STUDIES ON THE PATHOGENESIS OF FEVER*

XI. Quantitative Features of the Febrile Response to Leucocytic Pyrogen

By DONALD L. BORNSTEIN,‡ M.D., CARL BREDENBERG, 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, November 28, 1962)

The only method presently available for assaying the potencies of pyrogenic molecules is based on the febrile responses of suitably trained hosts (usually rabbits) to intravenous injections of the pyrogens. A preliminary study of the dose-response curve for rabbit leucocytic pyrogen has revealed a hyperthermic "ceiling" at which there is little sensitivity to dosage (1). A similar ceiling has been noted in the response of rabbits to pyrogenic bacterial endotoxin (2, 3). The bearing of these findings on the accuracy of the assay procedure has already been emphasized (1, 3).

The present report deals with a more comprehensive investigation of the febrile response as a function of dosage in the rabbit leucocytic pyrogen system. The quantitative relationships which obtain at relatively low dosages are further defined, the effects of large doses, multiple injections, and constant infusions are described, and the phenomenon of tolerance to leucocytic pyrogen is examined. The possible bearing which these data may have upon the manner in which the thermoregulatory systems of the host respond to endogenous pyrogen is briefly discussed.

Methods

The procedures used (*a*) in avoiding contamination of reagents and glassware with extraneous pyrogens, (*b*) in preparing leucocytic pyrogen (LP), (*c*) in assaying the activity of the pyrogen preparations by intravenous injection of rabbits, and (*d*) in measuring the febrile responses of the rabbits in arbitrary fever index units were the same as those described in previous publications (1, 3–5), except for the following:

Recording of Temperature Responses.—For purposes of comparison, fever indices (areas beneath curves in cm^2) were measured by planimetry of both 2 hour (FI_{120}) and 1 hour (FI_{60}) fever curves. Maximum temperature rises in degrees centrigrade (ΔT) were also tabulated.

Continuous Infusions.—Each continuous infusion was injected through an indwelling

* These studies were supported by grants from the Olin Mathieson Foundation, the Life Insurance Medical Research Fund, and the United States Public Health Service (E-3772).

‡ Present address: Massachusetts General Hospital, Department of Medicine.

polyethylene catheter[1] in a marginal ear vein. Pyrogen was infused only after a stable baseline temperature (±0.2°C) had been established for 60 minutes during a preliminary infusion of pyrogen-free saline.

Preparation of Leucocytic Pyrogen.—Pyrogen (LP) was prepared from leucocytes derived from acute peritoneal exudates. Sterile peritonitis was induced in rabbits under light pentothal anesthesia by means of an infusion of 400 ml of 0.85 per cent NaCl containing 0.1 per cent shellfish glycogen,[2] 20,000 units of penicillin G, and 0.25 gm of streptomycin. After 12 to 13 hours, the rabbits were sacrificed with pentobarbital administered intravenously, and the sterile exudate was collected in iced flasks containing 20 ml of 5 per cent disodium EDTA[3] per rabbit. The pooled exudate was filtered through gauze, its volume was measured, and samples were removed for cultures[4] and cell counts. The average yield of leucocytes per rabbit was 1.5 to 2.5 × 10^9. More than 90 per cent of the cells were granulocytes. Following centrifugation at 900 *G* for 20 minutes in the cold (4°C), the supernatant was decanted, the firm cell button was drained free of fluid, and the cells were resuspended in pyrogen-free saline (0.85 per cent) to make a final suspension of 70 × 10^6 leucocytes per ml. The saline-cell suspension was then incubated, with gentle continuous shaking, for 4 to 24 hours in a 37°C water bath. Finally, the incubated suspension was centrifuged in the cold, and the supernatant which contained the crude leucocytic pyrogen, was decanted, dialyzed against normal salt solution, and stored at 4°C until used. The dose of leucocytic pyrogen injected is expressed in all experiments as the number of leucocytes from which the pyrogen was derived. When small doses were injected, an appropriate amount of the stock solution was diluted with normal saline to a final volume of 5 ml for injection. Doses greater than 350 × 10^6 (see Fig. 4) were given either by increasing the volume of the standard solution injected or by concentrating the stock solution by means of lyophilization.

In clearance experiments, which involved the injection of doses of pyrogen (LP) derived from 1.7 and 3.5 × 10^9 leucocytes (Table II), the stock solution was concentrated by lyophilization to a point which permitted administration of the entire dose in a volume of less than 10 ml. All of the lyophilized preparations were dialyzed against cold pyrogen-free saline (0.85 per cent) before being injected. To increase the yield of pyrogen needed for these experiments, exudate cells harvested at 16 to 18 hours were incubated twice in normal saline, the supernatant of the first incubation being pooled with that of the second. The LP thus obtained was qualitatively indistinguishable from that prepared by the original procedure. The relative potency of each lot of LP was determined by the temperature responses of 4 non-tolerant rabbits to a dose of LP falling within the sensitive range of the dose-response curve (Figs. 1 and 2), namely that derived from 17.5 × 10^6 leucocytes.

Tolerance to endotoxin was produced by a week of daily injections (1.0 ml) of typhoid vaccine (6).[5] The test dose of the vaccine used in the tolerance experiments was 0.05 ml.

