STUDIES ON THE PATHOGENESIS OF FEVER

VI. The Interaction of Leucocytes and Endotoxin in Vitro*

By ROBERT D. COLLINS, M.D.,‡ and W. BARRY WOOD, Jr., M.D.

(From the Department of Microbiology, The Johns Hopkins University School of Medicine, and School of Hygiene and Public Health, Baltimore)

(Received for publication, August 14, 1959)

Endogenous pyrogen was first demonstrated in the serum of rabbits during the febrile response to intravenous typhoid vaccine (1). The concentration of the endogenous factor in the circulation correlated closely with the intensity of the fever, whereas the injected bacterial pyrogen was rapidly cleared from the blood stream. That the thermoregulatory centers of the brain were stimulated by the secondarily released endogenous pyrogen, rather than by the injected endotoxin itself, was suggested first, by the considerable latent period which preceded the fever and secondly, by the demonstration that endogenous pyrogen acts more directly than endotoxin upon the thermogenic centers (2). Confirmatory evidence supporting the role of endogenous pyrogen in the pathogenesis of endotoxin-induced fever has recently been published (3, 4).

Endogenous pyrogen is biologically indistinguishable from the pyrogenic factor extracted by Beeson and Bennett from polymorphonuclear leucocytes (5). Since leucocytes are known to be damaged by endotoxin both *in vivo* and *in vitro* (6–8), the hypothesis has been advanced that the febrile response following typhoid vaccine is caused by the thermogenic action of endogenous pyrogens released from injured leucocytes (1, 9).

Bennett and coworkers (10–12), however, have questioned the primary role of polymorphonuclear leucocytes in the pathogenesis of fever. Using animals made granulocytopenic with nitrogen mustard, they have shown: (*a*) that the fluid portion of virtually agranulocytic exudates contains a pyrogenic substance similar to leucocytic pyrogen (10), and (*b*) that the usual febrile response to bacterial pyrogen occurs in rabbits with severe neutropenia (11). These findings suggest that cells other than polymorphonuclear leucocytes may be capable of producing endogenous pyrogens, although all efforts to demonstrate them by direct extraction have thus far been unsuccessful (5, 13, 14).

Cranston *et al.* (15–18), on the other hand, have reported that when human polymorphonuclear leucocytes and endotoxin are incubated together *in vitro* for 3 hours, a

* This work was supported by a grant from the Life Insurance Medical Research Fund.

‡ Senior Research Fellow, United States Public Health Service. Present address: Department of Pathology, Vanderbilt University School of Medicine, Nashville.

pyrogenic substance is formed that acts like rabbit leucocytic pyrogen rather than the added endotoxin. Although they were unable to extract a pyrogenic factor directly from human leucocytes (19), their results suggest that the endotoxin may have stimulated the leucocytes to release an endogenous pyrogen. Their observations also suggest that leucocytes may be capable of inactivating endotoxin *in vitro*. This possibility is of particular interest inasmuch as the clearance of endotoxin *in vivo* is presently ascribed to action of the reticulo-endothelial system (20).

The present study was undertaken to investigate the interaction of rabbit leucocytes and endotoxin, particularly as it relates to the release of leucocytic pyrogen and to the possible concomitant inactivation of endotoxin. Experiments were performed with leucocytes from both normal and tolerant rabbits.

Methods

All glassware was rendered pyrogen-free by exposure to 180°C. for 2 hours. Normal saline was made from distilled water and was demonstrated to be non-pyrogenic. Sera from normal and tolerant rabbits were stored at 4°C. and were tested for sterility and pyrogenicity before being used.

Rabbits.—Male albino rabbits were obtained from a single dealer. The donor rabbits weighed 4 to 6 pounds. Those made tolerant were given at least seven daily intravenous injections of 1 ml. of undiluted typhoid vaccine. All recipient rabbits, which weighed 6 to 8 pounds, were housed in the constant temperature room (68–70°F.) where the temperature studies were performed. Normal recipients were used in groups of six and were "rested" after each test injection for at least 3 weeks in order to avoid the development of tolerance. The tolerant recipients were given 5.0 μg. of *Shigella flexneri* endotoxin in saline intravenously each day for 3 weeks, and were demonstrated to be tolerant before being used as test recipients.

