FURTHER STUDIES ON PASSIVE TRANSFER OF TOLERANCE TO PYROGENICITY OF BACTERIAL ENDOTOXIN

The Febrile and Leucopenic Responses

By HENRY H. FREEDMAN, Ph.D.

(From the Princeton Laboratories, Inc., Princeton)

(Received for publication, May 19, 1960)

In a previous report (1) we have described the passive transfer of tolerance to pyrogenicity of bacterial endotoxin. Such transfer was shown to depend upon the manner in which tolerance was induced in the donor animal, and this parameter has been further investigated. Both prolonged administration of the bacterial lipopolysaccharide, through 5 weeks, and daily injections of a large constant dose of endotoxin for a few days, permit demonstration of passive transfer, depending upon the magnitude of the test dose of the pyrogen in the recipient.

Intravenously injected doses of endotoxin which cause a fever also elicit an almost immediate peripheral granulocytopenia. It has been thought that development of tolerance to the fever-producing effect of the toxin, which is accompanied by refractoriness to various other responses, extends to the acute leucopenia (2–5). If tolerance to leucopenia regularly paralleled tolerance to pyrogenicity the hypothesis suggesting endogenous leucocytic pyrogen as the mediator of endotoxin-induced fever (4) would be strengthened. Not finding refractoriness to the drop in peripheral white cell count in our rabbits passively tolerant to the pyrogenicity of endotoxin, we have studied the changes in numbers of circulating leucocytes in endotoxin-treated animals. We have not found tolerance to the leucopenia under conditions yielding substantial tolerance to pyrogenicity. A search of the literature largely supports this finding that tolerance to the pyrogenic effect becomes manifest without change in the acute leucopenic response (6–12).

Following the demonstration by Grant and Whalen (13) of an endogenous pyrogen in the blood of rabbits injected with typhoid vaccine, an impressive series of experiments by Wood and coworkers (14–17) has led to the hypothesis that endotoxin-induced fever is a consequence of the action of the exogenous pyrogen upon the leucocytes, or other cells, causing release of endogenous pyrogen which then acts upon the thermoregulatory centers of the brain (4). From an equally impressive series of researches, Bennett and associates (9, 18–25) have shown that the fever seen after administration of endotoxin requires neither significant numbers of circulating leucocytes nor the presence of endogenous serum pyrogen, and have postulated a dual mechanism, involving a direct action of endotoxin, possibly accounting for the biphasic nature of the febrile response (3).

The present report describes the rapidly developing fever, of greater than normal intensity, seen after intravenous endotoxin in leucopenic rabbits. The results strongly support the second hypothesis: that the first phase, at least, of the characteristic biphasic febrile response is attributable to a direct action of the injected endotoxin upon the brain. It is further suggested that the lesser initial phase of fever, in the animal having normal numbers of circulating leucocytes, reflects a limitation of immediately available endotoxin by the

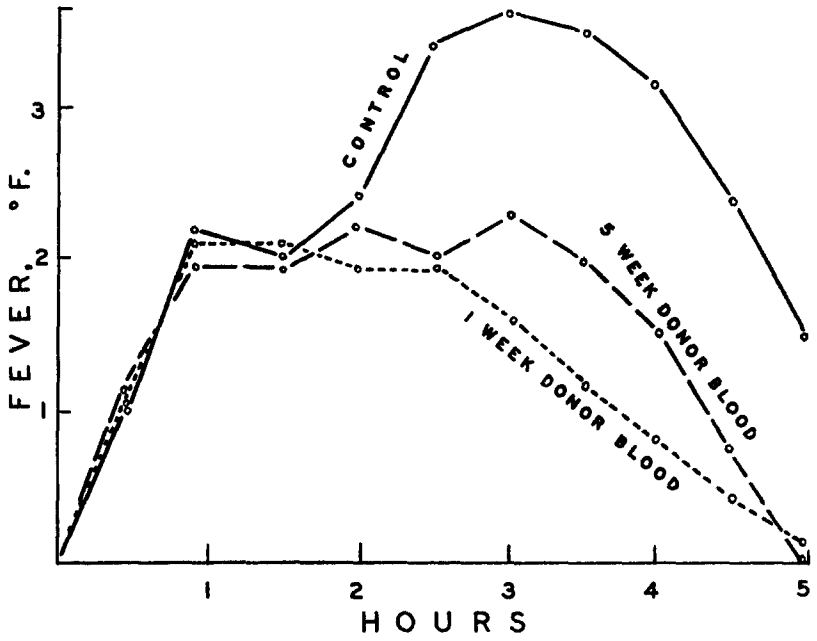

FIG. 1. Comparison of effectiveness of blood obtained by long and short term treatment for developing tolerant donors upon modification of febrile response of recipient rabbits given 2.5 μg. endotoxin.

rapid sequestration in the white blood cells, at the time of the acute leucopenia, described by Braude *et al.* (26). Evidence for development of tolerance to the pyrogenic effect of endotoxin, in the absence of significant numbers of white blood cells, is also presented.

Materials and Methods

The endotoxin used in these experiments was the lipopolysaccharide of *Salmonella typhosa* 0 901 (Difco). Rabbits were of mixed breed and both sexes, weighing 2 to 2.5 kg. Precautions against pyrogen contamination, collection of donor blood for serum or plasma, and measurement of the febrile response to endotoxin were as previously described (1). Total leucocyte counts were made on blood taken from the marginal ear vein.

Tolerant Donor Rabbits.—The various schedules employed for inducing tolerance with

endotoxin are described below in the appropriate experiments. In all instances endotoxin was injected into a marginal ear vein and donor blood was taken 20 to 24 hours after the last injection.

Normal Donor Rabbits.—These animals, from the same stock, provided serum or plasma as controls for the tolerant donors.

