STUDIES ON THE PATHOGENESIS OF FEVER WITH INFLUENZAL VIRUSES

II. The Effects of Endogenous Pyrogen in Normal and Virus-Tolerant Recipients*

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(Received for publication, November 22, 1957)

The intravenous injection of viruses within the influenza group is followed by a characteristic fever in the rabbit and by the appearance of a circulating pyrogen (1). This material is clearly separable from the injected virus and appears to be of endogenous origin. Since a close correlation exists between the duration of the febrile response and the presence of the pyrogen in the circulation, the release of endogenous pyrogen has been inferred to be an essential step in the pathogenesis of viral fever.

The present paper deals with a comparison of the action of the endogenous pyrogen in normal and in virus-tolerant animals.

Methods

General.—The Hickman strain of Newcastle disease virus (NDV) was employed exclusively. Techniques used for its growth and titration have been previously described (1). All methods pertaining to the selection of rabbits, recording of temperatures, and assay of circulating pyrogen are identical with those outlined in the preceding paper (1).

Rabbits designated as "virus-tolerant" received a single injection of 4 ml. NDV on the day prior to the experiment. By this means they were rendered refractory to the pyrogenic effect of reinjected virus (2).

Antibody Titration-Hemagglutination Inhibition (HI) Test.—The level of serum antibody was measured by inhibition of viral hemagglutination (HI). Serum was diluted to 1:10 and heated at 56°C. for 30 minutes to inactivate naturally occurring inhibitors (3). Serial twofold dilutions of serum were then made in physiological saline. Virus was diluted to 16 HA units. 0.2 ml. of virus was added to each tube containing 0.2 ml. diluted serum. 0.4 ml. of a 0.5 per cent suspension of washed chicken erythrocytes was then added. The tests were read at 45 minutes at 4°C.

Hyperimmune Serum.—Immune serum was obtained from 3 rabbits. Each donor was given an initial inoculation of 10 ml. NDV intravenously. One week later, the first of a course of 4 weekly intraperitoneal injections was administered with the same dose of virus. Two weeks after the last injection, or 6 weeks after the onset of immunization, the donors were bled. The HI titers of the individual sera before pooling were: 1:1280, 1:5120, and 1:1280.

* Supported by a grant from the United States Public Health Service (E-1564).

RESULTS

Comparison of the Response of Normal and Virus-Tolerant Recipients to Endogenous Pyrogen.—Pooled sera from donor rabbits injected with 4 ml. NDV and bled 4 hours later were used as a source of endogenous pyrogen.[1] This was given to two groups of recipients: one injected with virus the preceding day, and hence designated "virus-tolerant" (see Methods); the other, a control group which had received no previous injections.

Fig. 1 shows the mean fevers induced by endogenous pyrogen in both normal and virus-tolerant recipients. It is apparent that the pyrogenic response is different in these two groups. Noteworthy is the fact that in the larger dosage

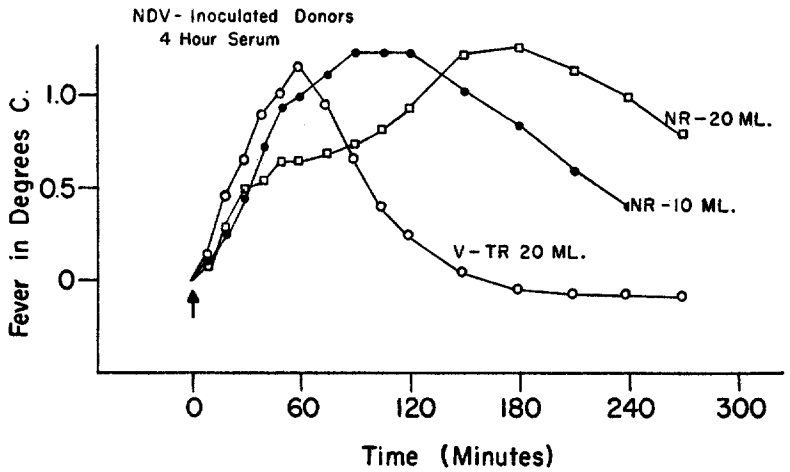

FIG. 1. Mean febrile responses of groups of 3 normal recipients to injection of 10 and 20 ml. endogenous pyrogen. Average response of 3 virus-tolerant recipients to the larger dose is shown for comparison. NR, normal recipient; V-TR, virus-tolerant recipient.