Bacterial Pyrogens.—The typhoid vaccine (2) was kindly supplied by Col. A. S. Benenson of the Walter Reed Army Institute for Research, Washington. Endotoxin, purified from *Shigella flexneri*, (type Z), was obtained from Dr. L. E. Cluff of the Department of Medicine, The Johns Hopkins University, and was diluted in normal salt solution to a concentration of 1 μg. per ml.

Production of Acute Inflammatory Exudates.—Polymorphonuclear leucocytes were obtained from exudates produced in rabbits by the intraperitoneal injection of saline as previously described (21). Each rabbit was tied on its back, anesthetized with intravenous pentobarbital, and shaved over its abdomen with electric clippers. A sharp, 18-gauge needle was inserted through a midline cutaneous incision which was made after the skin had been cleansed with acetone. Three hundred ml. of physiologic saline containing 0.25 gm. of streptomycin and 20,000 units of crystalline penicillin G were rapidly injected with a 50 ml. syringe. In order to increase peritoneal irritation, and thereby augment the yield of white cells, 0.2 to 0.3 ml. of pyrogen-free glass beads[1] were introduced through the same needle from a 1 ml. tuberculin syringe. The beads were injected a few at a time along with 0.5 ml. of pyrogen-free saline. After the needle had been withdrawn, the fluid in the abdomen was gently agitated, and the rabbit was returned to its cage without food or water.

Harvesting of Exudates.—After an interval of 15 to 16 hours, each rabbit was sacrificed by intravenous injection of 1 ml. of pentobarbital followed by 10 ml. of air. The abdominal surface was cleansed, and the peritoneal cavity was opened by a midline incision. The fluid

[1] Pavement marking beads, Minnesota Mining and Manufacturing Company, St. Paul, (Type 110).

exudate was removed by a 10 ml. pipette, and collected in a flask packed in ice. Clotting was prevented by the addition of heparin (0.2 mg./100 ml. exudate) to the flask. Bloody exudates were discarded. After a white cell count had been performed on the pooled exudate, the cells and supernatant fluid were separated by centrifugation at 625 *g* for 20 minutes at 4°C. Aliquots of 1.0×10^9 cells were resuspended in 20 ml. of saline, or 15 ml. of serum, and were stored in 40 ml. centrifuge tubes at 0°C. during the few minutes before use.

Preparation of Test Solutions.—Shortly before the start of the incubation period, the tubes containing the cells were transferred from the ice bath to a 37°C. water bath in which their temperatures were quickly raised to 37°C. by intermittent agitation. To each test suspension was added 0.5 ml. of endotoxin solution (0.5 μg. of endotoxin). A like volume of pyrogen-free saline was added to each control suspension. When the endotoxin was added to cells suspended in serum, it was first "potentiated" by incubation in 5 ml. of normal serum at 37°C. for 5 minutes (22, 23). To the control cell suspensions in serum, 5 ml. of serum containing no endotoxin was added. Both the saline and serum cell suspensions were gently agitated to insure complete mixing, and were then transferred to an incubator at 37°C. At the end of the test period, the supernatant fluid and cells were separated by centrifugation at 900 *g* for 10 minutes, and the cells were discarded. The supernatant fluids were stored at 4°C. until assayed for pyrogenicity.

Assay of Pyrogen.—Each sample of supernatant fluid was tested for pyrogenicity by intravenous injection into a recipient rabbit. The methods used in handling the rabbits and recording their temperatures have been previously published (1, 2). The nature of the pyrogen present (*i.e.* endotoxin, leucocytic pyrogen, or a mixture of both) was determined, as reported elsewhere (1), by comparing its activity in normal and tolerant recipients. The samples were tested in the following manner. Duplicate 20 ml. aliquots of supernatant were incubated with 10 ml. of either normal or tolerant serum for 30 minutes at 37°C. The aliquot containing normal serum was injected into a normal recipient, and the aliquot containing tolerant serum was injected into a tolerant recipient. The resulting pyrogenic responses were then compared.

