins to normal rabbits or dogs is followed within 60 to 120 minutes by the appearance in the serum of a fever-producing substance, presumably a product of the host's injured cells, and hence designated endogenous pyrogen (1-3). This serum pyrogen has been shown to differ from the originally administered endotoxin in the following respects:

(a) Intravenous injection of endogenous pyrogen into a normal animal results in a rapid, relatively brief, monophasic rise in body temperature. In contrast, the reaction to endotoxin is characterized by a more prolonged, biphasic febrile response after a latent period of 18 to 60 minutes.

(b) Administration of endogenous pyrogen produces as much fever in animals made "tolerant" to bacterial endotoxins by daily injection as in normal recipients.

(c) Daily injections of endogenous pyrogen do not elicit resistance to its fever-producing action.

These findings lend strong support to the hypothesis that endogenous pyrogen is generically distinct from bacterial endotoxin. However, the possibility that this fever-producing material is merely the endotoxin which has become modified by the plasma or its components is difficult to exclude completely (4-6).

The role of the endogenous fever-producing substance in the febrile response of the host to bacterial endotoxins is less clearly defined. Three observations seem pertinent in this regard:

(a) Rabbits or dogs made tolerant by repeated daily injections of endotoxins, continue to have mild fevers even though no endogenous pyrogen is demonstrable in the serum (7).

(b) Dogs made granulocytopenic with nitrogen mustard respond to the administration of endotoxin with brisk fevers in spite of the virtual absence of pyrogen in their serum (3).

(c) When bacterial endotoxins are placed directly into the subarachnoid space,*

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they give rise to marked elevations in body temperature which are not associated
with the appearance of endogenous pyrogen in the serum. This indicates that endo-
toxins are capable of acting directly upon nervous tissue and that an intermediate
circulating endogenous pyrogen is not essential to the production of fever by endo-
toxin. Furthermore, the intravenous administration of large doses of endotoxin to
dogs is followed within 15 minutes by the appearance in cerebrospinal fluid of a pyro-
gen which is biologically indistinguishable from the bacterial endotoxin given initially
(8).

These data suggest that the fever produced by bacterial toxins could be mediated
by two different mechanisms, the first a direct action of the toxin on the central
nervous system and the second related to cellular injury followed by the release of
an endogenous fever-producing substance. The relative importance of these two
mechanisms remains to be elucidated.

The studies reported in the present paper were designed to further define
the biological characteristics of endogenous pyrogen and to compare its activity
with that of bacterial endotoxins. The ability of endogenous pyrogen to pro-
duce leukopenia and to elicit the Shwartzman reaction were studied, the effect
of adrenal cortical hormones on the release of endogenous pyrogen was deter-
mined, and an attempt was made to evaluate the role of the endogenous serum
factor in the fever of the host given bacterial endotoxins under a variety of
conditions. In general, the results support the previous observations: (a) Endog-
enous serum pyrogen is a by-product of the administration of bacterial toxins,
and is distinctly different from these substances, and (b) it appears to play a
relatively minor role in the production of fever by bacterial endotoxins.

**Materials and Methods**

**Animals.**—Male and female mongrel dogs weighing 8 to 15 kilos were used. Male rabbits
of mixed breed and color weighing 2 to 3 kilograms were obtained from a single commercial
source. All animals were housed in air-conditioned rooms.

**Bacterial Endotoxins.**—A heat-killed vaccine from *Salmonella typhosa*, strain Ty-2 Felix
(obtained from Dr. Maurice Landy, United States Public Health Service, Bethesda), and
containing approximately $1 \times 10^{10}$ cells per ml., was used in experiments with dogs. In most
of the experiments with rabbits, a purified endotoxin obtained from *Shigella flexneri* type Z
(9) (obtained from Dr. L. E. Cluff) was utilized. A culture filtrate prepared from *Serratia marcescens*
was employed in the studies of the Shwartzman reaction.

**Preparation of Serum Pyrogens.**—Donor dogs were injected intravenously with one of the
pyrogens described above and were bled by cardiac or femoral arterial puncture under pento-
barbital anesthesia. Rabbits were exsanguinated by cardiac puncture without anesthesia.
Blood was allowed to clot at room temperature, stored at 4°C. overnight, and serum was
removed after centrifugation at 2000 r.f.m. for 2 hours. Serum was stored at 4°C. and was
tested within 1 week. All specimens were cultured in thioglycollate broth incubated at 37°C.
and at room temperature and were used only if bacteriologically sterile.