RESULTS

Responses of Rabbits to Small and Moderate Single Doses of Leucocytic Pyrogen.—

A group of 12 trained recipient rabbits was injected on different days with varying doses of LP. The doses injected were derived from leucocytes ranging in number from

[1] Deseret intercath, C. R. Bard, Inc., Murray Hill, New Jersey.

[2] Mann Research Labs, Inc., New York.

[3] Ethylenediaminetetraacetate, adjusted to pH 7.0.

[4] 5 ml aliquots of each pool of exudate were cultured in thioglycollate broth at 37°C for 1 week.

[5] Obtained through the courtesy of Col. A. S. Benenson, Walter Reed Army Institute for Research, Washington, D.C.

5×10^6 to 350×10^6.[6] Except as indicated by the section mark in Table I, two to four experiments were performed at each dose level. The average maximum temperature rise produced in each rabbit by each dose is recorded in the table.[7] The mean responses of the group to the various doses are plotted in Figs. 1 and 2. The values plotted on the ordinate of Fig. 1 are the maximum elevations of temperature (ΔT);

TABLE I

Maximal Rises in Temperature Observed in 12 Rabbits Following Varying Doses of Leucocytic Pyrogen

Rabbit	Dose of LP*						
	5×10^6	10×10^6	20×10^6	40×10^6	100×10^6	200×10^6	350×10^6
	ΔT (°C)‡	ΔT (°C)	ΔT (°C)	ΔT(°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)
1	0.4	0.85	0.95	1.28	1.47	1.35	1.35§
2	0.32	1.1	1.28	1.6	1.6	1.65	1.45§
3	0.24	0.65	0.97	1.08	1.23	1.43	1.55§
4	0.22	0.57	0.99	1.08	1.23	1.40§	1.4§
5	0.32	0.65	0.95	1.08	1.18	1.25§	1.1§
6	0.87	0.95	1.39	1.48	1.43	1.85§	1.35§
7	0.70	1.05	1.16	1.4	1.53	1.6§	1.5§
8	0.20	0.53	0.9	1.23	1.28	1.53	1.4§
9	0.15	0.68	0.75	1.0	1.08	1.23	1.15§
10	0.22	0.45	0.68	0.90	1.0	1.1§	0.95§
11	0.45	0.63	0.82	1.15	1.48	1.15§	1.2§
12	0.30	0.60	1.0	1.03	1.4	1.55	1.55§
Mean response of group‖....	0.37	0.73	0.99	1.19	1.33	1.40	1.33
SE of mean response.....	0.063	0.061	0.06	0.061	0.054	0.065	0.055

* Expressed in number of leucocytes from which dose of pyrogen was derived (see Methods).

‡ Each figure represents the average of 2 to 4 experiments made with each rabbit at each dose, except those figures marked with a section mark.

§ Figures are based on single experiments.

‖ Average of individual means, except where only single experiments were performed (see section mark).

those on the ordinates of Fig. 2 are the fever indices calculated from the 1 hour (FI_{60}) and 2 hour (FI_{120}) fever curves respectively.

The data recorded in Table I reveal that there was considerable variation in the *absolute* responses of the individual rabbits to a given dose of pyrogen.

[6] All of the leucocytes were derived from a *single* pool of peritoneal exudate cells.

[7] A characteristic fever curve for rabbits receiving the 350×10^6 dose is shown in Fig. 4 (lowest curve).

Despite these differences, the *relative* responses of each rabbit to the graded doses of pyrogen were fairly uniform.

Because of these differences in absolute sensitivity, a group of the same recipients should be used whenever possible in comparing the potencies of two or more preparations of pyrogen. Furthermore, if reproducible results are to be obtained, properly trained and selected recipients must be employed. An occasional rabbit, as shown in Fig. 3, will be found to be unacceptable as a

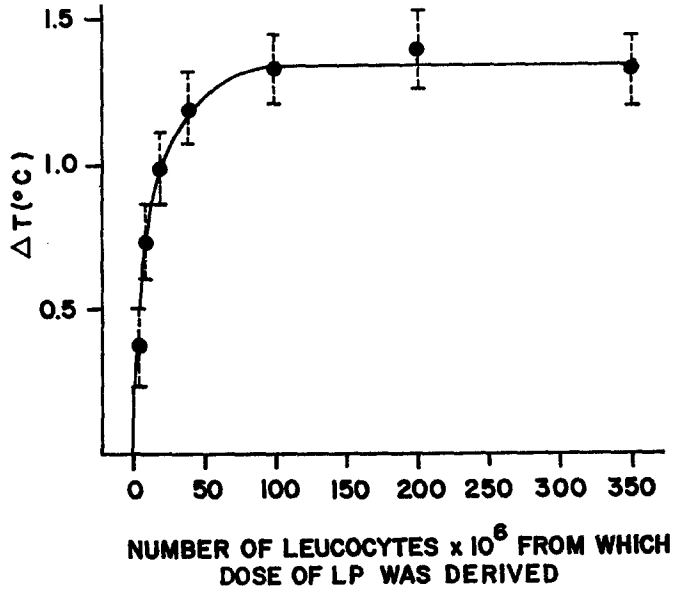

FIG. 1. The mean temperature rises observed in 12 rabbits given varying intravenous doses of LP. The average maximum fevers (see Table I) are plotted (ordinate) against the amount of pyrogen injected (abscissa). The dosages of the latter are expressed as the number of leucocytes from which the pyrogen was derived. The 95 per cent confidence limits (2.2 × SE) of the points on the curve are indicated by the vertical bars.

recipient because of its failure to respond in a reproducible fashion to repeated injections of the same dose of pyrogen. Such a rabbit should not be used as a recipient.