of 20 ml., endogenous pyrogen produced a distinct second peak beginning at 1½ hours, and reaching a maximum at 2½ to 3½ hours in the normal recipients, resembling the characteristic biphasic response to bacterial endotoxins or virus itself. With a dosage of only 10 ml. there was a prolonged monophasic response with the temperature still elevated 4 hours after injection. On the other hand, animals which had been given an injection of virus the preceding day responded to an injection of either dosage of endogenous pyrogen with briefer and more abrupt fever in which the temperature rapidly returned to normal by 2 to 2½ hours.

Dissociation of Fever and Tolerant Response to Endogenous Pyrogen.—It was thought advisable to determine what role fever might have in producing the modified response of the virus-tolerant recipient to endogenous pyrogen.

[1] Hereafter, as in the first paper of this series (1), the serum will be referred to simply as "endogenous pyrogen."

One group of rabbits was inoculated with 4 ml. NDV on the day preceding the experiment. To block the febrile response which is normally present, 0.9 gm. antipyrine (4.5 ml. of a solution containing 200 mg. per ml.) was given subcutaneously 1 hour before and either 1 or 2½ hours after the virus. By this means, the maximal mean elevation occurring in the virus-inoculated group was reduced to 0.35°C. A control group received the 2 injections of antipyrine at the same intervals.

The following day both groups of animals were given individual dosages of 15 ml. endogenous pyrogen. The results are shown in Fig. 2. Recipients pre-

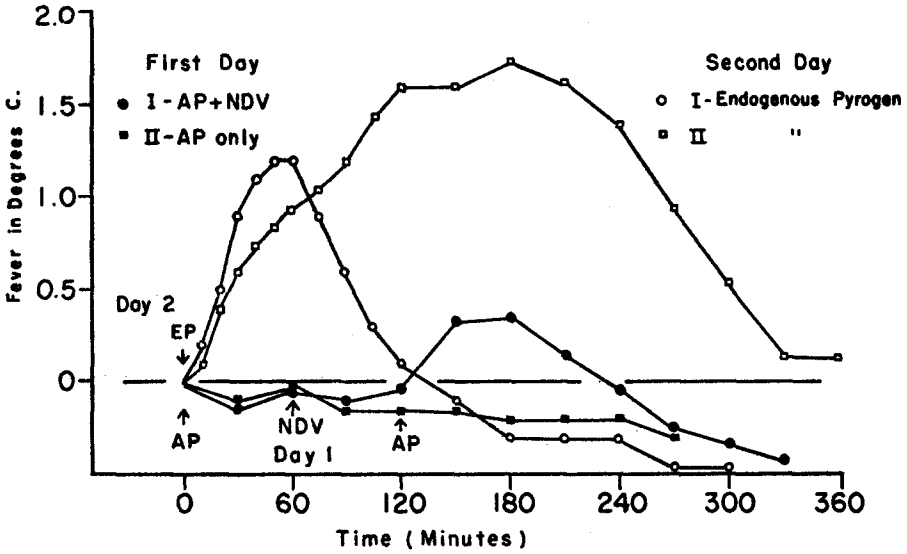

FIG. 2. Relation of fever to the development of tolerance to endogenous pyrogen. Average febrile responses of a group of 5 rabbits to 15 ml. endogenous pyrogen after suppression of virus fever on preceding day with antipyrine. Mean fever induced by endogenous pyrogen in 2 controls treated on previous day with antipyrine only is shown for comparison. AP, antipyrine; EP, endogenous pyrogen.

viously injected with virus all had monophasic fevers. The normal controls, on the other hand, given antipyrine only, had typical but somewhat exaggerated biphasic fever curves. The modified response of the virus-tolerant recipient to endogenous pyrogen, therefore, is independent of fever associated with the preceding injection of virus.

Effect of Sera on Pyrogenicity of Endogenous Pyrogen.—The second fever peak produced in the normal recipient by injection of 15 to 20 ml. of endogenous pyrogen suggested two possibilities: (*a*) The sera contained virus to which only the normal recipient would respond; (*b*) The virus-tolerant recipient had developed non-specific serum inhibitors capable of blocking the action of a second pyrogenic substance in the injected sera.