**Recipient Animals.**—Normal recipient dogs and rabbits for the testing of sera were injected
no oftener than once every 2 weeks to avoid the development of tolerance. Tolerant recipient
animals were given ten daily injections of endotoxin; febrile responses were recorded on the
1st and 10th days to confirm the presence of tolerance.
Recording of Temperatures.—Dogs were kept in cages unrestrained and food and water were withheld on the day of the experiment. Rectal temperatures were taken with ordinary clinical thermometers at 30 minute intervals, allowing at least 90 minutes for equilibration to take place. Animals with unstable base line recordings were not used. Temperatures were recorded for 4 hours following intravenous administration of test materials.

Rabbits were placed in metal stalls with loose-fitting collars and temperatures recorded at 30 minute intervals by means of a thermistor inserted into the rectum (telemetherometer, Yellow Springs Instrument Co., Yellow Springs, Ohio). A minimum of 2 hours was allowed for acclimatization before each experiment and animals whose temperatures fluctuated more than 0.2°C were discarded. All injections were made into a marginal ear vein and the course of body temperature was observed at 30 minute intervals for a minimum of 4 hours.

Fever curves were plotted on standard graph paper and a “fever index” was determined by planimetry (10).

Peritoneal Exudates.—In one experiment sterile exudates were employed. These were obtained by infusion of physiologic saline (0.85 per cent NaCl) intraperitoneal into rabbits. The procedure has been described in detail elsewhere (11).

Avoidance of Contamination by Bacterial Pyrogens.—All needles, glassware, and instruments were sterilized in hot air ovens at 170°C. for 3 hours. All serum and other injectables were cultured in thioglycollate medium and were discarded unless sterile. Physiologic saline solution (0.85 per cent NaCl) was tested in rabbits and was always free of pyrogenic contaminants.

Miscellaneous.—The adrenal cortical hormone employed in all studies was Δ1-hydrocortisone (generously donated by Dr. Andrew J. Moriarity of the Upjohn Company, Kalamazoo, Michigan). Thorotrast, containing 26 per cent thorium dioxide by volume, was supplied by the Testagar Corp., Detroit.

RESULTS

The Effect of 5 and 120 Minute Serum on the Level of Circulating Leukocytes in Normal Rabbits.—It is well known that administration of bacterial endotoxin elicits a profound granulocytopenia, followed by a leukocytosis with a predominance of immature forms (12). The addition of serum to endotoxin prior to injection enhances its leukopenic action (13). It seemed of interest, therefore, to compare the effects of endogenous pyrogen in serum collected 120 minutes after endotoxin injection on the level of circulating white blood cells with that of serum collected at 5 minutes, which is essentially a mixture of serum and endotoxin.

Groups of normal rabbits given 5.0 μg. of Shigella endotoxin were bled at 5 and 120 minutes. Ten ml. aliquots of pooled serum for each time interval were administered to 2 groups of six normal rabbits. Leukocyte counts were performed before injection and at intervals of 30, 60, 90, 150, and 240 minutes after the administration of serum. The results are detailed in Table I and summarized as the means between paired rabbits in Fig. 1. Administration of 5 minute serum resulted in a definite decrease in leukocytes which persisted for 4 hours. Injection of 120 minute serum was followed by a transient, mild decrease in leukocytes which then rose rapidly to high levels and remained elevated for at least 4 hours. While these results shed no light on the nature or mechanism of action of endogenous pyrogen, they demonstrate another difference in the effects of endogenous pyrogen and bacterial endotoxin.
Elicitation of the Shwartzman Phenomenon by Endogenous (120 Minute) and Exogenous (5 Minute) Pyrogen.—The relative potency of endogenous and exogenous pyrogen in eliciting the local Shwartzman reaction was also studied.

The abdominal wall of rabbits was shaved and 0.5 ml. of a 1:10 dilution of S. marcescens filtrate was injected intradermally as the "preparatory" dose in every animal. Twenty-four hours later, 10 ml. of the serum preparation to be tested were injected into the marginal ear vein. Forty-two recipient animals were employed.

Pooled serum from 5 groups of 6 normal rabbits was tested. The first group consisted of 6 normal rabbits; the second group was bled 5 minutes after receiving 1.0 ml. of S. marcescens filtrate intravenously; the third was bled 5 minutes after injection of 1.0 ml. of typhoid vaccine; the fourth was bled 2 hours after injection of 1.0 ml. of S. marcescens filtrate; the fifth was bled 2 hours after injection of 1.0 ml. of typhoid vaccine.

The results of this experiment are summarized in Table II and indicate that both endotoxin preparations, whether mixed with serum in vitro or in vivo (as 5 minute serum) were consistent in eliciting the Shwartzman reaction in prepared animals. In contrast, there were only two positive reactions among 18 rabbits given 120 minute serum as a provocative. These two reactions remain unexplained; they were not observed in further tests and the animals employed could not be shown to be unduly susceptible to the reaction. In general, however, there was again a consistently demonstrable difference in the activity of 5 minute and 120 minute serum of animals given endotoxin.