From the data summarized in Figs. 1 and 2 it is evident that essentially the same relationship of dosage to response is demonstrable, no matter whether the febrile reactions are recorded in terms of maximum elevations of temperature (ΔT) or as fever indices calculated from either 1 hour (FI_{60}) or 2 hour (FI_{120}) fever curves. In addition, the over-all dose-response relationship is characterized by: (*a*) a relatively steep portion of the curve where each increment of dosage results in a pronounced increase in response and (*b*) a hyperthermic ceiling at

which the response is insensitive to dosage. Only in the former dosage range is the assay method reliable.

Finally, as has already been emphasized in a previous report (1), the exact dose-response curve for a given lot of leucocytic pyrogen may not necessarily

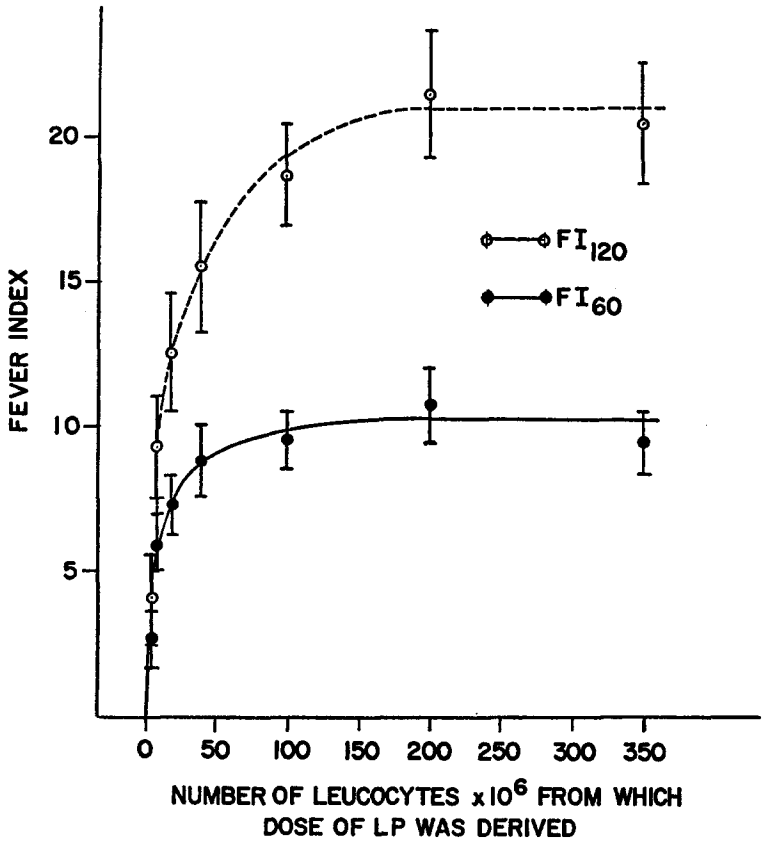

FIG. 2. The average fever indices, calculated from both the 60 and the 120 minute fever curves of the same 12 rabbits referred to in Table I and Fig. 1, are plotted (ordinate) against the dose of pyrogen (abscissa). The 95 per cent confidence limit (2.2 × SE) for each point is indicated as in Fig. 1.

apply to all similar preparations of LP, since the relative potencies of different lots may vary depending upon the origin of the cells and the manner in which they have been handled (1). If, however, the febrile response produced by each new lot of pyrogen is measured in multiple recipients at a single dose level in the sensitive range (*e.g.* 20×10^6), the potency of the new lot may be compared with that of the standard, and the appropriate correction factor may then be applied to the rest of the curve. The reliability of this procedure has been

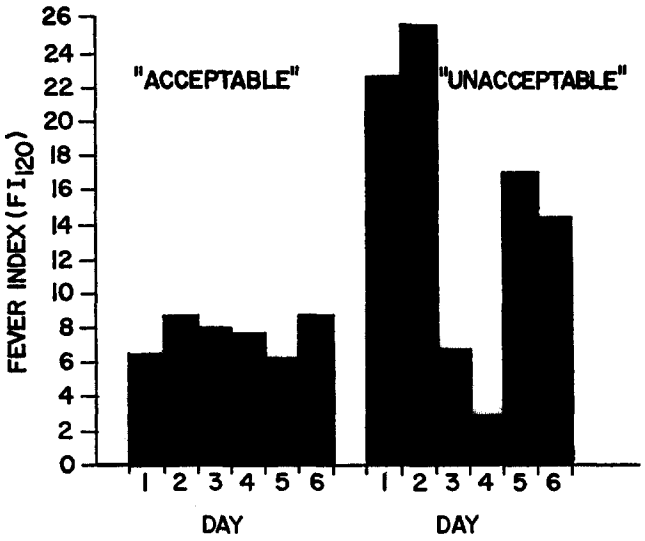

FIG. 3. Comparison of daily febrile responses of an "acceptable" and an "unacceptable" rabbit to injections of a constant dose of LP. The daily fever indices recorded are those measured from 2-hour fever curves (FI_{120}).