Recipient Test Rabbits.—Except as noted below, the test dose of endotoxin for quantitating the febrile response was 2.5 μg., intravenously. Preparation of test animals for, and management during, the testing procedure were as previously described (1). Plasma or serum was

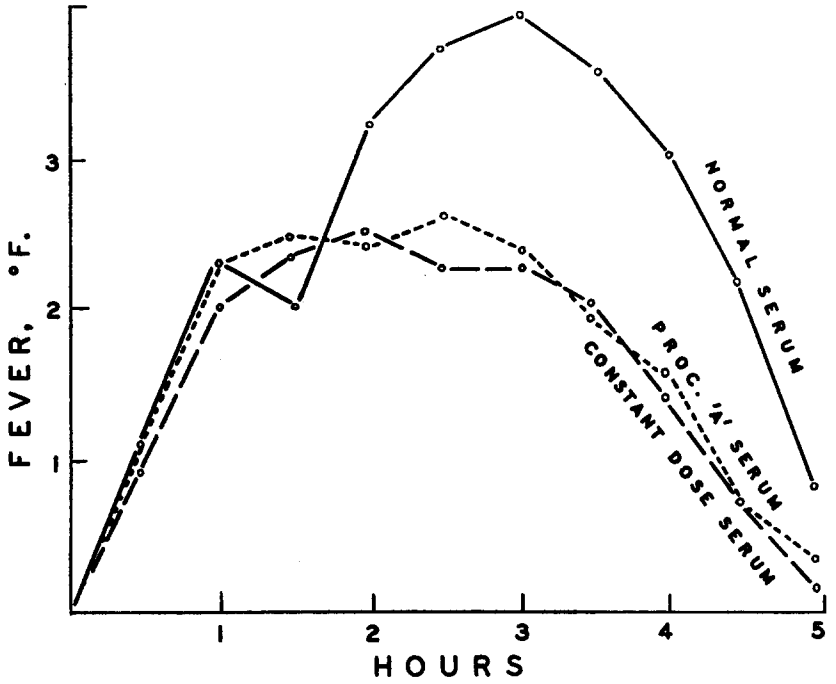

FIG. 2. Modification of febrile response of recipient rabbits to 2.5 μg. endotoxin by prior administration of serum from tolerant donors prepared by constant or stepped dose schedules.

given intravenously in 10 ml. volume, either 30 minutes before, or, in one series of experiments, mixed extemporaneously with, the endotoxin.

Leucopenic Test Rabbits.—The nitrogen mustard (HN2) used was methyl-bis[beta-chloroethyl]-amine HCl (Merck Sharp and Dohme) dissolved in pyrogen-free saline at 1 mg./ml. immediately before intravenous injection. Schedules for HN2 administration are given below; change in numbers of circulating leucocytes after HN2 treatment was followed by daily counts.

EXPERIMENTAL

Variation of Donor Schedule for Inducing Tolerance.—The effects of serum or plasma from donor rabbits made tolerant by various schedules of endotoxin treatment upon the febrile response to endotoxin of normal recipient rabbits

are shown in Figs. 1 to 3. For investigation of the effect of long term treatment of donors upon suitability for demonstration of passive transfer of tolerance to pyrogenicity, the following experiment was performed:

Two rabbits were each given a total of 25 daily intravenous injections of endotoxin over a 5 week period, starting with 1 μg. and increasing the dose in increments to 10 μg. One was bled for serum, the other for plasma, 24 hours after the last injection. Four normal recipients were given serum and 3 plasma 30 minutes before the standard 2.5 μg. test dose of endotoxin.

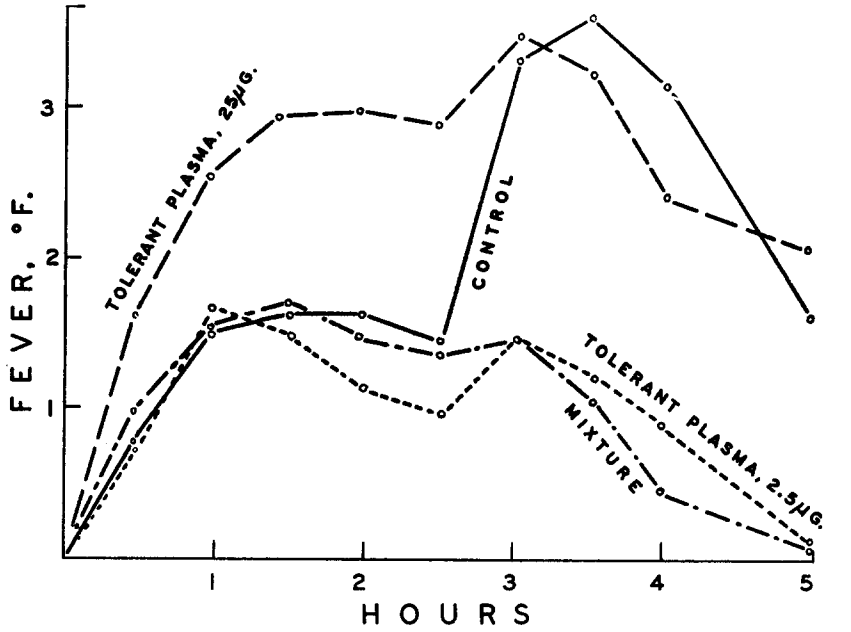

FIG. 3. Effect of magnitude of test dose of endotoxin upon alteration of the febrile response of recipient rabbits pretreated with plasma of tolerant donors, and response to mixture of tolerant plasma and endotoxin.

Three normal recipients were given plasma and 2 serum from tolerant donors prepared by the previously described "procedure A" (1): days 1 and 2, 2.5 μg.; days 3 and 4, 5.0 μg.; days 5 and 6, 10.0 μg. Six control rabbits received only the endotoxin. The results are shown in Fig. 1.