### Table I

<table>
<thead>
<tr>
<th>Animal and serum</th>
<th>Min. after injection</th>
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<tr>
<td></td>
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<tr>
<td>A-1 (5 min.)</td>
<td>5,350</td>
</tr>
<tr>
<td>A-2 “ “</td>
<td>5,950</td>
</tr>
<tr>
<td>A-3 “ “</td>
<td>6,300</td>
</tr>
<tr>
<td>A-4 “ “</td>
<td>4,775</td>
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<tr>
<td>Mean (120 min.)</td>
<td>7,100</td>
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</table>
Fig. 1. The effect of endogenous and exogenous serum pyrogen on the leukocyte count of normal rabbits. Points represent means in paired animals.

TABLE II

<table>
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<tr>
<th>Preparatory intradermal injection</th>
<th>Provocative intravenous injection</th>
<th>Total No. animals</th>
<th>No. positive</th>
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<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
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<td>4</td>
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<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
<td>10 ml. normal rabbit serum and 0.2 ml. <em>S. marcescens</em> filtrate</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
<td>10 ml. 5 min. serum from donors given 1.0 ml. <em>S. marcescens</em> filtrate</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
<td>10 ml. 5 min. serum from donors given 1.0 ml. <em>S. marcescens</em> filtrate</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
<td>10 ml. 120 min. serum from donors given 1.0 ml. <em>S. marcescens</em> filtrate</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>0.5 ml. 1:10 <em>S. marcescens</em> filtrate</td>
<td>10 ml. 120 min. serum from donors given 1.0 ml. <em>S. marcescens</em> filtrate</td>
<td>6</td>
<td>0</td>
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The Effect of Repeated Injections of Endotoxin, 5 Minute Serum, 120 Minute Serum, and Sterile Peritoneal Exudate in Rabbits.—Daily injection of bacterial pyrogen results in resistance or tolerance to its fever-promoting effect (10). In contrast, endogenous serum pyrogen or tissue pyrogens derived from sterile exudates fail to elicit tolerance when administered repeatedly (3, 14, 15). Multiple injections of sterile exudate given at intervals of a few hours produce repeated febrile spikes of equal amplitude (14). In view of the similarities between endogenous serum pyrogen and thermogenic substances derived from other tissues (1, 2), reactions of rabbits to multiple injections of endogenous pyrogen from serum and exudate were compared with those produced by bacterial endotoxin and 5 minute serum.

Eight normal rabbits were given 5.0 μg. of *Shigella* endotoxin and bled 5 and 120 minutes later. Pooled serum was used in all tests. Sterile peritoneal exudates were prepared in the manner already described. All recipient animals had been made tolerant by daily injection of 2.0 μg. of *Shigella* endotoxin. Tolerant animals were used in the experiments in order to accentuate changes in temperature evoked by mixtures of endotoxin and serum (6). Eight animals were used in each experiment. The top half of Fig. 2 illustrates the progressive development of refractoriness in animals given 7 successive injections of 5 minute serum during an 18 hour period. As shown in the lower half of Fig. 2 the responsiveness of rabbits to 0.5 μg. of *Shigella* endotoxin decreased in similar fashion. Despite their refractoriness to endotoxin, both groups were able to respond to injection of endogenous serum pyrogen or peritoneal exudate with brisk fevers.

In similar experiments (Fig. 3), eight rabbits received ten injections of 10 ml. of 120 minute serum containing endogenous pyrogen and a second group was given ten successive injections.
of 10 ml. of sterile exudate during an 18 hour period. No refractoriness to the pyrogenic action of either substance was evident.

These findings again confirm the similarity of endogenous serum pyrogen and other tissue pyrogens, and support the idea that both of these substances differ materially from bacterial endotoxin.

The Effect of Adrenal Cortical Hormones on the Release of Endogenous Pyrogen.—The observation that endogenous serum pyrogen fails to appear in the serum of leukopenic dogs is strong evidence for an important role of the leukocyte in the release of this substance (3). Atkins et al. found that abolition of the febrile response to endotoxin by adrenal steroids was unaccompanied by any modification of the leukopenia which accompanies administration of these bacterial products (16). In the following experiment, the influence of adrenal cortical hormone upon the production of endogenous pyrogen was tested.

Three normal dogs were given 50 mg. of prednisone-hemisuccinate intravenously 1 hour before, at the time of, and 1 hour after, the administration of 3.0 ml. of typhoid vaccine. One hour later (120 minutes after administration of typhoid) 150 ml. of blood was collected from each animal by femoral puncture. The animals were permitted to recover and 4 days later the experiment was repeated without the use of the steroid. Twenty ml. aliquots of serum obtained from each donor during the prednisone and control experiments were tested in 3 normal recipients; each recipient received paired sera from the same donor in criss-cross fashion.