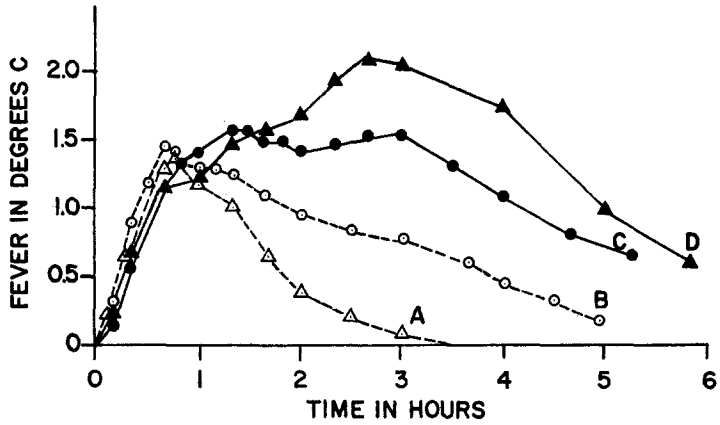

FIG. 4. Febrile responses produced by large doses of LP. Curves *A*, *B*, and *C* indicate the mean responses of 3 rabbits to doses derived from 3.50×10^8, 7.00×10^8, and 1.05×10^9 leucocytes respectively. Curve *D* depicts the mean response of 3 other rabbits to a dose obtained from 1.40×10^9 WBC.

checked on a number of complete titration curves obtained with different lots of leucocytic pyrogen.[8]

[8] Compare, for example, the points on the FI_{120} curve in Fig. 2 with those on the analogous curve of Fig. 1 in reference 1.

Responses to Single Large Doses of LP.—

When the dose of leucocytic pyrogen is increased to levels above 350×10^6, the maximum elevation of temperature in the 1st hour remains essentially unchanged, but the shape of the response curve is markedly altered (see Fig. 4). Whereas a dose of 700×10^6 merely causes a delayed defervescence, doses derived from a billion or more cells give rise to biphasic responses similar to those caused by large doses of endotoxin (7) (see Figs. 4 and 5). To make certain that such "double humped" response curves were not caused by contaminating endotoxin, doses derived from a billion leucocytes were injected into 4 recipients

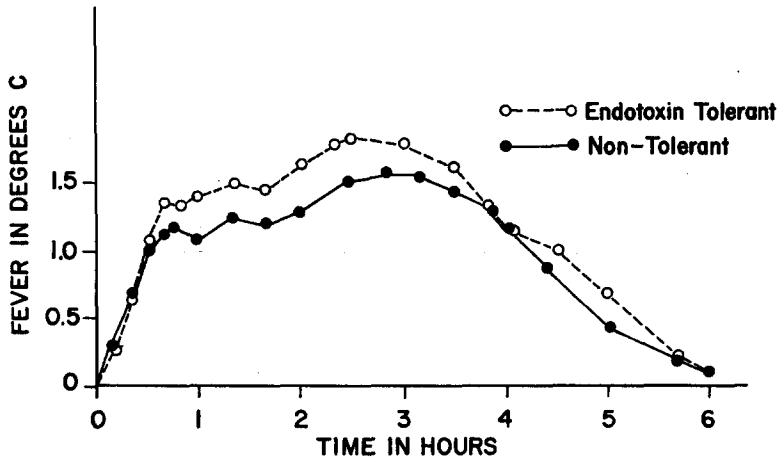

FIG. 5. Failure of tolerance to endotoxin to affect biphasic response to large dose of leucocytic pyrogen. Four rabbits, made tolerant to endotoxin by daily injections of typhoid vaccine, were each given a single dose of LP derived from 1.05×10^9 leucocytes. The mean fever response of the 4 rabbits (each of which had been shown the day before to be unresponsive to 0.05 ml of typhoid vaccine) is indicated by the broken curve. The mean febrile reaction to the same dose of LP, after the tolerance had subsided (4 weeks later), is shown by the solid curve.

previously made tolerant to a dose of 0.05 ml of typhoid vaccine. As shown in Fig. 5, the mean response of the 4 endotoxin-tolerant recipients was essentially identical with that exhibited by the same recipients 4 weeks later when the tolerance had lapsed. In both instances the mean fever curves were biphasic. It is thus clear that rabbits will respond with biphasic fevers to large doses of leucocytic pyrogen just as they do to large doses of endotoxin or of serum endogenous pyrogen (8).

Responses to Intermittent Injections of LP.—

If a small pyrogenic dose of LP, namely that derived from 40×10^6 leucocytes, is injected[9] every 15 minutes, the fever induced may be biphasic

[9] Venipuncture, under local anaesthesia, performed for each injection.

(Fig. 6 - upper curve) and may resemble that caused by a single large dose of LP (see Figs. 4 and 5). Once the rabbit's temperature has risen more than 1.5°C, its response to further injections becomes highly irregular. In fact there is apparently no response at all to some of the later injections. When the intervals