To test for the presence of virus, 15 ml. aliquots of sera containing endogenous pyrogen were incubated *in vitro* at 37°C. for 60 minutes with 5 ml. pooled immune sera (see Methods). Control studies had shown that the addition of this quantity of immune serum *in vitro* completely blocked the pyrogenic effect of 1 ml. NDV in 15 ml. normal serum. The combination of endogenous pyrogen and immune sera was then injected in individual doses of 20 ml. into 6 normal recipients.

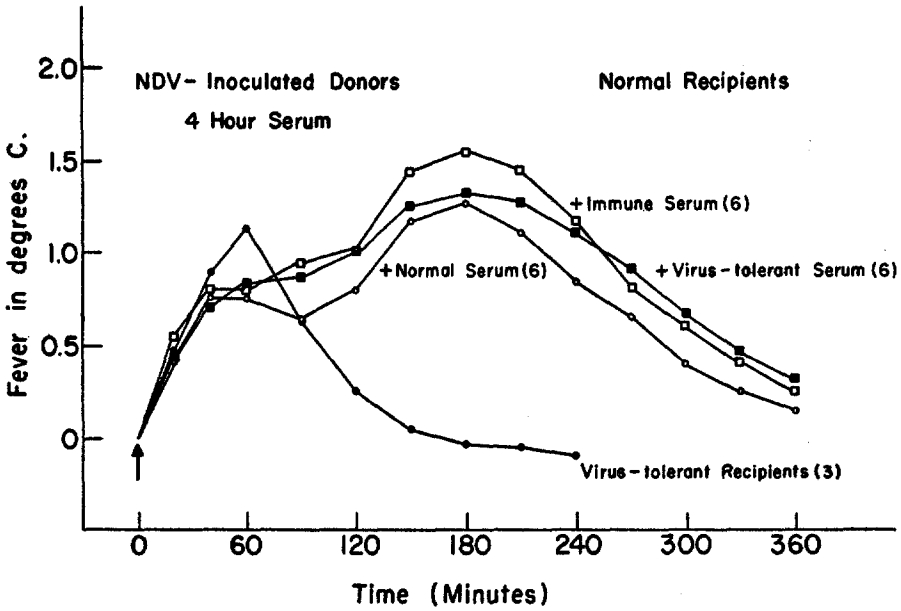

FIG. 3. Mean febrile responses of groups of normal recipients to 15 ml. endogenous pyrogen previously incubated with sera from normal, immune, or virus-tolerant animals. Average response of recipients to 20 ml. endogenous pyrogen after preceding inoculation of NDV is shown for comparison. Figures indicate number of rabbits used in each experiment.

The results are shown in Fig. 3. It is apparent that the biphasic response of the recipients was unmodified, indicating that the second fever peak was not due to virus contained in the injected sera.

A second experiment was designed to test for the presence of serum inhibitors in the animals which had been injected with virus on the preceding day. Individual doses of 15 ml. endogenous pyrogen were incubated with an additional 20 ml. of sera from animals which had been made tolerant to virus by prior injection of NDV. The mixture was then injected into each of another group of 6 normal recipients.

The addition of this serum likewise failed to modify the double peaked fever produced by endogenous pyrogen in the normal recipient (see Fig. 3). The failure of the virus-tolerant recipient to respond with a biphasic fever to the same dosage of endogenous pyrogen, therefore, appeared to be unrelated to the development of serum inhibitors.

Relation of Production of Endogenous Pyrogen to the Appearance of the Second Peak in Normal Recipients.—Since there appeared to be no evidence for the presence of a second pyrogenic substance in the sera containing endogenous pyrogen, the possibility was considered that endogenous pyrogen might itself be responsible for both the first and second peaks of fever in the normal recipi-