As is shown in Fig. 4, the adrenal hormone administered in this fashion was an excellent antipyretic, unlike smaller doses of cortisone which failed to suppress the febrile reaction to
endotoxin in rabbits when administered simultaneously with the toxin (17). Prednisone had no effect on the production of endogenous serum pyrogen by typhoid vaccine. In fact, serum obtained during hormone treatment produced more fever than that collected in the control study.

Fig. 4. The shaded areas at the top show the antipyretic action of intravenous prednisone hemisuccinate in dogs given endotoxin. The mean fever curves of recipient animals given 20 ml. aliquots of 120 minute serum from normal and prednisone-treated donors are shown below and indicate no suppression of endogenous serum pyrogen in the steroid-treated animals.

The Capacity of Endogenous Pyrogen to Serve as a Stimulus for Further Release of Endogenous Pyrogen.—The mechanism whereby the endogenous thermogenic substance that appears in serum in response to bacterial toxins produces fever is obscure. In order to shed some light on this problem an experiment was performed to ascertain whether endogenous pyrogen per se is capable of elicit-
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ing the release of a secondary tissue pyrogen. If this were so, it would imply that endotoxin fever is an "autocatalytic" process mediated by repetitive discharges of endogenous pyrogen.

The procedure used is illustrated in Fig. 5. Six dogs (A) were given 3.0 ml. of typhoid vaccine and were bled 120 minutes later. Fifty ml. of serum from each donor were administered to a second donor (B) who was bled at the height of the fever (usually 60 to 90 minutes after injection). Fifty ml. aliquots of the second donor's serum were injected into each of three normal recipients (C).

The composite temperature curves for each group of C recipients are depicted in Fig. 6. In only one of the six was there a significant rise in temperature (No. 3-45). It is conceivable that

![Diagram illustrating the procedure](image)

the serum from donor 3-45, although bacteriologically sterile, became accidentally contaminated with ordinary bacterial pyrogens. On the basis of these data, it is difficult to conclude that endogenous pyrogen is a "self-perpetuating" substance. However, this possibility merits further study, perhaps in smaller animals, in which dilution is a less important factor.

Ability of Animals with "Suppressed Tolerance" to Elaborate Endogenous Pyrogen.—Atkins and Wood demonstrated that rabbits made refractory to the thermogenic effect of endotoxin by daily injections were unable to elaborate endogenous pyrogen (1). These studies have been confirmed in dogs given relatively small doses of endotoxin (3). Because animals given sufficiently large doses of endotoxin will continue to have fever after many daily injections, the febrile response and production of endogenous pyrogen were studied under these conditions.
Six normal dogs (the "donors") were given 2.5 ml. of typhoid vaccine daily for 13 days, their temperature responses being recorded after each injection. On the 1st day, three animals were bled 2 hours after injection of the vaccine and the remaining three served as controls. The procedure was reversed on the 2nd day, the controls of the previous day being bled. The temperature curves and fever indices for the 2 days were averaged for each dog. Blood was collected on the 5th and 6th and on the 9th and 10th days in similar fashion. On the afternoon of the 12th day, each dog received 50 ml. of thorotrast intravenously, a procedure known to abolish tolerance. Blood was collected from three animals 2 hours after injection of vaccine on the 13th day, the other animals serving as controls. All dogs tolerated the frequent bleedings and daily injections of endotoxin well, and all survived the experiment.

Twenty ml. aliquots of unpoled serum from each bleeding were injected intravenously into 3 normal recipient dogs to determine the amounts of endogenous pyrogen present.
The results are summarized in Fig. 7. Of the six donors, three became partially tolerant, while the remainder showed no diminution in febrile responses throughout the period of testing. Figs. 8 and 9 illustrate examples of each type of donor response. No matter what the amount of fever shown by a donor, there was a marked diminution, and in most cases complete disappearance of endogenous pyrogen by the 5th day. Endogenous pyrogen was not present in detectable amounts in serum collected after this time despite the fact that all donors continued to respond to endotoxin injection with high fevers. There

<table>
<thead>
<tr>
<th>DAYS</th>
<th>1-2</th>
<th>3</th>
<th>5-6</th>
<th>7</th>
<th>9-10</th>
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<tr>
<td>THORO.</td>
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<td><img src="image11.png" alt="Graph" /></td>
<td><img src="image12.png" alt="Graph" /></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Six donors (top part of figure) were given daily injections of 2.5 ml. of typhoid vaccine, a dose large enough to suppress development of tolerance. Note that three animals developed no tolerance; the remaining three became slightly tolerant. Two-hour serum from all donors contained little or no endogenous pyrogen when tested in the same set of three normal recipients (bottom part of figure) after days 1 and 2. Administration of thorotrust caused slight return in endogenous pyrogen. Arrows depict mean fever indices.