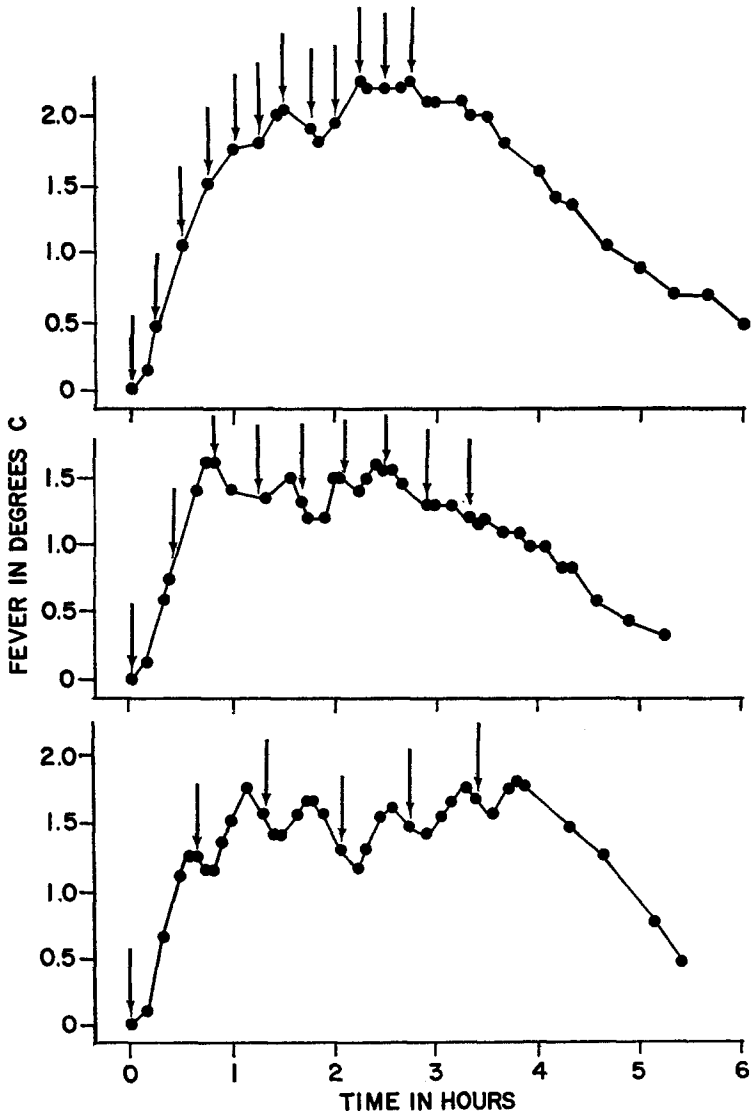

FIG. 6. Response to repeated injections (see arrows) of a constant dose (4.0×10^7 WBC) of LP administered at intervals of 15 (upper curve), 25 (middle curve), and 40 minutes (lower curve). (Note that third injection recorded in lower chart was given 5 minutes late.)

between injections are lengthened to 20 minutes and to 40 minutes (Fig. 6 - middle and lower curves), similar altered responses are noted at the elevated temperature levels. The general configuration of fever curves thus produced indicates that the thermoregulatory response to a given dose of pyrogen is definitely modified once the fever has become established.

Response to Constant Infusions of LP.—

Fig. 7 depicts the responses of 2 rabbits which received constant infusions of the same lot of LP. The 1st rabbit (upper curve) received per minute a dose

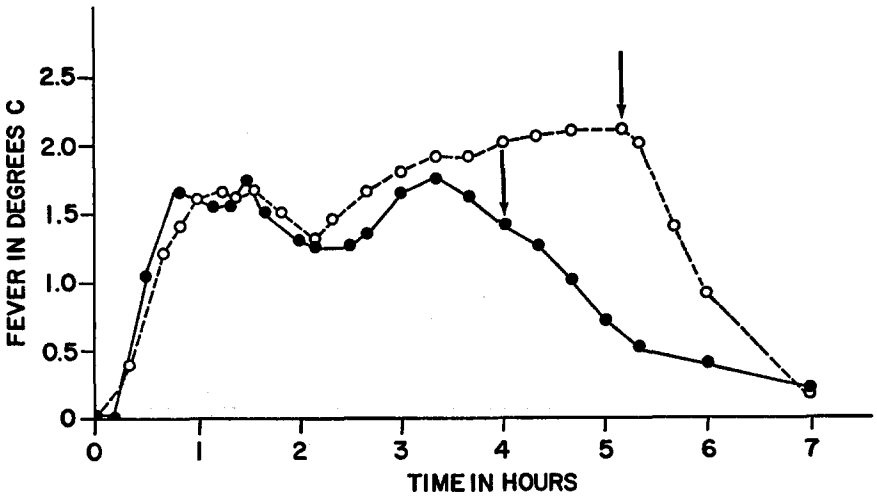

FIG. 7. Two typical biphasic responses to continuous intravenous infusions of LP. The broken curve depicts the reaction of a rabbit which received, during each minute, the amount of LP derived from 1.0×10^7 leucocytes. The solid curve indicates the response of a rabbit receiving a slightly smaller per minute dose (8.0×10^6 WBC). The arrows indicate the times at which the continuous infusions were terminated.

derived from 1×10^7 cells; the 2nd rabbit (lower curve) received that derived from 8×10^6 cells. It will be noted that both rabbits developed biphasic fevers despite the fact that the pyrogen was being injected continuously at a constant rate. Five other rabbits were similarly treated at approximately the same dosage level; 4 exhibited biphasic responses, the 5th developed a prolonged monophasic fever. The occurrence of the biphasic responses suggests one of three possibilities: (*a*) that the reactivity of the thermoregulatory mechanisms to the constant infusion of LP becomes temporarily depressed when the fever first reaches a magnitude of approximately 1.5°C (as in the intermittent injection experiments), or (*b*) that a second wave of endogenous pyrogen is released (presumably from the leucocytes of the host) once the injected LP has attained a sufficient concentration in the blood, or (*c*) that both phenomena are involved.