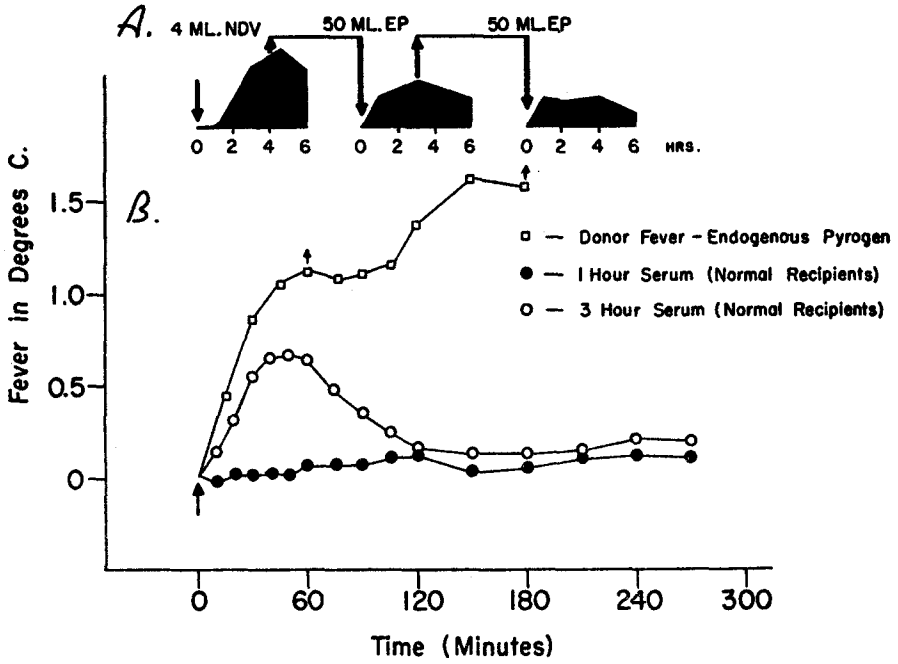

FIG. 4. Serial transfer of endogenous pyrogen. Mean fever of donor rabbits given 30 ml. endogenous pyrogen. Donors were divided into 2 groups and bled at 1 hour or 3 hours as indicated by upright arrows. The average febrile responses of normal recipients are shown to 15 ml. pooled sera from each interval. Sera from 2 donors bled at 1 hour were pooled and given to 4 recipients; the 3 hour sera were pooled from 3 donors and given to 3 recipients. Results of 2 serial transfers of pooled 50 ml. endogenous pyrogen are shown in shadowgraphs. Each curve represents an average of 3 animals.

ent. It was postulated that in addition to its direct pyrogenic effect, the injected endogenous pyrogen had stimulated the release of the recipient's own endogenous pyrogen and thereby caused the appearance of a second febrile response.

An injection of 30 ml. endogenous pyrogen was given to each of a group of normal animals. Following the injection, the animals were divided into two groups and bled at 1 and 3 hours, at the height of the first and second peaks of fever respectively. The sera from the 1 and 3 hour intervals were pooled separately and then, in turn, given to two new groups of normal recipients.

The results of this second serial transfer of serum are shown in Fig. 4. The pooled serum, obtained 1 hour after the injection of endogenous pyrogen, was

non-pyrogenic. In contrast, serum taken at 3 hours produced brief febrile responses in the normal recipients. Similar results were obtained in an earlier experiment in which smaller doses of sera were used in the two transfers.

These findings suggest that the injection of endogenous pyrogen produces two separate effects, one direct and the other indirect, in the normal recipient. (*a*) There is an initial febrile response, which is presumably due to the direct action of endogenous pyrogen on the thermoregulatory center. Since the injected material is rapidly diluted in the recipient's circulation, no endogenous pyrogen is transferable at 1 hour during the first fever peak. (*b*) Following this, a second fever peak appears as a result of the release or activation of endogenous pyrogen in the normal recipient. The endogenous pyrogen *produced by the recipient* is responsible for the presence of transferable endogenous pyrogen during the second peak of fever at 3 hours.

The virus-tolerant recipient, on the other hand, responds only to the initial direct action of endogenous pyrogen. The absence of a second peak indicates that there is no further activation of endogenous pyrogen (Fig. 1).

With larger injections of endogenous pyrogen, a serial transfer of the double peak may be demonstrated in normal recipients (see shadowgraph, Fig. 4). In this experiment, two successive transfers of 50 ml. endogenous pyrogen were performed after the injection of the first group of animals with NDV.

