STUDIES ON TUBERCULIN FEVER*

I. The Mechanism of Fever in Tuberculin Hypersensitivity

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Despite extensive recent investigation, the pathogenesis of fever is incompletely understood. It has been postulated that many injected pyrogenic agents cause fever indirectly by liberating a product of tissue injury which, in turn, acts directly upon the hypothalamic thermoregulatory center (1).

In rabbits, a fever-promoting material has been obtained from both sterile exudates and saline extracts of circulating granulocytes (2). Furthermore, the presence of a serum pyrogen, indistinguishable in its biologic properties from granulocytic pyrogen, has been demonstrated during fevers induced by a number of agents including endotoxins, viruses and bacterial infections (3–6). These findings have suggested that the release of an endogenous pyrogen from damaged cells, including granulocytes, is an essential step in the pathogenesis of a variety of experimental fevers.

The fever elicited by old tuberculin (O.T.) in animals infected with B.C.G. (7) provides an appropriate system for the study of this hypothesis, for there is considerable evidence indicating that O.T. selectively damages tuberculin-sensitive cells, including granulocytes (8–16). Furthermore, passive transfer of tuberculin hypersensitivity has only been unequivocally accomplished with white cells or their products (17–19). Since the leukocyte thus appears to be involved in both the pathogenesis of certain fevers and tuberculin hypersensitivity, it seems possible that this cell may play a role in the fevers induced by injection of O.T. in sensitive animals. Moreover, the hypersensitive system is unique in that it involves a specific change in the state of the host which is believed to be directly responsible for the toxic effects of injected antigen. Consequently, the demonstration of a circulating endogenous pyrogen in such an experimental model would seem to provide additional evidence that this substance is derived from host tissues and is not simply a modification of the injected agent as has been suggested in endotoxin-induced fever (20, 21). Evidence has recently been presented, however, that many of the toxic effects of tuberculin in sensitive animals may be due to the hyperreactivity of such animals to trace amounts of endotoxin contained in O.T. (22).

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In the present paper an effort is made to determine whether the febrile response to O.T. is a specific property of the hypersensitivity system or due to the presence of contaminating endotoxins.

Materials and Methods

General.—Male and female rabbits of several breeds, weighing 3 to 5 kg., were used in all experiments. They were housed in an air-conditioned room. Experiments were conducted in an adjacent room maintained at 65° to 70°F.

All glassware and needles were sterilized by dry heat at 170°C. for 2 hours to inactivate any contaminating pyrogens (23). Physiologic saline solution was made with distilled water and tested at intervals for pyrogenicity.

Temperature Recording.—Rabbits were placed in wooden boxes with openings for the head and rectum. To insure a consistent response, recipients used for the first time were kept in these boxes for 5 to 6 hour periods on the 2 days immediately preceding each experiment. Animals previously used as recipients were boxed 1 day prior to each experiment.

On the day of an experiment the animals were left in boxes for 2 hours before injection. During the 2nd hour, temperature readings were taken at 15 minute intervals to establish a baseline. After injection, temperatures were recorded every 15 minutes for the 1st hour and at 30 minute intervals thereafter for a total of 5 hours. In some instances when no significant response was elicited, readings were taken for only 4 hours.

Rectal temperatures were measured with the Foxboro rabbit scanning switch and fever recorder. Animals showing a variation of over 0.3°C. in the hour preceding injection or an initial temperature higher than 40°C. were not used.

Sensitization of Animals.—The Phipps strain of B.C.G. was used exclusively.[1] Cultures were maintained by alternate passage on Lowenstein slants and in Dubos media. For inoculation, 2-week-old colonies were scraped from slants, ground in a mortar and pestle, and suspended in sterile saline to give a concentration of 5 mg. per ml. One-half ml. of the suspension was injected intradermally into each flank of the recipient rabbit. Experiments were performed 3 weeks after inoculation.

Old Tuberculin.—Samples of old tuberculin (O.T.) were obtained from various sources. Except where otherwise indicated, however, O.T. from the Massachusetts Department of Public Health (Lot No. 50C) was used exclusively.[2] This material contained 2000 mg. of O.T. per ml., and was diluted 1:100 with pyrogen-free saline before each experiment. All sensitive recipients received an intravenous injection of 100 mg. (in 5 ml. saline), an amount which produced no observable toxic effects other than fever.

Skin Tests.—Tuberculin tests were performed intradermally by injecting 10 mg. of O.T. (0.5 ml. of a 1:100 dilution) in an abdominal site. After 24 hours, about 80 per cent of sensitized recipients developed an oval circumscribed area of erythema at the injection site, with minimal central edema. The lesion was recorded as "millimeters of erythema" since induration was not a prominent feature. Necrosis and central pallor, evidence of marked sensitivity, never developed in these reactions. Animals with positive skin tests were designated "tuberculin-sensitive." After infection with B.C.G., most rabbits gradually lost their skin sensitivity over a period of 8 to 10 weeks. Such rabbits were readily resensitized in the same manner and used again in subsequent tests.

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[1] Obtained through the courtesy of Dr. Gardner Middlebrook, National Jewish Hospital, Denver.

[2] Obtained through the courtesy of James A. McComb, D.V.M., Director, Biologic Laboratories, Department of Public Health, Jamaica Plain, Massachusetts.

Endotoxin.—Typhoid vaccine (monovalent reference standard NRV -LS No. 1), made from *Salmonella typhosa* V-58, was used as the bacterial pyrogen.[3] Animals were rendered pyrogen-tolerant by at least 7 daily injections of 1.5 ml. of a 1:10 dilution of the vaccine in pyrogen-free saline (24).