Evidence that the second peak of the biphasic response to serum endogenous pyrogen (EP) may be due to a second wave of circulating pyrogen, generated within the host as a result of the stimulus received from the first wave of injected pyrogen, has been reported by Atkins *et al.* (8–11).

Tolerance to LP.—

When large single doses of LP (derived from a billion or more cells) are injected on several successive days, a pronounced change in the fever response, characterized by disappearance of the "second hump" (see Fig. 8), becomes apparent on the 2nd day. This altered response persists over the next 24 to 48

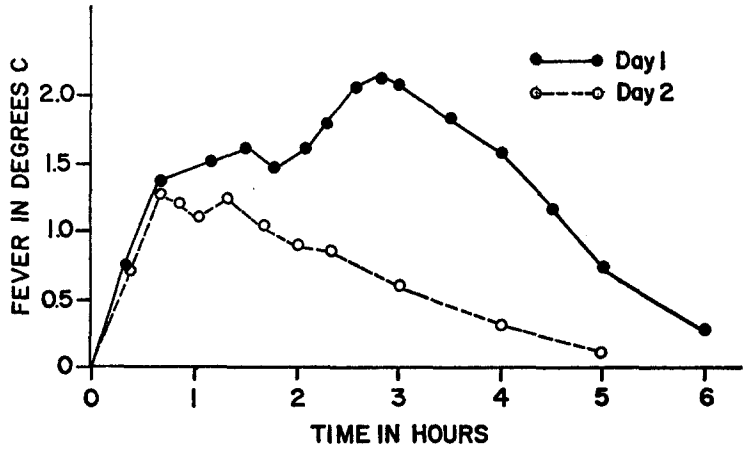

FIG. 8. Tolerance to large dose of LP. Solid curve shows biphasic response of rabbit to initial single injection of LP obtained from 1.4×10^9 leucocytes. Broken curve depicts monophasic response to the same dose given the next day.

hours. After an interval of 2 days or more of rest, the same dose of LP again produces a biphasic response indistinguishable from that caused by the initial injection.

This type of transient refractoriness to large doses of LP is similar to that which follows the production of biphasic fever with substantial doses of other pyrogens, including EP (8). Having concluded that the second peak of the biphasic response is due to the mobilization of additional EP from the tissues of the host, Atkins (8–11) has postulated that the transient tolerance is caused by the failure of this mobilization to occur after the maximal response to the first injection.

Mechanism of Transient Tolerance.—

Because of the depression of thermoregulatory responsiveness noted when the temperature was elevated in both the intermittent injection (Fig. 6) and

continuous infusion experiments (Fig. 7), the possibility was entertained that a similar depression might result from the massive dose of LP needed to induce transient tolerance. Accordingly, experiments were performed to test the comparative reactivities of the tolerant and non-tolerant rabbits to a standard small dose of LP. The dose selected for these tests was known to be in the sensitive range of the dose-response curve for normal rabbits, namely that derived from 20×10^6 leucocytes (see Figs. 1 and 2). When the responses of 12 recipients were compared before and during tolerance, the tolerant response was lower than the control response in every animal (Fig. 9). Statistical analysis of the data for the group as a whole revealed the difference to be significant ($p < 0.001$).[10]

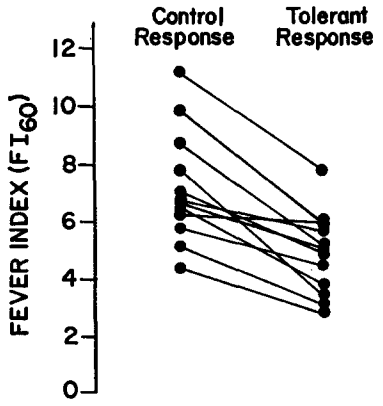

FIG. 9. Depressed responsiveness of LP tolerant rabbits to small dose of LP. Left hand dots show initial responses (FI_{60}) of 12 rabbits to a dose of LP derived from 20×10^6 leucocytes. Right-hand dots indicate responses to same dose after the rabbits had been made tolerant to LP (see Fig. 8). The solid lines connect the two responses of each rabbit.

In order to test the hypothesis advanced by Atkins (*vide supra*) concerning the cause of transient tolerance, it was necessary, first, to determine whether a second wave of circulating pyrogen could be demonstrated following the injection of a single large dose of LP, as observed in the EP system (8–11). Accordingly, the amounts of pyrogen in the circulations of non-tolerant rabbits were measured by passive transfer tests (5) at various intervals following a large single dose of LP. Particular attention was paid to the amount present at 2 hours when the second rise in the fever curve usually begins (see Fig. 8).[11]

[10] Where test doses in the insensitive range of the dose-response curve (Figs. 1 and 2) were used, no change in responsiveness could be demonstrated unless, of course, the challenge dose was large enough to produce a biphasic fever in normal rabbits, in which case the resulting response in the tolerant animals was monophasic, as shown in Fig. 8.

[11] Although in these experiments the dose of LP was slightly larger than in the experiment summarized in Fig. 8, the configurations of the fever curves were essentially the same.