The serial transfer of a second fever peak appears to be evidence for a self-activating system. A large initial injection of endogenous pyrogen stimulates the production of a proportionately large amount in the recipient. The transfer of a sufficient dosage of the recipient's endogenous pyrogen in turn releases endogenous pyrogen in a new recipient.

Leukocyte Responses of Normal and Tolerant Recipients to an Injection of Endogenous Pyrogen.—The intravenous injection of bacterial pyrogen produces a biphasic fever which is associated with a prompt polymorphonuclear leukopenia and the appearance of a circulating endogenous pyrogen. It has been postulated that the endogenous pyrogen may be derived from leukocytes injured by the endotoxin (4–6). Since virus-induced endogenous pyrogen produces a biphasic febrile response, which also appears to be associated with the release of an endogenous pyrogen in the normal recipient, it seemed pertinent to determine whether these findings would be preceded by a similar decrease in circulating leukocytes. The virus-tolerant recipient, on the other hand, responds to an injection of endogenous pyrogen with a brief monophasic fever which is not accompanied by further liberation of endogenous pyrogen. It was inferred, therefore, that changes in circulating leukocytes would not be expected and that these animals would serve as controls.

Nine rabbits were given an injection of 4 ml. NDV on the day preceding the experiment and were hence designated virus-tolerant. The experimental group consisted of 9 normal animals. On the day of the experiment, preliminary leukocyte counts were made and an

average of 2 successive values exhibiting a variation of less than 2,000 cells was used as a base line. All recipients were then injected with 20 ml. endogenous pyrogen. Serial counts were made immediately following inoculation and thereafter at 30 minute intervals for 4 hours. All counts were obtained on free-flowing blood from a succession of fresh cuts made distally on the marginal ear vein opposite the one used for injection. Rabbits with preliminary counts of less than 5,000 or more than 16,000 were eliminated. Counting was done with Tren-

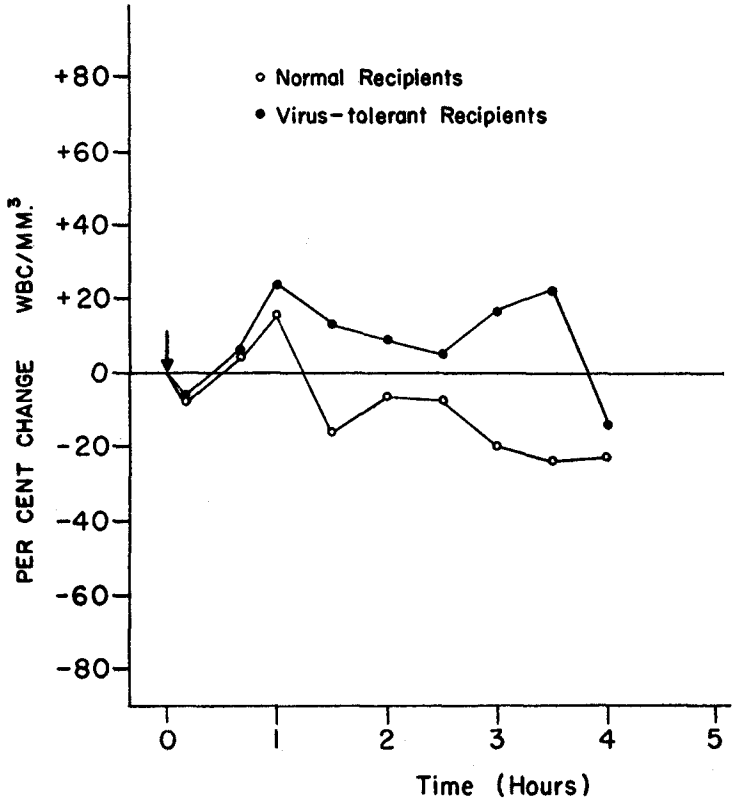

FIG. 5. Mean deviation of total leukocyte count of 9 normal and 9 virus-tolerant recipients after injection of 20 ml. endogenous pyrogen.

ner (N.B.S.) automatic pipettes and Spencer bright-line chambers utilizing the four corner squares.

The mean variations in total leukocyte counts of the two groups of animals after inoculation of endogenous pyrogen are shown in Fig. 5. The maximal changes and times of occurrence are presented in Table I.