Since leucocytic pyrogen is not altered by incubation with either normal or tolerant serum (24), and is equally active in normal and endotoxin-tolerant rabbits (5), similar fever curves indicate that all of the pyrogen in the test solution is of the leucocytic type. When endotoxin is present in the test solution, on the other hand, the febrile response of the normal recipient is greater than that of the tolerant recipient. This difference is greatly enhanced by prior incubation of the two test samples with normal and tolerant serum respectively (1, 23), as described above. Thus, the response of the tolerant recipient may be taken as a measure of the amount of leucocytic pyrogen present in the solution, whereas the difference between the responses of the normal and the tolerant recipients may be assumed to be due to endotoxin.[2]

Calculation of Fever Index.—All fever curves were charted on 1/20 inch graph paper, with 20 lines on the vertical representing each degree centrigrade and 20 lines on the horizontal representing each hour. The area beneath each 3 hour fever curve was measured by means of a compensating planimeter, as previously described (21). This figure, designated the "fever index," is a measure of both the height and the duration of the fever. The mean value for each sample was determined by averaging the individual indices calculated on usually three or more recipients.

RESULTS

I. Characterization of Peritoneal Exudates.—Saline-bead peritonitis was established in 81 normal and 44 tolerant rabbits. The animals were sacrificed

[2] The dose of endotoxin used in these studies was such that even if potentiated, it was essentially non-pyrogenic when previously incubated with tolerant serum and injected into a tolerant recipient (see Fig. 1 and section II, Results).

in groups at 16 hours, and their exudates were pooled before the volumes were measured and the white counts determined. The average white cell counts and volumes of exudate obtained from normal and tolerant donors are shown in Table I. Of the white cells present, 90 to 95 per cent were found by differential count to be polymorphonuclear leucocytes. Five ml. of each pooled exudate were cultured in thioglycollate broth. All exudates were sterile.

TABLE I

Volumes and Leucocyte Counts of Peritoneal Exudates from Normal and Tolerant Rabbits

Rabbits	No. of rabbits	Average volume of exudate per rabbit	Average number of leucocytes (per mm.^{3})	Average total leucocytes per rabbit
		ml.		
Normal.....	81	85	7534	6.4×10^8
Tolerant.....	44	78	8432	6.6×10^8

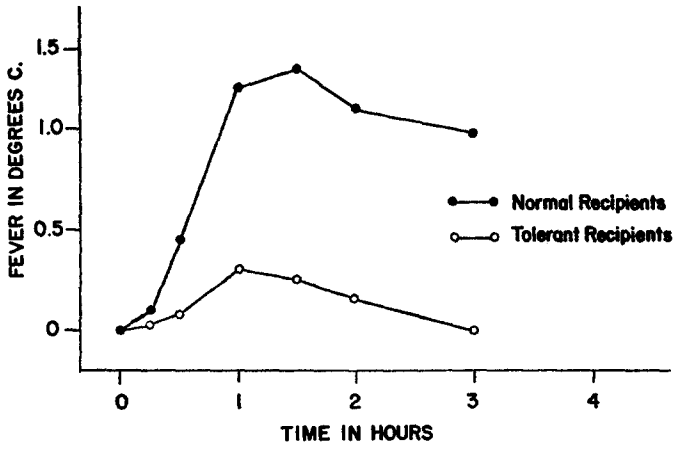

FIG. 1. Comparison of the mean febrile responses of 3 normal and 3 tolerant recipients to aliquots of 0.5 μg. of endotoxin "potentiated" by incubation for 30 minutes with 10 ml. of normal serum. The aliquots injected into each of the tolerant recipients were incubated with tolerant serum to attain maximal suppression of the pyrogenic reaction to the potentiated endotoxin.

II. Effectiveness of Tolerance Test in Differentiating Endotoxin from Leucocytic Pyrogen.—To determine whether the pyrogenic effect of the dose of endotoxin used in the present studies, namely 0.5 μg. can be differentiated from that of leucocytic pyrogen regardless of whether the endotoxin has, or has not, been previously "potentiated,"[3] the following experiment was devised.