As shown in Table II the LP originally injected was cleared with great rapidity from the blood stream. Nevertheless the temperatures of non-tolerant rabbits injected with this amount of LP continue to rise (see Fig. 8), despite the absence of detectable pyrogen in the circulation at 30 and 60 minutes after the inocula-

TABLE II

Amounts of Transferable Pyrogen in Sera of Rabbits Following Single Large Doses of Leucocytic Pyrogen (LP)*

State of rabbit	Dose of LP	Volume of serum transferred	Amounts of transferable pyrogen						
			Time following intravenous injection, min.						
			5	10	15	30	60	90	120
		ml							
Non-tolerant	LP from 1.7 × 10^9 WBC	10	5.4	4.5‡	3.7	0	0		0
			4.8	5.4‡	0.8‡	0	0		0
			4.3	4.8	0.6‡	0	0		5.3‡
			7.6		2.9				5.7‡
			4.3						0
									0
		25				0			
						0			
		30					0		
Non-tolerant	LP from 3.5 × 10^9 WBC	20					0		5.3
							0		5.9
							0		6.0
							0		5.9
Tolerant	LP from 3.5 × 10^9 WBC	20							0
									0
									0

* Expressed in 60-minute fever indices (FI_{60}) measured from febrile responses of non-tolerant rabbits receiving passively transferred serum intravenously (see Methods). Zero (0) indicates no significant fever ($\Delta T < 0.3°C$).

‡ FI_{60} averaged from fevers measure in two recipients.

tions. At 2 hours, however, when the secondary rise in temperature begins, transferable pyrogen was again demonstrable in two out of six experiments. Because of this suggestive finding, a second set of experiments was performed in which the dose of injected LP and the volume of serum transferred were both doubled. Under these conditions (see Table II), all of the 2 hour samples of serum contained appreciable quantities of transferable pyrogen, whereas no pyrogen was detectable in the 1 hour samples.

In accordance with Atkins' postulate, the second wave of transferable pyro-

gen was not demonstrable at 2 hours (Table II) during the monophasic febrile response of the tolerant rabbits.

From the results of these two types of experiments it is evident: first, that the tolerant rabbits are less reactive to small doses of LP, and secondly, that the tolerant animals, when given a large dose of LP, mobilize less EP from their own tissues than do non-tolerant rabbits. The precise mechanisms which account for these apparently distinct forms of unresponsiveness have not been defined.

DISCUSSION

The present study of the quantitative dose-response relationships which obtain when rabbits are injected intravenously with *small* or *moderate* single doses of leucocytic pyrogen has revealed two important points pertaining to the reliability of current methods of pyrogen assay.

1. The absolute sensitivities of individual rabbits to the same dose of a single lot of pyrogen may vary over a considerable range, even when the animals are properly trained and care is taken to exclude inconsistent recipients. The relative responses of each rabbit, however, to changes in dosage of the same pyrogen are fairly uniform (Table I). Therefore, to minimize the errors introduced by the variations in absolute responsiveness, comparative assays should be performed with the same group of recipients.

2. The dose-response relationship to LP is characterized by a hyperthermic ceiling at which the intensity of the febrile reaction is insensitive to dosage (Figs. 1 and 2). Therefore, quantitative measurements made in the dosage range of the ceiling may be grossly misleading. Apparent misinterpretations of data resulting from this error have been discussed elsewhere (3).

The biphasic febrile reaction which characteristically results from a single injection of bacterial endotoxin was originally considered to be a hall-mark of endotoxin fever (12). More recently, serum endogenous pyrogens, obtained from animals with a variety of experimental fevers, have been shown to produce similar biphasic responses when given in sufficiently large doses (9–11, 13). It is not surprising, therefore, that *large* single doses of LP likewise cause biphasic fevers (Figs. 4 and 5).

Concerning the mechanism of the secondary rise in temperature in biphasic fevers there has been much controversy (8). Petersdorf, Bennett, and Keene (14, 15) have suggested that in endotoxin fever the first peak is due to the direct action of the endotoxin on the central nervous system and that the second peak is caused by the mobilization of endogenous pyrogen from the tissues. Manifestly, this hypothesis cannot be applied to the biphasic fevers caused by the injection of endogenous pyrogens which contain no demonstrable endotoxin (9–11, 13, 16). As already mentioned, Atkins and Huang (9) have reported evidence that the presence of a sufficiently large amount of EP in the circula-

tion may cause the release of additional EP from the tissues (probably leucocytes) of the recipient. Accordingly, it has been suggested (8, 9, 11) that in biphasic fevers, whether caused by large intravenous doses of endotoxins, viruses, or EP, the secondary rise in temperature is due to a second wave of EP mobilized in response to the first wave which causes the primary fever.[12] The results of the present studies indicate that the same mechanism of EP mobilization may contribute to the biphasic response which results from the injection of large doses of LP.

It has also been shown, however, in the present experiments that responsiveness to the injected pyrogen may be depressed when the fever is relatively high (Fig. 6). Therefore, it is conceivable that the biphasic fevers produced by constant infusions of LP (Fig. 7) may be due: (*a*) to a change in the responsiveness of the thermoregulatory mechanisms as the fever progresses, (*b*) to the eventual generation from the tissues of a second wave of EP, or (*c*) to a combination of both of these factors.