Long term treatment of the donors allowed demonstration of passive transfer of tolerance to the pyrogenic effect of the subsequent dose of endotoxin. Such treatment was clearly no more effective than the brief schedule, adding further evidence that the phenomenon is not attributable to specific antibody to the lipopolysaccharide (1, 28). Transfer from donors given a small constant daily dose of pyrogen was next examined:

Two rabbits were given 5 μg. of endotoxin daily for 7 days, and serum taken on day 8. Four recipients were injected with serum of these donors 30 minutes before the test dose of

pyrogen, 4 were injected with serum from donors prepared by "procedure A," and 3 controls were given normal rabbit serum before the endotoxin. The fevers observed in the test rabbits are described in Fig. 2.

The constant small dose regimen was at least as effective as the stepped dose schedule, "A." It was decided to try a large constant dose, and to investigate the effect of the magnitude of challenging dose of endotoxin in the recipient:

Six rabbits were injected with 25 μg. of endotoxin daily for 7 days, and blood for citrated plasma was taken on day 8. Plasma from individual donors was given to paired recipients, 3 receiving the standard 2.5 μg. test dose of endotoxin 30 minutes later and 3 receiving a 25 μg. challenging dose. Three normal controls were given 2.5 μg. of endotoxin only. Included in the experiment were 2 recipients injected with 10 ml. of plasma from donors made tolerant by "procedure A" mixed extemporaneously with 2.5 μg. of the pyrogen. The results are given in Fig. 3.

TABLE I

Acute Leucopenic Response to 2.5 μg. Endotoxin in Control Rabbits Suffering Normal Febrile Response and in Rabbits Passively Tolerant to Pyrogenic Effect

Group	No. of Rabbits	Mean white cell count/mm.³ and range	
		Pre-injection	1 hr. post-injection
Controls*	18	10,775 (9,500–13,550)	6,055 (3,450–8,450)
Normal donor Serum or plasma*	12	10,680 (9,400–12,000)	7,240 (5,200–9,500)
Tolerant donor Serum or plasma‡	43	10,850 (8,300–14,200)	7,160 (3,850–10,000)

* Normal febrile pattern.

‡ Tolerant febrile pattern.

It is seen that qualitative differentiation of the febrile response from that observed in control animals is possible with the smaller test dose. The larger dose, equal to that given the donors, resulted in a prolonged, biphasic, fever. The simultaneous administration of tolerant donor plasma and endotoxin yielded a typical tolerant pattern.

Acute Leucopenia in Recipient Rabbits.—Total peripheral while cell counts were made before and 1 hour after 2.5 μg. of endotoxin in normal control rabbits and in rabbits pretreated with normal rabbit serum or plasma, both groups exhibiting a normal biphasic fever (1), and in recipient rabbits exhibiting a tolerant febrile course resulting from prior adinistration of tolerant donor serum or plasma. These data were obtained in the animals used in the studies on passive transfer reported here and previously (1). In Table I are given the data demonstrating equivalent leucopenia in all groups, despite the difference

in magnitude and time course of fever following passive transfer from tolerant donors. At no time was any correlation found between intensity of fever suffered and degree of leucopenia. The assumption that the leucopenia reflects damage to leucocytes with release of endogenous pyrogen, the latter mediating endotoxin-induced fever (*vide infra*), is not consistent with these findings.

Failure to Develop Tolerance to Leucopenic Response in Pyrogen-Tolerant Rabbits.—Next investigated were the pre- and post-injection white cell counts in rabbits given daily injections of endotoxin to induce tolerance to the pyrogenic effect:

Six rabbits were given endotoxin intravenously as follows: days 1 to 3, 2.5 μg.; days 4 and 5, 5.0 μg.; days 6 and 7, 10.0 μg. The usual tolerance to pyrogenicity was observed, with disappearance of the second phase of fever on the 2nd day. Total white cell counts were made daily before and 1 hour after injection of the toxin, and in Table II are given the results for days 1 to 3 and 7.

TABLE II

Acute Leucopenic Responses in Rabbits Given Daily Injections of Endotoxin. Responses on Days 1, 2, 3 and 7 of Treatment

Day*	Mean white cell count/mm.³ and range	
	Preinjection	1 hr. postinjection
1	11,110 (9,550–12,550)	7,170 (5,100–11,800)
2	11,570 (9,500–13,500)	6,510 (4,500–9,300)
3	11,750 (10,650–13,450)	7,810 (5,750–12,100)
7	11,410 (9,900–12,550)	7,460 (5,100–9,000)

* Days 1 to 3, 2.5 μg.; days 4 to 5, 5.0 μg.; days 6 to 7, 10.0 μg.

No change in acute leucopenic response was found despite the usual modification of fever pattern. This result has been consistently obtained in similarly prepared donor rabbits. The 2 donor animals treated with endotoxin for 5 weeks (*vide supra*) responded irregularly during the last week, but at least a slight fall in count was usually seen. Doubtless, with sufficiently long treatment the leucopenic response may disappear, but this does not constitute parallel behavior to the development of tolerance to pyrogenicity.

Time-Course of Febrile and Leucopenic Responses.—In Fig. 4 are shown the changes in body temperature and leucocyte count with time following a 2.5 μg.

dose of endotoxin in 6 normal rabbits. No indication of a biphasic leucopenia was seen. When a 10 μg. dose was used, the mean white cell counts before and at hourly intervals after endotoxin were: 9,230; 5,070; 4,000; 7,150; and 9,550/mm.^{3}; the time course of fever was essentially the same as with the lesser dose.