[3] Since the "potentiation" of endotoxin in normal serum results from interaction with protein (25), it is conceivable that a similar interaction might occur with leucocytic protein (26).

Each of six aliquots of 0.5 μg. of endotoxin was "potentiated" by being added to 10 ml. of normal serum and incubated at 37°C. for 15 minutes (22). Three of the aliquots were incubated with an additional 5 ml. of normal serum per aliquot at 37°C. for 30 minutes, and the remaining three were incubated with 5 ml. of tolerant serum per aliquot. Each of the aliquots incubated with normal serum was injected into a normal recipient, and each of the aliquots containing tolerant serum was injected into a tolerant recipient. The average febrile responses for the two groups are shown in Fig. 1.

It is apparent that the pyrogenic effect 0.5 μg. of endotoxin, even when "potentiated," is markedly depressed in the "tolerant system" as compared to the "normal system."

When tested by the same method ("tolerant system"), 0.5 μg. of endotoxin in saline ("unpotentiated") caused no fever at all. Thus, the pyrogenic effect of endotoxin can be readily distinguished from that of leucocytic pyrogen by the method outlined, regardless of whether the endotoxin is in the "potentiated" or the "unpotentiated" form in the supernatant fluids prepared from leucocytes

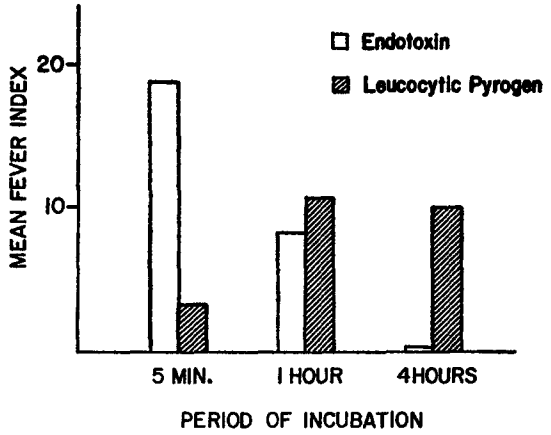

FIG. 2. Release of leucocytic pyrogen and inactivation of endotoxin resulting from the *in vitro* incubation of "normal" leucocytes and endotoxin in salt solution. Each mean fever index recorded in this and the following 2 charts, (Figs. 3 and 4), was calculated from the febrile responses of 3 recipients.

III. Incubation of Endotoxin with Normal Leucocytes in Saline.—

Aliquots of 1.0×10^9 normal leucocytes were resuspended in 20 ml. of pyrogen-free saline, and were incubated with 0.5 μg. of endotoxin for 5 minutes at 37°C. At the end of the incubation period, the cells and supernatant of each aliquot were separated by centrifugation and the supernatant fluids were pooled. Duplicate samples of the pooled fluid were then incubated with normal and tolerant serum, and were injected into normal and tolerant recipients, respectively. Similar aliquots of cells incubated with endotoxin for 1 hour and for 4 hours were also treated in the same manner.

The results expressed in mean fever indices (Fig. 2) reveal that endotoxin gradually disappears from the supernatant fluid, so that by the end of the 4

hour period of incubation, all of the pyrogen remaining in the fluid is of the leucocytic type.

The apparent disappearance of the endotoxin is of particular interest. It is postulated that one of two factors, or both, may have been involved. The endotoxin may have become attached to the leucocytes and thus have been removed by centrifugation, or the leucocytes may have released into the supernatant fluid a substance capable of inactivating endotoxin. The second of these two possibilities was tested by the following experiment.