* denoted by 6 different symbols.
Fig. 8. Shaded areas indicate temperature record of a donor dog who became slightly tolerant to the pyrogenic effect of 2.5 ml typhoid vaccine daily. Note the disappearance of endogenous pyrogen from the serum, indicated by the solid curves in the lower portion of the figure.

was no spontaneous recovery of the capacity to elaborate endogenous pyrogen during the period of daily injections. Reticuloendothelial "blockade" with throrotrasr was followed by the reappearance of small amounts of endogenous pyrogen on the 13th day, but the effect of administering this agent on the
magnitude of fever in the donor and the ability to elaborate endogenous serum pyrogen was not striking.

These results show clearly that dogs can respond to administration of bacterial endotoxin with high fevers that bear no relationship to the amount of endogenous pyrogen detectable in the circulating blood. The dissociation of
febrile response and serum content of pyrogen demonstrated in this experiment is similar to that which occurs in leukopenic dogs (3).

Rate of Depletion of Endogenous Pyrogen.—In the foregoing experiment it was demonstrated that dogs given daily injections of 2.5 ml. of typhoid vaccine produced no detectable serum pyrogen after the 5th or 6th day. There was also some indication that serum collected on the 2nd day was less thermogenic than that obtained on the 1st day (Fig. 7). In order to study this further, the following experiment was done.

Three dogs were given 5.0 ml. of typhoid vaccine on 2 successive days and blood was collected 2 hours after injection on each day. Three other animals received the same amount of vaccine on each day but were not bled, their temperature responses being recorded for 4 hours after injection, instead of only 2 hours as in the donor animals. Serum obtained from each donor on the 1st and 2nd days was tested in 20 ml. amounts in groups of three normal recipients. Fever indices of both the donors and controls given typhoid vaccine were computed for the first 2 hours after injection and for the second 2 hours.

There was a consistent sharp drop in the amount of endogenous pyrogen in the serum of donors on the 2nd day. The temperature responses of the donors, which were measured for only 2 hours, remained the same (in one animal, it increased on the 2nd day). The total amount of fever in the control animals was slightly less on the 2nd day but the reduction was entirely a result of decrease in the temperature response during the 3rd and 4th hours after injection, fever indices for the first 2 hours remaining the same. These results are diagrammed in Fig. 10, which shows clearly that the reduction in the amount of endogenous pyrogen detectable in serum collected two hours after injection of endotoxin on the 2nd day bears no relationship to the febrile response during the first 2 hours but is strikingly correlated with a reduction in fever during the 3rd and 4th hours. These findings suggest that the early pyrogenic action of endotoxin is independent of fluctuations in the serum content of endogenous pyrogen and support the suggestion that a direct action of the toxin is responsible for the early rise in body temperature.

The Relationship between Depletion of Endogenous Pyrogen and Circulating Leukocytes.—In view of the evidence summarized earlier, which links endogenous serum pyrogen to granulocytes and the suggestion by Atkins et al. that the leukopenic response to endotoxin is an indication of leukocyte injury and release of endogenous pyrogen from these cells (3), the response of the circulating granulocytes was measured and serum was assayed for endogenous pyrogen in dogs given endotoxin on successive days.

Three normal dogs were given 3.0 ml. of typhoid vaccine and peripheral leukocyte counts were performed immediately before injection and 5, 30, 60, and 120 minutes thereafter. The animals were bled 2 hours after administration of the toxin. On the following day, the procedure was repeated. Twenty ml. aliquots of serum from each donor were tested in groups of three normal dogs. The results are summarized in Fig. 11. As in the foregoing experiment,
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Fig. 10. Three donor dogs were given 5.0 ml. of typhoid vaccine on 2 successive days and were bled on each day after 2 hours. Three control animals were also given vaccine but not bled. Note that there was a considerable diminution of endogenous pyrogen on the 2nd day when 20 ml. aliquots from each donor were tested in the same group of three recipients (shaded curves). There was no decrease in the first 2 hours of the donors' fever (open bars) on the 2nd day; but all animals manifested considerable diminution in the second half of the febrile response on the 2nd day, suggesting that endogenous pyrogen is active in the later part of the temperature response to endotoxins.

There was a marked decrease in the amount of endogenous pyrogen in serum collected on the 2nd day with relatively little change in the donor's febrile response. This diminution in the pyrogen content of the serum, however, was not accompanied by a detectable change in the magnitude of the leukopenic response to endotoxin. Every animal manifested a profound drop in circulating white blood cells on both days, the leukopenia appearing within 5 minutes and persisting for at least 2 hours (Table III).
Fro. 11. Three donors (cross-hatch) were given 3.0 ml. of typhoid vaccine on successive days and bled 120 minutes later on each day. There was a marked decrease in endogenous pyrogen on day 2 (black bars). This bore no relationship to the leukopenia which occurred in response to the vaccine (bottom half of chart).