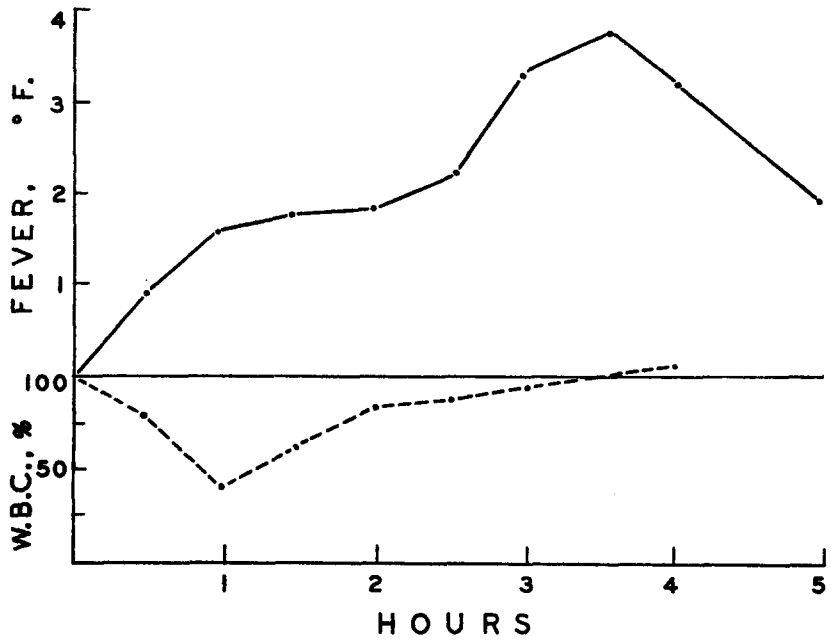

FIG. 4. Time course of febrile and leucopenic responses of normal rabbits given 2.5 μg. endotoxin.

Febrile Response of Leucopenic Rabbits.—Two schedules of nitrogen mustard administration were used to prepare leucopenic test animals:

Three normal rabbits were given HN2 intravenously as follows: day 1, 2 mg.; day 4, 1 mg.; day 6, 2 mg. On the 7th day counts were 825,525, and 600/mm.^{3} Each was then given 2.5 μg. of endotoxin, as were 3 normal control rabbits, and the fevers were recorded. The HN2 treated animals were given another 2.5 μg. of endotoxin on day 8, when counts were 925,625, and 1950/mm.^{3}, respectively. The results are given in Fig. 5.

The leucopenic rabbits suffered a rapidly developing, exaggerated fever following the first dose of pyrogen. The white cell counts 1 hour after the endotoxin were 1550, 650, and 475/mm.^{3}, respectively. The first animal, with a mean pre- and postinjection count of 1200/mm.^{3}, had a lesser fever than the other 2 with mean counts of 590 and 540/mm.^{3}. The pattern of fever to the second dose of endotoxin, 24 hours later, was typical of the tolerant response to a second dose seen in rabbits with normal numbers of circulating leucocytes.

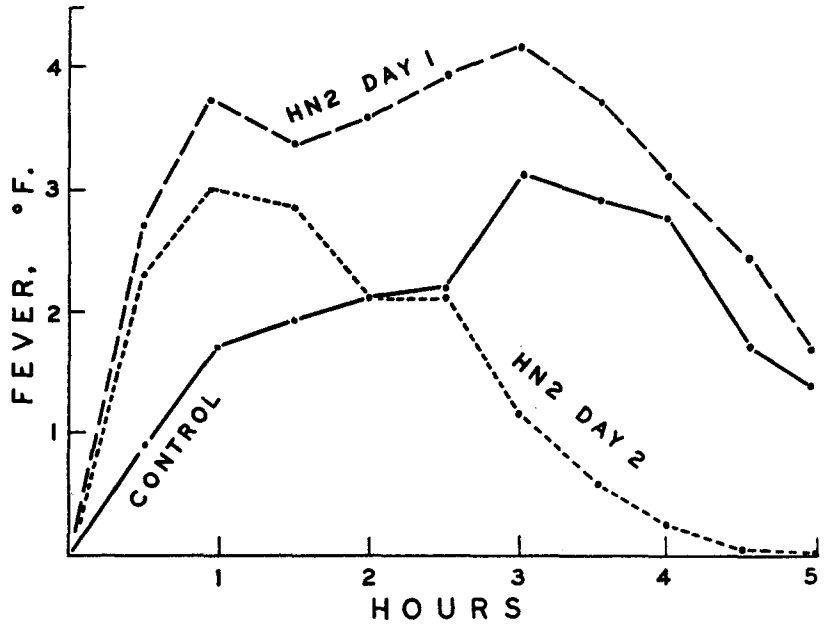

FIG. 5. First and second day febrile responses of severely leucopenic rabbits given 2.5 μg. endotoxin.

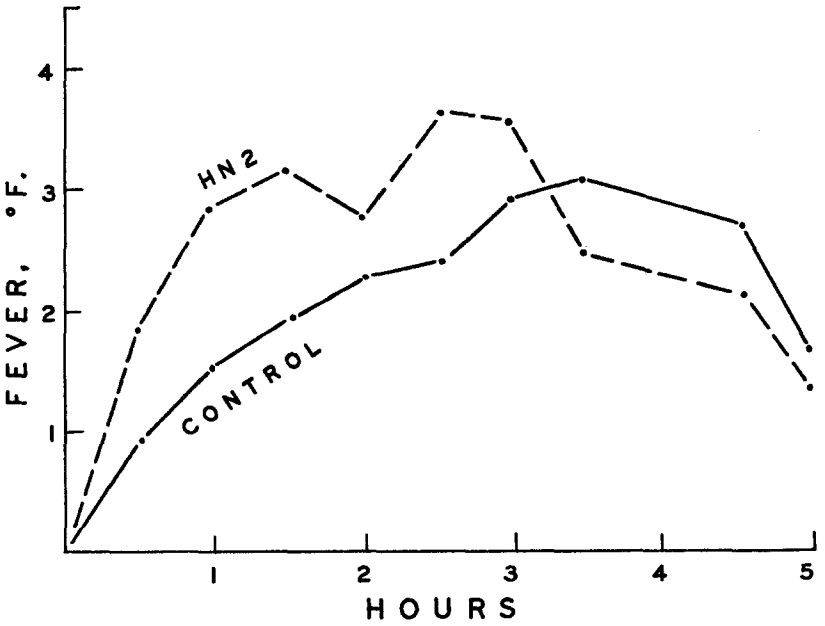

FIG. 6. Febrile response to 2.5 μg. endotoxin of mildly leucopenic rabbits.