From the graph and the accompanying table the following facts are evident.

(*a*) There was a considerable fluctuation in the individual responses within each group, both with respect to degree of leukopenia or leukocytosis and the time at which these appeared.

(*b*) The mean maximal values for both leukopenia and leukocytosis were comparable in the two groups and were of small magnitude.

(*c*) Only one-half of the recipients in each group showed a rise or a fall in white cells that exceeded 30 per cent of the preinoculation value. With the exception of a single animal in the virus-tolerant group, none of the recipients developed a significant leukopenia followed by leukocytosis as characteristically seen after the injection of bacterial endotoxins (7, 8). There does not appear to be a significant difference in the leukocytic responses of these two groups of

TABLE I

Leukocytic Responses of Normal and Virus-Tolerant Recipients to Intravenous Injection of Endogenous Pyrogen

Normal	Maximum fall		Maximum rise		Virus-tolerant	Maximum fall		Maximum rise	
	Per cent	Hrs.	Per cent	Hrs.		Per cent	Hrs.	Per cent	Hrs.
1	40	1½	0	—	1	34	1½	54	3
2	50	2½	0	—	2	2	3	54	½
3	16	1½	79	1	3	31	2	14	1
4	7	2½	156	½	4	38	1	26	½
5	28	½	24	2½	5	21	2½	10	1½
6	41	½	2	2½	6	29	½	8	2
7	46	3	19	¼	7	24	½	87	3½
8	23	2	16	3	8	28	¼	139	1
9	24	¼	68	1	9	25	¼	54	3
Mean.....	30		40		Mean....	26		49	

Preinoculation mean leukocyte counts: Normal, 10.5 ± 1.0 × 10^3. Virus-tolerant, 11.2 ± 0.9 × 10^3.

recipients to endogenous pyrogen, therefore, despite the appearance of circulating pyrogen and a second fever peak in the normal recipient.

DISCUSSION

The disparity of the pyrogenic effects of viral-induced endogenous pyrogen in normal and virus-tolerant recipients was an unexpected finding. The biphasic response of the normal recipient to an injection of 15 ml. or more of endogenous pyrogen resembled that occurring with bacterial endotoxin. It is of interest that studies of an endogenous pyrogen derived from polymorphonuclear leukocytes showed that this material produced only a monophasic fever even when large dosages were given (9). Other experiments have been reported with pyrogens of presumably endogenous origin, obtained both from the circulations of donors made febrile by the injection of typhoid vaccine (4, 5) and from the thoracic duct lymph of animals with pneumococcal peritonitis (10). In the amounts used,

these substances have also produced identical brief monophasic fevers when injected into either normal or endotoxin-tolerant recipients.[2] On the other hand, two separate series of events follow the injection of virus-induced endogenous pyrogen in the normal recipient. First, there appears to be a direct stimulation of the thermoregulatory center resulting in the initial fever peak. This phase is present in both the normal and virus-tolerant recipient. Second, in the normal recipient, there is a release of additional endogenous pyrogen which, in turn, produces the second fever peak. The monophasic response produced by injected endogenous pyrogen in the animal which has received virus on the preceding day indicates that there is no activation of endogenous pyrogen in this recipient.

The data, suggest, therefore, that a sufficient dosage of endogenous pyrogen is self-activating in the non-tolerant recipient. The failure of endogenous pyrogen obtained in other situations to produce a second peak may be due to quantitative considerations only. In support of this possibility is the observation that when the dosage of serum containing endogenous pyrogen from endotoxin-injected donors is increased to 40 ml., normal recipients respond with typical biphasic fevers (13). Similar results have also been recently obtained with the transfer of large volumes of sera from animals developing fever with two experimentally induced infections (16). Canine peritoneal exudates likewise appear capable of inducing a biphasic fever when injected in dogs (reference 17, see shadowgraphs) suggesting, in contrast to earlier findings (9), that endogenous pyrogen derived from granulocytes may be self-activating.