The complexities of pyrogen tolerance have been comprehensively reviewed by Atkins (8, 11), and their relationship to the present experiments have already been discussed. That rabbits do not become tolerant to repeated small doses of either LP or EP (14), as they do to endotoxin (18), has been clearly established. Because of this fact it is possible to use the same trained recipients from day to day in assaying the potencies of endogenous or leucocytic pyrogens, provided they are given in small or moderate doses. When large enough doses of either are injected to cause a biphasic response, an immediate and transient refractoriness develops characterized by: (*a*) a disappearance of the second peak in the biphasic fever curve (8, 11) and (*b*) a diminished responsiveness to the intravenous injection of small doses of EP (19) or LP in the sensitive range of the dose-response curve. Clearly, the occurrence of this kind of tolerance makes it inadvisable, in quantitative assay procedures, to use recipients which have recently received doses of pyrogen large enough to cause biphasic fevers.

SUMMARY

Although the *absolute* febrile responses of trained individual rabbits injected intravenously with small to moderate doses of leucocytic pyrogen vary over an appreciable range, the *relative* responses of each rabbit to changes in dosage are satisfactorily reproducible.

The quantitative dose-response relationship is characterized by a hyperthermic ceiling at which the intensity of the febrile reaction is relatively constant over a wide dosage range. Only at lower dose levels, where the dose-re-

[12] In the endotoxin and virus models the first wave of EP results from the action of the injected exogenous pyrogens on the tissues (presumably leucocytes) of the host (3, 5, 7, 8, 17); in the EP model it is obviously due to the injected EP (9).

sponse curve is reasonably steep, is the magnitude of the fever produced proportional to the amount of pyrogen injected.

When sufficiently large doses of LP are injected, the hyperthermic ceiling is exceeded. The fevers thus induced are biphasic in character and, in this way, resemble the usual response to bacterial endotoxin.

Similar biphasic fevers result from continuous infusions of relatively low concentrations of LP at a constant rate.

Repeated intermittent injections of moderate doses of LP likewise cause prolonged biphasic fevers, but, once the fever has become established, the reaction to each individual injection becomes markedly depressed.

When large doses of LP are injected at daily intervals, the characteristic biphasic response occurs only following the first injection. Thereafter a state of tolerance intervenes in which the late secondary rise in temperature fails to occur. This form of tolerance lasts as long as the daily injections are continued but subsides within a few days after the injections are stopped.

During the transient tolerance the rabbit's responsiveness to small doses of LP (in the sensitive range of the dose response curve) is depressed. In addition, the amount of endogenous pyrogen mobilized from the tissues by a large dose of LP is not as great as that generated in a normal rabbit.

The relations of these findings to biphasic fevers, tolerance, and the accuracy of the conventional method of pyrogen assay are briefly discussed.

The authors gratefully acknowledge the able technical assistance of Ruth Gay Robbins.

BIBLIOGRAPHY

1. Kaiser, H. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. IX. The production of endogenous pyrogen by polymorphonuclear leucocytes, *J. Exp. Med.*, 1962, **115**, 27.
2. Keene, W. R., Silberman, H. R., and Landy, M., Observations on the pyrogenic response and its application to the bioassay of endotoxin, *J. Clin. Inv.*, 1961, **40**, 295.
3. Gillman, S. M., Bornstein, D. L., and Wood, W. B., Jr., Studies on the pathogenesis of fever. VIII. Further observations on the role of endogenous pyrogen in endotoxin fever, *J. Exp. Med.*, 1961, **114**, 729.
4. 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.
5. 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.
6. 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.
7. Wood, W. B., Jr., Studies on the cause of fever, *New England J. Med.*, 1958, **258**, 1023.

8. Atkins, E., Pathogenesis of fever, *Physiol. Rev.*, 1960, **40,** 580.
9. Atkins, E., and Huang, W. C., Studies on the pathogenesis of fever with influenzal viruses. II. Effects of endogenous pyrogen in normal and virus-tolerant recipients, *J. Exp. Med.*, 1958, **107,** 403.
10. 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.
11. Moses, J. M., and Atkins, E., Studies on tuberculin fever. II. Observations on the role of endogenous pyrogen in tolerance, *J. Exp. Med.*, 1961, **114,** 939.
12. Bennett, I. L., Jr., and Beeson, P. B., The properties and biologic effects of bacterial pyrogens, *Medicine*, 1950, **29,** 365.
13. 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.
14. Petersdorf, R. G., Keene, W. R., and Bennett, I. L., Jr., Studies on the pathogenesis of fever. IX. Characteristics of endogenous serum pyrogen and mechanisms governing its release, *J. Exp. Med.*, 1957, **106,** 787.
15. Petersdorf, R. G., and Bennett, I. L., Jr., Experimental approach to the mechanism of fever, *Arch. Int. Med.*, 1959, **103,** 991.
16. Bennett, I. L., and Beeson, P. B., Studies on the pathogenesis of fever. II. Characterization of fever-producing substances from polymorphonuclear leucocytes and from fluid of sterile exudates, *J. Exp. Med.*, 1953, **98,** 493.
17. Atkins, E., and Huang, W. C., Studies on the pathogenesis of fever with influenzal viruses. I. Appearances of endogenous pyrogen in blood following intravenous injection of virus, *J. Exp. Med.*, 1958, **107,** 383.
18. Beeson, P. B., Tolerance to bacterial pyrogens. I. Factors influencing its development, *J. Exp. Med.*, 1947, **86,** 29.
19. Atkins, E., personal communication.