IV. Inactivation of Endotoxin by Cell-Free Leucocytic Extract.—

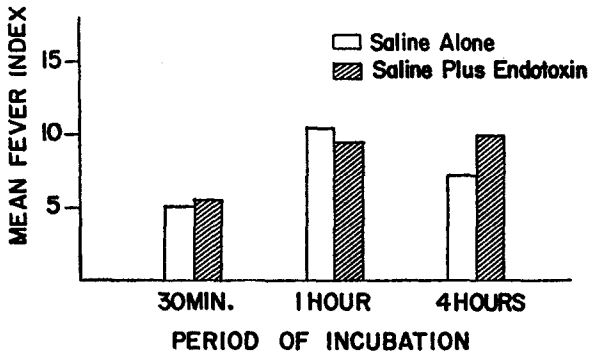

FIG. 3. Comparison of pyrogen-stimulating effects of endotoxin in normal saline and of normal saline alone. Note that incubation with saline alone acts as a potent stimulus for the release of pyrogen from leucocytes.

Aliquots of 3.5×10^8 leucocytes were incubated in 20 ml. of normal saline for 24 hours, at which time the supernatant fluids were separated from the cells by centrifugation. Duplicate samples of the supernatant were incubated with 0.5 μg. of endotoxin for 5 minutes and for 4 hours, respectively. Aliquots of the two samples were then tested for pyrogenicity in both the normal and tolerant test systems.

Although most of the endotoxin added was demonstrable after 5 minutes' incubation, none was detectable by the end of 4 hours. Thus, it is concluded that leucocytes are capable of shedding a substance which inactivates endotoxin.

V. The Effect of Endotoxin upon the Release of Leucocytic Pyrogen in Saline.— Since leucocytic pyrogen became demonstrable in the saline preparations of leucocytes and endotoxin, an experiment was designed to determine whether its release was accelerated by the endotoxin.

Leucocytic suspensions containing 1×10^9 cells were incubated with and without endotoxin (0.5 μg.) for periods of 30 minutes, 1 hour, and 4 hours. Each of the supernatants from the two samples were then incubated with 10 ml. of tolerant serum, and were injected into tolerant recipients.

Comparison of the pyrogenic responses shows (Fig. 3) that the release of the leucocytic pyrogen was just as rapid in the saline alone as it was in the saline plus the endotoxin.

VI. The Effect of Endotoxin upon the Release of Leucocytic Pyrogen in Serum.—Because incubation of leucocytes in saline alone caused them to discharge promptly their pyrogen, the experiment was repeated with leucocytes suspended in fresh serum. As shown by the open bar graph in Fig. 4, very little leucocytic pyrogen was released in the serum alone, even when the cells were incubated for 4 hours.[4] The addition of 0.5 μg. of "potentiated" endotoxin,

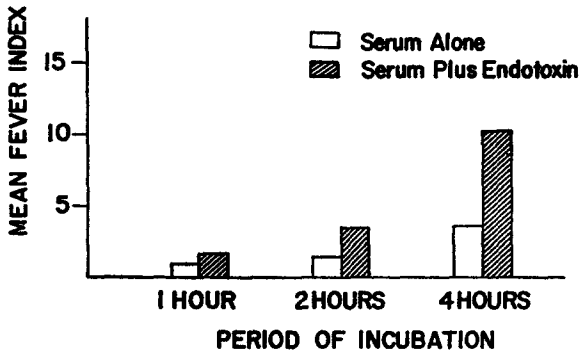

FIG. 4. Stimulating effect of endotoxin upon the release of pyrogen from leucocytes incubated in fresh serum. Note that relatively little leucocytic pyrogen is released during the incubation in serum alone.

on the other hand, significantly stimulated the discharge of leucocytic pyrogen (cross-hatched bar graph), particularly when the incubation was prolonged for 4 hours. That this amount of endotoxin, even when "potentiated," was a weaker stimulus in serum than was the saline alone is indicated by the relatively small amount of leucocytic pyrogen present at 1 hour. (Compare Figs. 3 and 4). Nevertheless, the results clearly demonstrate that endotoxin is capable of stimulating the release of leucocytic pyrogen in serum.