The white counts at 1 hour were 575, 450, and 1150/mm.^{3}, respectively. This consistent fall in count was accompanied by lower fevers than on the 1st day when there was no net drop in white count.

Next, 2 rabbits were given a single injection of 5 mg. of HN2. Four days later the white cell counts were 2,100 and 2,270/mm.^{3}. These, and 2 normal control rabbits, were given the 2.5 μg. test dose of endotoxin, and the observed fevers are shown in Fig. 6.

Again, a greater than normal fever was obtained, but the febrile response was less severe than in the more severely leucopenic rabbits described in the preceding experiment. The counts at 1 and 3 hours after endotoxin were 1,600 and 950/mm.^{3}, and 3,850 and 2,300/mm.^{3}, respectively.

DISCUSSION

Passive transfer of tolerance to pyrogenicity of bacterial endotoxin (1) has been demonstrated with donor animals made tolerant by a variety of dose and time schedules. That serum from donors treated with endotoxin for several weeks was no more effective than that obtained from animals given a brief series of injections is further evidence that the phenomenon is not mediated by specific antibody to the toxin. The importance of the magnitude of the test dose of pyrogen in the recipient is emphasized by the experiments with plasma from donors given a large constant dose of endotoxin: recipients tested with the same large dose, as in Cluff's experiment (10), subsequent to administration of tolerant donor plasma suffered typical biphasic fevers, whereas testing with a smaller dose permitted qualitative differentiation of the febrile response from that seen in control animals. Rabbits injected with extemporaneously mixed lipopolysaccharide and tolerant donor plasma showed tolerance to the pyrogenic effect. Protection by tolerant plasma against lethality of endotoxin in the mouse (27) is not obtained by this procedure (28). The two test systems differ greatly in challenging dose of toxin: 2.5 μg. in the rabbit as against 600 μg. in the mouse. In the latter case, irreversible changes produced quickly by the lethal dose may require earlier stimulation of recipient animal defense mechanisms (1, 28).

Passive transfer of non-specific resistance may have broader significance than is revealed by experiments with bacterial endotoxin alone. Attention is directed to recent findings by Reichard *et al.* (29) of passive transfer of acclimatization to ordinarily lethal shock produced by tumbling trauma, and by Gledhill (30) of protection against virus infection by serum of endotoxin injected animals, this not explained by interference. Investigation in recipient animals of other aspects of the wide spectrum of non-specific resistance achieved with endotoxin treatment (31) is being pursued in our laboratory.

The leucopenic response to lipopolysaccharide was not modified in recipient rabbits by prior treatment with either normal or endotoxin-tolerant donor plasma, and the degree of leucopenia did not correlate with the magnitude of the fevers observed.

Olitzki *et al.* (5) found that normal rabbit serum, given simultaneously with vaccine, or up to 48 hours before, prevented the leucopenia in normal rabbits. The opposite was described by Farr and Lequire (32) who found an enhanced leucopenia (and fever) in normal rabbits when the pyrogen was injected in normal rabbit serum instead of in saline. Although inactivation of various properties of endotoxin by incubation in serum has been described by many workers (see Landy *et al.*, (33)), the basis for refractoriness to leucopenia produced by normal serum, from a non-refractory donor, given some time before the vaccine is not easily understood.

From the studies on peripheral leucocyte counts in rabbits made tolerant by daily intravenous injections of bacterial endotoxin it is clear that tolerance to the granulocytopenia does not develop as does tolerance to pyrogenicity. This is most readily seen after 2 or 3 daily injections of endotoxin by which time the second, major, phase of fever has disappeared. Smith (6) and Beeson (7) described repeated leucopenia with daily administration of typhoid vaccine, but believed the responses were of lesser magnitude toward the ends of their experiments. Morgan (8) and Smiddy and Fine (11) noted that tolerance to the drop in white cell count did not occur as it did to the pyrogenic effect. Cluff (10) found the same degree of fall in numbers of circulating leucocytes on the 10th as on the 1st day of endotoxin treatment. Petersdorf *et al.* (9) showed there was no difference in the leucopenic responses seen on the 1st and 2nd days of vaccine treatment despite alteration of the febrile response to the second injection. Palmer and Herion (12) have recently described a tendency toward a biphasic leucopenia following intravenous pyrogen in the dog, corresponding to the biphasic fever, with no change in the initial leucopenia in the tolerant subject. We have not been able to obtain any evidence for a biphasic white cell response in our rabbits. Working with the guinea pig, Olitzki *et al.* (5) found essentially complete disappearance of the granulocytopenia within a few days. Their counts were made at 2 hours after injection of vaccine. The studies reported herein confirm that refractoriness to the acute leucopenia is not ordinarily obtained in rabbits exhibiting tolerance to the pyrogenicity of bacterial lipopolysaccharide. Pertinent here is Westphal's study (34) using graded doses of lipopolysaccharide. Fevers were obtained with minute doses in normal rabbits, without concomitant leucopenia, this latter response appearing only as the dose of endotoxin was increased to several hundred times the minimum pyrogenic dose.