The demonstration that viral-induced endogenous pyrogen is a self-activating substance would seem to offer an interpretation of the biphasic fever seen with sufficient dosages of virus. It may also explain the characteristic double-peaked fever produced by a large injection of endotoxin. Although there is good evidence that endotoxins can induce fever by a direct action on the thermoregulatory center (18), it is not established whether they do so in the usual doses injected intravenously. Using a technique of intracarotid injection, King and Wood have shown that *circulating* endotoxin appears to act by a less direct mechanism than endogenous pyrogen (19).

The apparent inability of virus-induced endogenous pyrogen to produce significant changes in circulating leukocytes of either normal or tolerant recipients provides evidence for two important points regarding the nature and mode of action of this substance.

[2] Pyrexin, a pyrogenic material isolated by Menkin from sterile exudates (11) and more recently from serum (12), though producing a biphasic fever, appears to be a different substance. Recipients tolerant to endotoxin, which react like normal recipients to the endogenous pyrogen described here (13), show a markedly decreased response to pyrexin as compared with controls (9). This fact, as well as the marked heat stability of pyrexin (11) suggests a similarity, if not identity, with bacterial or tissue polysaccharides (14), not evident in the properties of endogenous pyrogen described by other investigators (15).

First, despite the considerable fever occurring with the dosage of endogenous pyrogen employed (see Fig. 1), neither group developed a consistent early leukopenia or late leukocytosis. Furthermore, the mean maximal values attained in regard to either fall or rise in circulating leukocytes are far below those usually seen with amounts of bacterial polysaccharides which produce a comparable degree of fever (see Table I). The injection of a sufficient dosage of endotoxin causes a prompt leukopenia of 50 to 70 per cent within the first 1½ hours followed usually by leukocytosis in 3 to 5 hours (14, 20, 21). This effect is markedly potentiated when endotoxin is present in serum (22). Results with the recently isolated tissue polysaccharides are generally similar and in the few cases when leukopenia was absent, a brisk leukocytosis subsequently developed as seen with small doses of endotoxin (14). These results would seem, therefore, to indicate that endogenous pyrogen is not identical with either bacterial or tissue polysaccharides.[3]

Second, the leukocytic responses of the two groups of recipients to the injected endogenous pyrogen were similar despite the occurrence of a high second peak of fever in the normal group (Fig. 1). If the development of leukopenia is an essential step in the release of endogenous pyrogen following inoculation of endotoxin, then the lack of change in circulating granulocytes after the injection of viral-induced endogenous pyrogen would make it appear unlikely that this cell is the main source of the endogenous pyrogen which is liberated and causes the second peak in normal recipients. Likewise, animals given virus show no change in the number of granulocytes during the latent period *prior* to the appearance of endogenous pyrogen (23, 24). In this regard, it is of interest that leukopenia induced by nitrogen mustard does not modify the response of recipients to virus-induced endogenous pyrogen (1), suggesting further that the release of this substance is not primarily dependent upon circulating leukocytes.

The evidence presented indicates that the altered reactivity of the virus-tolerant recipient to an injection of endogenous pyrogen cannot be ascribed either to development of serum inhibitors or to fever accompanying the injection of virus on the preceding day. In addition, since sera containing endogenous pyrogen do not have detectable amounts of virus (1), the difference in the response of the normal and virus-tolerant recipient to endogenous pyrogen is not due to tolerance to virus *per se*. It appears that the further release of endogenous pyrogen in the tolerant recipient may have been prevented by the mobilization of endogenous pyrogen on the preceding day of virus injection.

[3] Corroborative evidence for this conclusion is the fact that when rabbits are given virus, the appearance of endogenous pyrogen in their sera is not followed by a fall in circulating polymorphonuclear leukocytes (23, 24).

SUMMARY

Observations have been made on the fever-inducing properties of an endogenous pyrogen found in the circulation of rabbits after the intravenous inoculation of Newcastle disease virus (NDV).

When endogenous pyrogen was given to a normal recipient, a biphasic fever was produced which simulated that seen with bacterial endotoxins. With the use of a technique of serial passive transfer, it has been shown that the "double-humped" response results from two separate actions of the injected pyrogen. The first of these appears to be a direct stimulation of the thermoregulatory centers. The second involves the release of further endogenous pyrogen in the normal recipient to cause, in turn, the second fever peak. Since the injection of endogenous pyrogen did not produce a significant change in the number of circulating leukocytes, it is inferred that this substance is different from either bacterial or tissue polysaccharides.