**TABLE III**

Leukocyte Counts of Dogs Given 3.0 ml. Typhoid Vaccine on 2 Successive Days

<table>
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<tr>
<th>Dog</th>
<th>Day</th>
<th>WBC base line</th>
<th>Min. after typhoid vaccine</th>
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<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
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<td></td>
<td>1-95</td>
<td>15,800</td>
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<td>3,900</td>
<td>24,800</td>
<td>950</td>
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<td>2,800</td>
<td>7,800</td>
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These findings cannot be interpreted as indicating that the granulocyte is not a source of endogenous serum pyrogen. They suggest strongly, however, that the leukopenic reaction to injected endotoxin is independent of the formation of endogenous pyrogen, although in certain circumstances, they are sequential phenomena.
The Effect of Reticulo-Endothelial "Blockade" on the Release of Endogenous Pyrogen.—Atkins and Wood found that the serum of animals tolerant to the fever-producing effect of endotoxin contains no detectable endogenous pyrogen (1). The explanation suggested for this was that the injected endotoxin is removed from the blood so rapidly in tolerant animals (18, 19) that tissue injury is minimal and endogenous pyrogen is not released. This idea is supported by the finding that abolition of tolerance by injection of thorotrast, a procedure which lengthens the clearance time of the endotoxin, is followed by restoration of the ability to produce endogenous serum pyrogen (2). The data already presented in the present report indicate that failure of endogenous pyrogen to appear in the serum of animals given daily injections of endotoxin precedes by several days the full development of tolerance to the fever-producing action of endotoxin. The following experiment was performed in an attempt to determine whether the decrease in serum content of endogenous pyrogen in animals given daily injections of endotoxin is a reflection of decreasing tissue injury by the toxin, that is, a lessened stimulus to the release of endogenous pyrogen. Another explanation could be that animals become unable to elaborate endogenous pyrogen because of some refractoriness of the mechanisms responsible for its release or because of temporary exhaustion of its sources.

Twelve rabbits were given four daily injections of 5.0 µg of Shigella endotoxin. On the afternoon of the 4th day, six animals were given 5.0 ml of thorotrast, and on the 5th day they received 5.0 µg of toxin. The other six rabbits were given 50 µg of toxin on the 5th day. On day 6, all animals again received daily injections of 5.0 µg of endotoxin and these were continued through the 15th. On the afternoon of that day, six animals received 5.0 ml of thorotrast, and on the following day were challenged with 5.0 µg of Shigella endotoxin. The remaining six animals were given 50 µg of toxin on the 16th day. The febrile response of half of the animals was recorded on days 1, 4, 5, 15, and 16. From the other 6 animals, blood was collected 120 minutes after injection of endotoxin on day 1, day 4, day 5 (two groups, one treated with thorotrast, and the other with 50 µg of toxin), day 15, and day 16 (2 groups). The serum collected on each day was pooled and 10 ml aliquots were tested in four to six normal recipient rabbits.

The results of the experiment are recorded in Fig. 12. On day 1, animals had high, biphasic fevers and there was a considerable amount of transferable pyrogen in the serum. On day 4, the donors were somewhat tolerant, the second febrile peak had disappeared, and little or no endogenous serum pyrogen was detectable. On day 5, in animals given thorotrast, the endotoxin produced high fever, there was a reappearance of the second peak, and endogenous serum pyrogen was again easily demonstrable. The administration of 50 µg of Shigella endotoxin on day 5 led to a similar increase in fever and appearance of endogenous serum pyrogen. By day 15, all animals were again tolerant and serum produced little or no fever in test animals. Thorotrast again restored the animals' ability to respond with high fever and endogenous serum pyrogen was demonstrable. While the administration of 50 µg toxin on day 16 was less effective than thorotrast in restoring fever to its previous height and the amount of endogenous serum pyrogen was less, there was still an increase in both over the findings on day 15.
These studies support the idea that failure of endogenous serum pyrogen to appear is the result of less stimulus to its production. Because of the increasing rate at which endotoxin is cleared from the circulation as tolerance develops, each successive daily injection is, in a sense, a smaller "target" dose.