The response of the polymorphonuclear leucocyte to intravenous endotoxin has played an important role in studies on the pathogenesis of fever. The presence of an endogenous pyrogen in the blood of normal animals given endotoxin intravenously (13–15), differing in properties and action from the exogenous pyrogen (9, 16, 35), is well established. The recognition of the polymorphonuclear leucocyte as the source of serum pyrogen is equally well established: leucocytic pyrogen (18, 20–22) appears to be identical with the circulating endogenous pyrogen, and animals made severely leucopenic by treatment with a nitrogen mustard prior to injection of endotoxin lack endogenous pyrogen (24, 25). No other tissue source has been found (20, 22).

The many arguments for (4, 15, 17) and against (3, 9, 24, 25) the hypothesis that endogenous serum pyrogen is the final mediator of endotoxin-induced fever have been eloquently stated, and need not be repeated here. Our finding that leucopenic rabbits respond to a test dose of endotoxin with a rapidly developing, supernormal fever re-

inforces the existing arguments against this hypothesis based upon the previous finding by Bennett and coworkers (19, 25) that the leucopenic animals, lacking endogenous pyrogen, still suffer a normal course of fever from intravenous endotoxin. The exaggerated, rather than normal, fevers exhibited by our HN2-treated animals are probably explained by the difference in experimental design. In the earlier work each animal served as its own control, having been tested first with pyrogen, and, then, one (19) or two (25) weeks later being made granulocytopenic and retested. Our HN2-treated rabbits had not previously received endotoxin, for, in our experience, a first dose of pyrogen produces a degree of tolerance to the pyrogenic effect of a second, equal, dose for up to 3 weeks.

Thus the endotoxin-induced leucopenia, assumed to reflect the sticking of these cells to vascular endothelium (36) with concomitant injury and release of endogenous pyrogen (4), still occurs in rabbits showing altered febrile responses characteristic of endotoxin tolerance.[1] That the leucocytes of the tolerant animal respond to endotoxin *in vitro* with release of as much pyrogen as do those from the normal donor has been demonstrated (17). The suggestion that, *in vivo*, the leucocytes of the tolerant animal do not contribute as much endogenous pyrogen because of rapid removal of the endotoxin by the more active reticuloendothelial system (RES) (17) is not consistent with the equivalent leucopenia confirmed here. Endogenous leucocytic pyrogen cannot be assumed to be removed by the stimulated RES, for this pyrogen is fully active in the tolerant animal and tolerance to its pyrogenicity does not develop. It is of great interest that Penner and Klein (37), using pairs of dogs whose circulatory systems were surgically altered so that the circulation serving the head of each dog was in continuity with the body of the other for each pair, found that injection of toxin into a femoral vein resulted in prolonged leucopenia only in the opposite circulation. Proof of the integrity of the separate circulations is inherent in the results and the conclusion appears inescapable that the leucopenia is a secondary effect following cerebral stimulation and is mediated by the central nervous system.

In distribution studies with labelled endotoxin Braude *et al.* (26) showed that following a massive 6 mg. dose the greatest initial concentration per unit mass of tissue was in the buffy coat, reaching a maximum at about 1 hour. With intravenous injection of a few micrograms of endotoxin one would reasonably expect, on *a priori* grounds, that the concentration in the circulating white cells relative to other tissues would be even greater. Actually, the immediate clearance of sublethal doses is not very different from normal in tolerant animals (38). Others (39, 40) have described the rapid, temporary, localization of intravenously administered endotoxin, *e.g.*, within the spleen, lungs, and lumen of vessels, with subsequent gradual decrease and apparent return to the blood stream. Cortisone-treated rabbits hold endotoxin in the spleen

---

[1] An endotoxin from a Gram-positive organism (*Staphylococcus*, kindly supplied by Dr. C. W. Christensen, Difco) has produced the typical biphasic febrile course seen after injection of the Gram-negative endotoxins, but the response of the circulating leucocytes has differed qualitatively. Instead of a maximum leucopenia at 1 hour, the approximate time of the peak of the first phase of fever, the decrease in total white cell count did not begin until 1.5 to 2 hours, when the initial fever had ended. Maximum leucopenia (50 per cent of preinjection count) occurred at 3 hours, at which time maximum fever had already developed (43).

and lungs and survival time is increased (40). The tolerant animal temporarily traps endotoxin in the lungs, presumably in the pulmonary capillaries, at the time of the acute leucopenia, with release about an hour later (38, 41). It is worth considering that the exaggerated fevers in our leucopenic rabbits occur because of the absence of sufficient numbers of leucocytes to temporarily sequester the injected toxin, resulting in a more rapid and intense effect upon the brain. Indeed, the objection (4) that endotoxin only "spills over" into the spinal fluid when very large doses are given may be true precisely because of the limitation imposed by sequestration in the circulating white cells. When endotoxin is injected by the intrathecal route sensitivity to pyrogenicity is several thousand times greater than that seen with intravenous administration and there is no peripheral leucopenia (24, 42).[2]

We may postulate, then, that the role played by the leucocytes in endotoxin-induced fever results in limiting and delaying the febrile response, by restricting the blood concentration of endotoxin that may act upon the brain. The tolerant animal, with its exalted RES activity, may clear endotoxin, as it reappears in the blood, at a rate sufficient to prevent setting off the second phase of fever, but the normal animal may not, accounting for the typical biphasic response. The results of our studies on passive transfer (1, 28) are consistent with this hypothesis.

Preliminary experiments (43) have yielded results supporting this postulate. These studies, involving serial injection of heterologous endotoxins in normal rabbits and in rabbits following passive transfer from endotoxin-tolerant donors, suggest a contributory role for specific antibody in the early tolerant febrile response, within the framework of Braude's findings (38, 41), which, in itself, cannot account for tolerance.