VII. Effect of Endotoxin upon Leucocytes Obtained from Tolerant Donors.—Inasmuch as the fever response of tolerant rabbits to a moderate dose of endotoxin is appreciably less than that of normal rabbits (20), it appeared of interest to compare the effects of endotoxin upon "normal" and "tolerant" leucocytes. Accordingly, the experiment described in section *III* was repeated using leucocytes obtained from rabbits previously made tolerant to endotoxin (see Table I). The results, summarized in Fig. 5, were essentially the same as those obtained with normal leucocytes (Fig. 2). Comparison of Figs. 2 and 5

[4] The effectiveness of serum in preventing the release of leucocytic pyrogen was found to diminish rapidly upon storage at 4°C.

reveals that the tolerant leucocytes produced just as much pyrogen as did normal leucocytes. Although the 5 minute and 1 hour data suggest that the tolerant cells may have inactivated the endotoxin more rapidly than the normal cells, the difference was not consistent in repeated experiments.

Likewise, when the tests were performed in normal serum (as described in section *VI*), the results with leucocytes from tolerant and normal rabbits were indistinguishable. As previously demonstrated with normal leucocytes (Fig. 4), the addition of endotoxin to the serum suspensions accelerated the release of leucocytic pyrogen. It is evident, therefore, that endotoxin in serum causes leucocytes from normal and tolerant rabbits to release essentially the same amount of pyrogen.

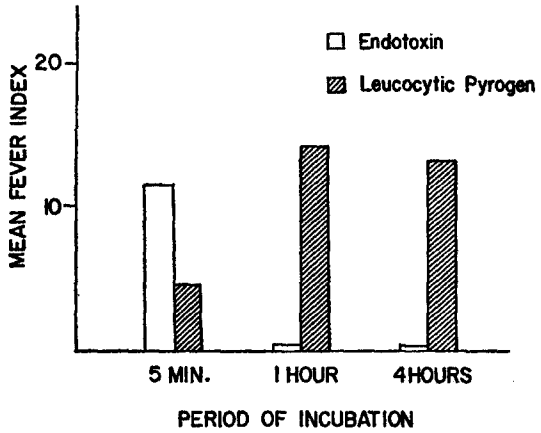

FIG. 5. Same experiment as in Fig. 2, except that leucocytes from tolerant, rather than normal, rabbits were used as the source of leucocytic pyrogen.

DISCUSSION

Injury to polymorphonuclear leucocytes causing them to release endogenous pyrogen has been postulated to be a basic mechanism in the pathogenesis of fever (1, 9, 27–29). In addition to the established observation that endotoxin damages leucocytes (6–8), the present *in vitro* studies clearly show that endotoxin stimulates the release of leucocytic pyrogen. Whether other cells are capable of releasing a similar pyrogen *in vivo* is, at present, unknown. However, it should be noted that all of the basic tenets of the cell-injury hypothesis, as previously advanced (1, 9), have now been confirmed in the case of endotoxin fever: (*a*) endotoxin has been observed to produce leucocytic injury both *in vivo* (6) and *in vitro* (7, 8); (*b*) serum-suspended leucocytes damaged by endotoxin have been demonstrated to release leucocytic pyrogen; (*c*) a transferable endogenous pyrogen, indistinguishable from leucocytic pyrogen, has been found in the circulation during endotoxin-induced fever; (*d*) both it

and leucocytic pyrogen have been shown to act directly upon the central nervous system in initiating the febrile response (2).

Although the mechanisms governing the release of leucocytic pyrogen have not yet been systematically investigated, it is evident from the present studies that mere exposure of the cells to so called "physiological" saline rather than to serum will cause them to shed their fever-producing factor. Furthermore, it is apparent that the amount of endotoxin employed in the present experiments was a relatively weak stimulus *in vitro*. Very little pyrogen, for example, was released in the serum-endotoxin preparations before 2 hours of incubation. It is of interest that this time interval is comparable to the period of incubation which Cranston *et al.* (16) found necessary for the production of a rapidly acting pyrogen in human blood samples exposed to endotoxin. Despite these *in vitro* time relationships, it seems unlikely that the *in vivo* release of pyrogen stimulated by endotoxin proceeds at such a slow rate. The onset of fever in rabbits occurs 30 to 45 minutes after the intravenous injection of bacterial pyrogen. Similarly in man the latent period is seldom more than an hour. These apparent descrepancies between the *in vitro* and the *in vivo* observations may possibly be accounted for on quantitative grounds alone. Regardless of their explanation, they in no way negate the demonstration that endotoxin stimulates leucocytes to release pyrogen *in vitro*.