**DISCUSSION**

The failure of serum collected 2 hours after intravenous injection of bacterial endotoxin to elicit significant leukopenia or to provoke the local Shwartzman reaction adds two points to the list of differences between endogenous serum pyrogen and bacterial endotoxin or endotoxin-serum mixtures. It could be argued that larger amounts of endogenous pyrogen might produce leukopenia or elicit the Shwartzman reaction and that the observations described are a result of dosage. However, the pyrogenic activities of the quantities of endotoxin, "5 minute" serum, and "120 minute" serum used in these experiments were essentially the same and, at present, the only basis for quantitative estimation of these substances is their ability to produce fever in test animals.
When the body temperature response was recorded in rabbits given repeated injections of endogenous pyrogen or endotoxin at intervals of 2 to 3 hours, a third and even more striking difference became apparent. Animals became completely refractory to the fever-producing action of endotoxin after four or five injections but retained their ability to respond to injection of endogenous serum pyrogen or sterile exudate with fever. In contrast, animals given multiple doses of endogenous pyrogen continued to react with febrile spikes of comparable magnitude as long as injections were continued.

These findings, taken with previously described differences in the biologic effects of serum pyrogen and endotoxin, mentioned at the beginning of this paper, constitute an impressive list in support of the hypothesis of Atkins and Wood (1, 2) that the fever-producing substance in the serum of animals given endotoxin is a new substance. It cannot yet be said that the evidence completely rules out the possibility that endogenous serum pyrogen is a greatly modified moiety of the exogenous endotoxin originally injected intravenously into the animals. Even the observations of Braude et al. (19) showing that the blood of normal animals given endotoxin tagged with radiochromium is devoid of radioactivity long before peak amounts of endogenous pyrogen appear, do not meet the argument that “modification” of endotoxin in the body that confers upon it the properties of endogenous pyrogen may involve a splitting of the bacterial lipopolysaccharide that divests the active portion in serum of its isotopic marker. The present evidence bearing upon this question can be summarized fairly by stating that with the single exception of a similarity in susceptibility to destruction by heating (5), endogenous pyrogen and endotoxin have been found to elicit clearly different reactions by every method that has been employed in comparative testing.

Source of Endogenous Pyrogen.—The suggestion by Atkins and Wood (1, 2) that polymorphonuclear leukocytes and possibly other tissues injured by endotoxin release endogenous pyrogen into the circulation is supported by comparative studies of the properties of serum pyrogen and fever-producing substances in extracts of granulocytes or in sterile peritoneal exudates.

In their ability to produce brief monophasic fevers after a relatively short latent period and to produce as much fever in animals that are tolerant to endotoxin as in normal animals, in their failure to produce tolerance when injected daily, and in their susceptibility to heating (13–6), endogenous serum pyrogen and leukocyte extracts are the same. Furthermore, the demonstration in the present study that both will provoke repeated febrile reactions when given at short intervals and that the rapid development of “refractoriness” to bacterial endotoxin leaves an animal with no impairment of ability to react to either substance with fever is an additional point of similarity between the two. These findings, taken with the previously reported observation that
endogenous pyrogen appears in negligible amounts in the serum of leukopenic animals given endotoxin (3), all point to the granulocyte as an important source of endogenous serum pyrogen.

Intravenous injection of endotoxin is regularly followed by peripheral granulocytopenia and the decrease in circulating leukocytes always precedes the febrile response to the endotoxin. It is reasonable to surmise that the fall in leukocytes may reflect injury to these cells and that this injury may culminate in release of endogenous pyrogen into the serum (16). When animals are made tolerant to endotoxin fever by daily injections, there is a progressive decrease in the amounts of endogenous pyrogen detectable in their serum and also, the leukopenic response is less marked although a fall in white cells is usually still detectable. This parallel decrease of endogenous serum pyrogen, leukopenia, and febrile response as tolerance develops is not necessarily indicative of a causal relationship. The increased clearance of endotoxin by the fixed phagocytes of the reticulo-endothelial system in tolerant animals appears to neutralize its toxic effects in some manner with the result that there is a decrease not only of those already mentioned, but others, including the provocation of the Shwartzman reaction, the production of hemorrhagic necrosis in tumors, the depletion of liver glycogen and hemodynamic alterations, specifically a fall in arterial blood pressure. It is entirely possible that decrease in leukopenic action of the toxin in tolerant animals is coincidental with the decrease in febrile response, without being the cause of the decreased fever. Braude and his colleagues (19) have found that a portion of intravenously injected endotoxin is taken up by circulating leukocytes; this interesting observation, however, does not constitute proof that endotoxin injures leukocytes directly, that the ensuing leukopenia is dependent upon ingestion of toxin by leukocytes, or that leukopenia and leukocyte injury are related phenomena. In this connection, Berthrong and Cluff (20) and Stetson (21) both observed failure of leukocytes exposed to endotoxin in vitro to show the alterations (decreased migration, clumping) that characterize the in vitro behavior of leukocytes removed from an animal immediately after injection of endotoxin. These findings seem to indicate that endotoxin's effect upon leukocytes may be mediated in vivo by some predominantly indirect mechanism. Kerby and Barrett (22) found that exposure of normal polymorphonuclear leukocytes to endotoxin in vitro caused the release of a lysozyme-like substance from the cells. These experiments leave no doubt about the ability of endotoxin to alter leukocytes by direct action. However, the fact that leukocytes from subjects that had been given adrenal steroids were not found to release lysozyme when exposed to endotoxin in vitro makes it very unlikely that release of this enzyme parallels the events under discussion, since Atkins et al. (16) have made a special point of the fact that the leukopenic action of endotoxin is not modified by administration of adrenal steroids and the studies reported here indicate
that steroids do not prevent the appearance of serum pyrogen. For the present, then, it appears that there is no substantial evidence to support any relationship between the leukopenic action of endotoxin and the possible release of endogenous pyrogen from granulocytes.