Remaining to be explained, of course, are the presence of endogenous serum pyrogen after a first injection of endotoxin and the decrease in endogenous pyrogen seen in developing tolerance. The latter point may be answered by the work of Petersdorf and Bennett (25) who found that from 10 to 100 times the pyrogenic dose of endotoxin was needed to demonstrate endogenous pyrogen (and that purified endotoxin was a weaker stimulus for endogenous pyrogen than was vaccine). A second dose of endotoxin may elicit less endogenous pyrogen because it is, in effect, a lesser dose. While there is great temptation to refer the observed endotoxin fever to the serum pyrogen present in particular circumstances, things that look alike need not be alike. Worth recalling, here, is that leucocytic pyrogen has been demonstrated (21) in white cells taken from the blood stream of normal afebrile animals.

---

[2] The pyrogenic effect of submicrogram doses of endotoxin injected into the lateral cerebral ventricle of the cat has recently been described; this route of administration was not subject to tolerance (44).

SUMMARY

The effect of various schedules for inducing tolerance to bacterial endotoxin in donor rabbits upon suitability for demonstration of passive transfer of tolerance to pyrogenicity in normal recipients has been investigated. Long-term treatment of donors, through 5 weeks, is no more effective than a brief series of injections, adding further evidence that tolerance is not attributable to specific antibody to the endotoxin. Qualitative differentiation of the febrile pattern of passively tolerant recipients from that seen in control animals depends upon the magnitude of the test dose of pyrogen.

Passively tolerant rabbits respond to endotoxin with an acute leucopenia equivalent to that seen in controls suffering a full biphasic fever. Animals given daily injections of endotoxin continue to show the acute leucopenia, despite the early modification of the course of fever characteristic of endotoxin tolerance. The assumption that the leucopenia reflects damage to the leucocytes, with release of endogenous pyrogen, is not consistent with these findings. Rabbits rendered leucopenic by nitrogen mustard and then given endotoxin exhibit a rapidly developing fever of greater than normal intensity, the exaggeration of the febrile response being proportional to the severity of the induced leucopenia.

The implications of these findings for the pathogenesis of endotoxin-induced fever are discussed. The evidence supports the hypothesis that endotoxin produces fever by direct action rather than by release of endogenous leucocytic pyrogen. It is postulated that the lesser fever, in animals having normal numbers of circulating leucocytes, reflects a limitation of available endotoxin by the known rapid sequestration in the white blood cells at the time of the acute leucopenia. It is further suggested that the biphasic febrile response of the normal rabbit results from reinoculation of the blood stream by the temporarily sequestered endotoxin, the RES of the tolerant animal clearing the released endotoxin at a rate sufficient to prevent triggering the second phase of fever.

It is a pleasure to acknowledge the skilled technical assistance of Miriam Graff.

BIBLIOGRAPHY

1. Freedman, H. H., Passive transfer of tolerance to pyrogenicity of bacterial endotoxin, *J. Exp. Med.*, 1960, **111,** 453.
2. Thomas, L., The physiological disturbances produced by endotoxins, *Ann. Rev. Physiol.*, 1954, **16,** 467.
3. Bennett, I. L., Jr., and Cluff, L. E., Bacterial pyrogens, *Pharmacol. Rev.*, 1957, **9,** 427.
4. Wood, W. B., Jr., Studies on the cause of fever, *New England J. Med.*, 1958, **258,** 1023.

5. Olitzki, L., Avinery, Sh., and Bendersky, J., The leucopenic action of different micro-organisms and the antileucopenic immunity, *J. Immunol.*, 1941, **41,** 361.
6. Smith, E. V., The effects of injections of toxic extracts of the typhoid bacillus on the blood picture in rabbits, *Am. J. Hyg.*, 1939, **29,** 15.
7. Beeson, P. B., Tolerance to bacterial pyrogens. I. Factors influencing its development, *J. Exp. Med.* 1947, **86,** 29.
8. Morgan, H. R., Immunologic properties of antigenic material isolated from *Eberthella typhosa*, *J. Immunol.*, 1941, **41,** 161.
9. Petersdorf, R. G., Keene, W., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. IX. Characteristics of endogenous pyrogen and mechanisms governing its release, *J. Exp. Med.*, 1957, **106,** 787.
10. Cluff, L. E., Studies on the effect of bacterial endotoxin on rabbit leucocytes. II. Development of acquired resistance, *J. Exp. Med.*, 1953, **98,** 349.
11. Smiddy, F. G., and Fine, J., Host resistance to hemorrhagic shock. X. Induction of resistance by shock plasma and by endotoxins, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 558.
12. Palmer, J. G., and Herion, J. C., Relation of leucocyte changes and fever following injection of typhoid vaccine in non-tolerant and tolerant dogs, *Am. J. Med.*, 1959, **27,** 319.
13. Grant, R., and Whalen, W. J., Latency of pyrogen fever. Appearance of a fast-acting pyrogen in the blood of febrile animals and in plasma incubated with bacterial pyrogen, *Am. J. Physiol.*, 1953, **173,** 47.
14. Atkins, E., and Wood, W. B., Jr., Studies on the pathogenesis of fever. I. Presence of transferable pyrogen in the blood stream following the injection of typhoid vaccine, *J. Exp. Med.*, 1955, **101,** 519.
15. Atkins, E., and Wood, W. B., Jr., Studies on the pathogenesis of fever. II. Identification of an endogenous pyrogen in the blood stream following the injection of typhoid vaccine, *J. Exp. Med.*, 199, **102,** 499.
16. King, M. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. IV. The site of action of leucocytic and circulating endogenous pyrogen, *J. Exp. Med.*, 1958, **107,** 291.
17. Collins, R. D., and Wood, W. B., Jr., Studies on the pathogenesis of fever. VI. The interaction of leucocytes and endotoxin *in vitro*, *J. Exp. Med.*, 1959, **110,** 1005.
18. Beeson, P. B., Temperature-elevating effect of a substance obtained from polymorphonuclear leucocytes, *J. Clin. Inv.*, 1948, **27,** 524.
19. Bennett, I. L., Jr., and Cluff, L. E., Influence of nitrogen mustard upon reactions to bacterial endotoxins: Shwartzman phenomenon and fever, *Proc. Soc. Exp. Biol. and Med.*, 1952, **81,** 304.
20. Bennett, I. L., Jr., and Beeson, P. B., Studies on the pathogenesis of fever. I. The effect of injection of extracts and suspensions of uninfected rabbit tissues upon the body temperature of normal rabbits, *J. Exp. Med.*, 1953, **98,** 477.
21. Bennett, I. L., Jr., and Beeson, P. B., Studies on the pathogenesis of fever. II. Characterization of fever-producing substances from polymorphonuclear leucocytes and from the fluid of sterile exudates, *J. Exp. Med.*, 1953, **98,** 493.
22. Bennett, I. L., Jr., Studies on the pathogenesis of fever. III. Failure to demonstrate