The findings of Cranston *et al.* (15–19) are of particular interest in relation to the present study. The rapidly acting pyrogen which they described in endotoxin-treated human blood behaved precisely like leucocytic pyrogen (18). Why they failed to extract a pyrogen directly from human leucocytes is at present not clear (19). Extraction of pyrogen from relatively large numbers of rabbit leucocytes has been most successful when the cells have been incubated for 24 hours in 10 to 20 volumes of saline (21, 24), a method apparently not employed by Cranston and his group (19). Furthermore, the quantitative difficulties involved in the transfer of pyrogen, which have been repeatedly emphasized (27–30), may be particularly great in experiments involving human subjects. In view of the observations already made, it appears probable that human leucocytes will eventually yield an extractable pyrogen.

With the greatly simplified experimental model used in the present studies (involving only leucocytes, serum, and endotoxin), it was possible to duplicate *in vitro* the release of endogenous pyrogen and the inactivation of endotoxin, both of which occur *in vivo* following the injection of bacterial pyrogen (1). It would seem reasonable to assume, therefore, that circulating polymorphonuclear leucocytes constitute at least one source of endogenous pyrogen in the host. Whether granulocytes play a significant part in clearing the circulation of injected endotoxin is not known. The data thus far available suggest that their role in clearance may be subsidiary to that of the reticulo-endothelial system (20).

The results of the present study indicate that leucocytes from tolerant

donors, when stimulated by endotoxin, release the same amount of pyrogen as normal cells. This finding is in keeping with previous observations: (*a*) that tolerant animals react normally to endotoxin after reticulo-endothelial blockade by thorotrast (20), (*b*) that administration of large doses of endotoxin may cause a "break-through" of tolerance and produce maximal fever (4), and (*c*) that rabbits tolerant to influenzal virus exhibit a normal febrile reaction when given typhoid vaccine (31).

Finally, the demonstration that release of pyrogen from leucocytes incubated in fresh serum is relatively slow adds further indirect support to the concept that release of leucocytic pyrogen *in vivo* is brought about by leucocytic injury (1, 9).

SUMMARY

Study *in vitro* of the interaction of bacterial endotoxin with rabbit polymorphonuclear leucocytes has resulted in the following findings:

1. Incubation of endotoxin and leucocytes in saline results in: (*a*) the release of leucocytic pyrogen, and (*b*) the inactivation of endotoxin.

2. Cell-free extracts of leucocytes also inactivate endotoxin.

3. Incubation of leucocytes in "physiological" saline causes rapid discharge of leucocytic pyrogen. In contrast, relatively little pyrogen is released by leucocytes incubated in fresh serum.

4. The release of leucocytic pyrogen in serum is markedly stimulated by the presence of endotoxin.

5. Leucocytes obtained from tolerant rabbits interact with endotoxin in essentially the same manner as leucocytes from normal rabbits.

The pertinence of these findings to the pathogenesis of fever and to related information concerning human leucocytes has been discussed.

BIBLIOGRAPHY

1. 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.*, 1955, **102,** 499.
2. 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.
3. 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.
4. Petersdorf, R. G., Keene, W. R., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. IX. Characteristics of endogenous serum pyrogen and mechanism governing its release, *J. Exp. Med.*, 1957, **106,** 787.
5. 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.