Role of Endogenous Pyrogen in Endotoxin Fever.—The present studies indicate clearly that fever can occur in response to intravenous injection of bacterial endotoxin in the absence of detectable amounts of endogenous serum pyrogen. An “absence of endogenous serum pyrogen in detectable amounts” does not necessarily mean that an animal’s serum is devoid of the material. However, if endogenous serum pyrogen is the only cause of fever after injection of endotoxin or, indeed, if it is a major cause of fever, it is reasonable to expect that it would be detectable in essentially comparable concentrations in the serum on every occasion that the same animal responds to the same dose of endotoxin with the same amount of fever. A previous study reported dissociation of the febrile response to endotoxin and the level of circulating endogenous pyrogen in granulocytopenic dogs (3). In the experiments reported here, the use of daily injections of endotoxin in amounts large enough to suppress the development of detectable tolerance to fever produced a similar, clearcut dissociation of fever and circulating pyrogen.

The present study adds nothing to ideas of the mechanism of the fever produced by endotoxin in the absence of circulating endogenous pyrogen. There is considerable evidence that endotoxin can act directly on the central nervous system. Intrathecal injection of tiny amounts produces high fever in rabbits and in dogs; this fever is unaccompanied by leukopenia, formation of endogenous pyrogen, or tolerance (8). It is reasonable to suggest that fever in animals in the absence of serum pyrogen is produced by the endotoxin itself.

Endogenous pyrogen probably exerts its greatest effect upon body temperature during the later phases of the febrile response to endotoxin. This is suggested by the observation that the level of endogenous serum pyrogen correlates well with the amount of fever during the 3rd and 4th hours but seems unrelated to the temperature response during the 2 hours immediately following injection of bacterial toxin. More convincing is the demonstration that the second peak of the fever curve in rabbits is closely related to the presence of detectable endogenous serum pyrogen. The serum of rabbits that responded to endotoxin with biphasic fevers invariably contained pyrogen and the disappearance of the second peak in the course of daily injections was consistently accompanied by disappearance of endogenous pyrogen.

While many aspects of the febrile response to bacterial endotoxin remain to be clarified, it seems to the authors that a concept of endotoxin fever as involving a direct action on the nervous system supplemented by an indirect effect mediated by tissue endogenous pyrogens is most acceptable at present.

Such phenomena as the “latent” period between injection of endotoxin and
onset of fever, the increase in tolerance that continues after endogenous pyrogen disappears, and the mechanism of the rapidly developing refractoriness to endotoxin that comes on within a few hours are currently under investigation.

SUMMARY

The "endogenous serum pyrogen" that appears in the circulating blood after a single intravenous injection of endotoxin does not produce leukopenia in normal animals, fails to provoke the local Shwartzman reaction, and elicits no "tolerance" when injected daily. Suppression of the febrile response to endotoxin by prednisone does not prevent the appearance of pyrogen in the blood.

Animals given large amounts of endotoxin daily continue to respond with high fevers despite failure of endogenous serum pyrogen to appear in detectable amounts after the first two or three injections. Analysis of the response to daily injections shows clearly that the fever during the first 2 hours after administration of endotoxin is unrelated to levels of endogenous serum pyrogen; in contrast, the magnitude of the fever after the 2nd hour correlates well with endogenous pyrogen in some instances. The leukopenic response to endotoxin could not be correlated with the appearance of endogenous serum pyrogen.

The differences between endotoxin and endogenous pyrogen and the similarities between leukocyte extracts (sterile exudates) and endogenous pyrogen are summarized and discussed. Dissociation of the febrile response to bacterial endotoxin and levels of endogenous serum pyrogen are discussed and it is concluded that a mechanism involving both direct and indirect action of endotoxins offers the best explanation for the pyrogenic action of these bacterial products.

BIBLIOGRAPHY