fever-producing substances in the tissues of leuckopenic rabbits, *Bull. Johns Hopkins Hosp.*, 1956, **98,** 1.

23. Bennett, I. L., Jr., Studies on the pathogenesis of fever. IV. Further observations on the production of fever in rabbits by the fluid of sterile exudates, *Bull. Johns Hopkins Hosp.*, 1956, **98,** 7.
24. Bennett, I. L., Jr., Petersdorf, R. G., and Keene, W. R., The pathogenesis of fever: evidence for direct cerebral action of bacterial endotoxins, *Tr. Assn. Am. Physn.*, 1957, **70,** 64.
25. Petersdorf, R. G., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. VIII. Fever-producing substances in the serum of dogs, *J. Exp. Med.*, 1957, **106,** 293.
26. Braude, A. I., Carey, F. J., and Zalesky, M., Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of *E. coli* endotoxin labelled with radioactive sodium chromate, *J. Clin. Inv.*, 1955, **34,** 858.
27. Freedman, H. H., Passive transfer of protection against lethality of homologous and heterologous endotoxins, *Proc. Soc. Exp. Biol. and Med.*, 1959, **102,** 504.
28. Freedman, H. H., RES and passive transfer of endotoxin tolerance, Conference on the Reticuloendothelial system (RES), *Ann. New York Acad. Sc.*, 1960, **88,** 99.
29. Reichard, S. M., Gordon, A. S., and Tessmer, C. F., Humoral modification of RES function, Conference on the Reticuloendothelial System (RES), *Ann. New York Acad. Sc.*, 1960, **88,** 213.
30. Gledhill, A. W., Sparing effect of serum from mice treated with endotoxin upon certain murine virus diseases, *Nature*, 1959, **183,** 185.
31. Shilo, M., Nonspecific resistance to infections, *Ann. Rev. Microbiol.*, 1959, **13,** 255.
32. Farr, R. S., and LeQuire, V. S., Leucocytic and pyrogenic effects of thyphoid vaccine and augmentation by homologous sera, *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 661.
33. Landy, M., Trapani, R.-J., and Shear, M. J., Inactivation of endotoxin by a humoral component. IV. Alteration in the immunological properties of typhoid endotoxin, *J. Exp. Med.*, 1959, **110,** 731.
34. Westphal, O., Pyrogens, *in* Polysaccharides in Biology, (G. F. Springer, editor), New York, Josiah Macy, Jr. Foundation, 1957, 115.
35. Petersdorf, R. G., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. VI. The effect of heat on endogenous and exogenous pyrogen in serum of dogs, *Bull. Johns Hopkins Hosp.*, 1957, **100,** 197.
36. Berthrong, M., and Cluff, L. E., Studies of the effect of bacterial endotoxins on rabbit leukocytes. I. Effect of intravenous injection of the substances with and without induction of the local Shwartzman reaction, *J. Exp. Med.*, 1953, **98,** 331.
37. Penner, A., and Klein, S. H., The pathogenesis of experimental dysentery intoxication. Production of the lesions by cerebral circulation of the toxin, *J. Exp. Med.*, 1952, **96,** 59.
38. Carey, F. J., Braude, A. I., and Zalesky, M., Studies with radioactive endotoxin.

III. The effect of tolerance on the distribution of radioactivity after intravenous injection of *Escherichia coli* endotoxin labelled with Cr^{51}, *J. Clin. Inv.*, 1958, **37,** 441.

39. Seligman, A. M., Shear, M. J., Leiter, J., and Sweet, B., Chemical alteration of polysaccharide from *Serratia marcescens*; tumor-necrotizing polysaccharide tagged with radioactive iodine, *J. Nat. Cancer Inst.*, 1948, **9,** 13.
40. Cremer, N., and Watson, D. W., Influence of stress on distribution of endotoxin in RES determined by fluorescein antibody technic, *Proc. Soc. Exp. Biol. and Med.*, 1957, **95,** 510.
41. Ribble, J. C., Zalesky, M., and Braude, A. I., Distribution of Cr^{51}-labelled endotoxin in cortisone-treated mice, *Bull. Johns Hopkins Hosp.*, 1959, **105,** 272.
42. Keene, W. R., Pathogenesis of fever: fevers produced by intrathecal injection of endotoxin, *Bull. Johns Hopkins Hosp.*, 1956, **99,** 103.
43. Freedman, H. H., data to be published.
44. Sheth, U. K., Clark, W. G., Rosenstein, R., and Borison, H. L., Pyrogenic effect of *S. typhosa* lipopolysaccharide injected into the lateral cerebral ventricle in cats, *Fed. Proc.*, 1960, **19,** 44.