6. Smith, M. R., and Wood, W. B., Jr., unpublished data.
7. Berthrong, M., and Cluff, L. E., Studies on the effect of bacterial endotoxins on rabbit leucocytes. I. Effect of intravenous injection of the substances with and without induction of the local Shwartzman reaction. *J. Exp. Med.*, 1953, **98,** 331.
8. Kerby, G. P., and Barrett, J. A., Jr., The effect of hydrocortisone and of Piromen *in vitro* on leukocytes of patients receiving A.C.T.H. and cortisone therapy, *J. Clin. Inv.*, 1954, **33,** 725.
9. Wood, W. B., Jr., The pathogenesis of fever, *Am. J. Med.*, 1955, **18,** 351, (editorial).
10. Bennet, I. L., Jr., and Beeson, P. B., Studies on the pathogenesis of fever. II. Characterization of fever-producing substances from polymorphonuclear leukocytes and from the fluid of sterile exudates, *J. Exp. Med.*, 1953, **98,** 493.
11. Bennett, I. L., Jr., and Cluff, L. E., Influence of nitrogen mustard upon reactions to bacterial endotoxins: Schwartzman phenomenon and fever, *Proc. Soc. Exp. Biol. and Med.*, 1952, **81,** 304.
12. Bennett, I. L., Jr., Petersdorf, R. G., and Keene, W. R., Pathogenesis of fever: evidence for direct cerebral action of bacterial endotoxins, *Tr. Assn. Am. Physn.*, 1957, **70,** 64.
13. Beeson, P. B., Temperature-elevating effect of a substance obtained from polymorphonuclear leucocytes, *J. Clin. Inv.*, 1948, **27,** 524 (abstract).
14. Bennett, I. L., Jr., Studies on the pathogenesis of fever. III. Failure to demonstrate fever-producing substances in the tissues of leukopenic rabbits, *Bull. Johns Hopkins Hosp.*, 1956, **98,** 1.
15. Gerbandy, J., Cranston, W. I., and Snell, E. S., The initial process in the action of bacterial pyrogens in man, *Clin. Sc.*, 1954, **13,** 453.
16. Cranston, W. I., Goodale, F., Jr., Snell, E. S., and Wendt, F., Role of leucocytes in the initial action of bacterial pyrogens in man, *Clin. Sc.*, 1956, **15,** 219.
17. Goodale, F., Jr., Snell, E. S., Wendt, F., and Cranston, W. I., Inactivation of a bacterial pyrogen by human serum and plasma, *Clin. Sc.*, 1956, **15,** 491.
18. Snell, E. S., Goodale, F., Jr., Wendt, F., and Cranston, W. I., Properties of human endogenous pyrogen, *Clin. Sc.*, 1957, **16,** 615.
19. Pickering, G., Regulation of body temperature in health and disease, *Lancet*, 1958, **1,** 59.
20. Beeson, P. B., Tolerance to bacterial pyrogens: Role of reticuloendothelial system, *J. Exp. Med.*, 1947, **86,** 39.
21. King, M. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. III. The leucocytic origin of endogenous pyrogen in acute inflammatory exudates, *J. Exp. Med.*, 1958, **107,** 279.
22. 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.
23. Farr, R. S., Clark, S. L., Jr., Proffit, H. E., and Campbell, C. H., Some humoral aspects of the development of tolerance to bacterial pyrogens in rabbits, *Am. J. Physiol.*, 1954, **177,** 269.
24. Collins, R. D., unpublished data.

25. Cluff, L. E., A study of the effect of serum on the immunological reaction of a bacterial endotoxin, *J. Exp. Med.*, 1956, **103,** 439.
26. Rafter, G. W., Collins, R. D., and Wood, W. B., Jr., Studies on the pathogenesis of fever. VII. Preliminary purification of leucocytic pyrogen, to be published.
27. Wood, W. B., Jr., Studies on the cause of fever, *New England J. Med.*, 1958, **258,** 1023.
28. Wood, W. B., Jr., The role of endogenous pyrogen in the genesis of fever, *Lancet*, 1958, **2,** 53.
29. Wood, W. B., Jr., The genesis of fever in infectious diseases, *in* Immunity and Virus Infection, (Victor A. Najjar, editor), New York, John Wiley and Sons, Inc., 1959, 144–162.
30. King, M. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. V. The relation of circulating endogenous pyrogen to the fever of acute bacterial infections, *J. Exp. Med.*, 1958, **107,** 305.
31. Bennett, I. L., Jr., Wagner, R. R., and LeQuire, V. S., The production of fever by influenzal viruses. II. Tolerance in rabbits to the pyrogenic effect of influenzal viruses, *J. Exp. Med.*, 1949, **90,** 355.