In rabbits rendered tolerant by a previous injection of virus the second fever peak failed to appear and the response to endogenous pyrogen was monophasic. Evidence indicates that the absence of a second fever peak in the tolerant recipient was not due to rise in temperature on the preceding day of virus injection or to the development of either serum inhibitors or tolerance to virus itself.

It is postulated that prior mobilization of endogenous pyrogen by virus may have modified the ability of the tolerant recipient to liberate further amounts of this substance in response to an injection of endogenous pyrogen.

BIBLIOGRAPHY

1. Atkins, E., and Huang, W. C., Studies on the pathogenesis of fever with influenzal viruses. I. The appearance of an endogenous pyrogen in the blood following intravenous injection of virus, *J. Exp. Med.*, 1958, **107,** 383.
2. 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,** 335.
3. Ginsberg, H. S., and Horsfall, F. L., Jr., A labile component of normal serum which combines with various viruses. Neutralization of infectivity and inhibition of hemagglutination by the component, *J. Exp. Med.*, 1949, **90,** 475.
4. Atkins, E., and Wood, W. B., Jr., Studies on the pathogenesis of fever. I. The presence of transferable pyrogen in the blood stream following the injection of typhoid vaccine, *J. Exp. Med.*, 1955, **101,** 519.
5. 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.
6. Wood, W. B., Jr., The pathogenesis of fever, *Am. J. Med.*, 1955, **18,** 351, (editorial).

7. Bennett, I. L., Jr., and Beeson, P. B., The properties and biologic effects of bacterial pyrogens, *Medicine*, 1950, **29**, 365.
8. Thomas, L., The physiological disturbances produced by endotoxins, *Ann. Rev. Physiol.*, 1954, **16**, 467.
9. Bennett, 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.
10. Bennett, I. L., Jr., Studies on the pathogenesis of fever. V. The fever accompanying pneumococcal infection in the rabbit, *Bull. Johns Hopkins Hosp.*, 1956, **98**, 216.
11. Menkin, V., Chemical basis of fever with inflammation, *Arch. Path.*, 1945, **39**, 28.
12. Menkin, V., Pyrexin, the pyrogenic factor of inflammatory exudates, and its relation to some bacterial pyrogens, *J. Lab. and Clin. Med.*, 1955, **46**, 423.
13. Atkins, E., and Huang, W. C., Studies on the pathogenesis of fever with influenzal viruses. III. The relation of tolerance to the production of endogenous pyrogen, *J. Exp. Med.*, 1958, **107**, 415.
14. Landy, M., and Shear, M. J., Similarity of host responses elicited by polysaccharides of animal and plant origin and by bacterial endotoxins, *J. Exp. Med.*, 1957, **106**, 77.
15. Wood, W. B., Jr., Fever, *Scient. Am.*, 1957, **196**, 62.
16. 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.
17. Petersdorf, R. G., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. VII. Comparative observations on the production of fever by inflammatory exudates in rabbits and dogs, *Bull. Johns Hopkins Hosp.*, 1957, **100**, 277.
18. 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.
19. 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.
20. Atkins, E., Allison, F., Jr., Smith, M. R., and Wood, W. B., Jr., Studies on the antipyretic action of cortisone in pyrogen-induced fever, *J. Exp. Med.*, 1955, **101**, 353.
21. Bennett, I. L., Jr., and Beeson, P. B., The effect of cortisone upon reactions of rabbits to bacterial endotoxins with particular reference to acquired resistance, *Bull. Johns Hopkins Hosp.*, 1953, **93**, 290.
22. Farr, R. S., and LeQuire, V. S., Leukocytic and pyrogenic effects of typhoid vaccine and augmentation by homologous plasma, *Proc. Soc. Exp. Biol and Med.*, 1950, **75**, 661.
23. Wagner, R. R., Bennett, I. L., Jr., and LeQuire, V. S., The production of fever by influenzal viruses. I. Factors influencing the febrile response to single injections of virus, *J. Exp. Med.*, 1949, **90**, 321.
24. Harris, S., and Henle, W., Lymphocytopenia in rabbits following intravenous injection of influenzal virus, *J. Immunol.*, 1948, **59**, 9.