Serum.—Blood was obtained by cardiac puncture. Most animals used in serum transfer experiments were exsanguinated. To avoid convulsions, they were lightly anesthetized with veterinary nembutal prior to bleeding. Serum obtained by the same procedure from uninjected control animals was demonstrated to be non-pyrogenic.

The blood was allowed to clot in an Erlenmeyer flask for approximately 1 hour at room temperature. The clot was then ringed and the flask stored overnight at 4.0°C. On the following day, serum was separated and cleared by centrifugation at 2000 R.P.M. for 20 minutes. All sera were stored at 4.0°C. until used, usually within 1 week of bleeding. Prior to injection, serum was incubated at 37°C. for 30 minutes.

Fever Charts.—Fever curves were plotted on ⅙ inch graph paper, with five lines on the ordinate representing 1°C. and six lines on the abscissa for each hour. In most experiments the area beneath each 5 hour fever curve was measured with a compensating planimeter[4] to determine the fever index, a numerical expression of both the height and duration of fever (25). Since most tuberculin fever curves failed to return to the baseline by 5 hours, a perpendicular line was drawn from the 5 hour temperature to the baseline in order to define the area. In cases in which no definite fever resulted, the algebraic sum of the areas above and below the baseline was calculated resulting in a negative value in some instances.

RESULTS

Pyrogenicity of Tuberculin.—Because of the possibility that the old tuberculin used in the following experiments was contaminated with trace amounts of bacterial endotoxins, undetectable in small doses, it was thought advisable to challenge unsensitized animals with a much larger dose than that administered to hypersensitive recipients. Accordingly, normal and pyrogen-tolerant animals (see Methods) were each given 1 ml. (2000 mg.) of pooled undiluted O.T. intravenously. The results are shown in Fig. 1.[5]

The failure of this large dose to cause fever indicates that the present lot of O.T. was free of significant endotoxin contamination by the usual standards of measurement.

By comparison, the average febrile response of tuberculin-sensitive rabbits to the intravenous injection of only 100 mg. of O.T. in saline is shown in the

[3] This vaccine had a bacterial count of approximately 500 million per ml. and a nitrogen content of 0.03 mg. per ml. ± 10 per cent. It was obtained through the kindness of Dr. Abram S. Benenson from the Immunology Division, Army Medical Service Graduate School, Washington, D. C.

[4] Keuffel and Esser compensating planimeter No. 4236M.

[5] In preliminary experiments, an occasional rabbit in both pyrogen-tolerant and normal groups responded to 1000 mg. of O.T. with moderate fever. Most animals, however, remained afebrile. Several other lots of O.T. obtained from Eli Lilly and Co. were found to be consistently pyrogenic, however, when given to normal recipients in dosages of as little as 100 mg. The short latent period and the diminished response of tolerant recipients suggest that fever in these instances was due, in part at least, to the presence of bacterial endotoxins.

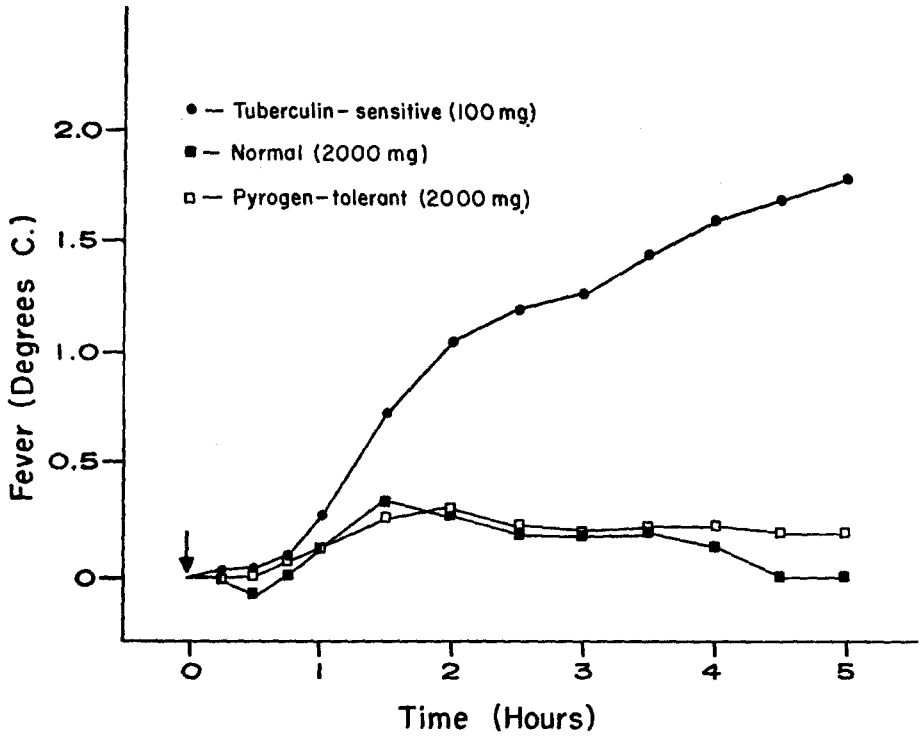

FIG. 1. Mean febrile response of 4 tuberculin-sensitive recipients to 100 mg. O.T. Average temperature variation of 3 normal and 3 pyrogen-tolerant recipients given 2000 mg. O.T. is shown for comparison.

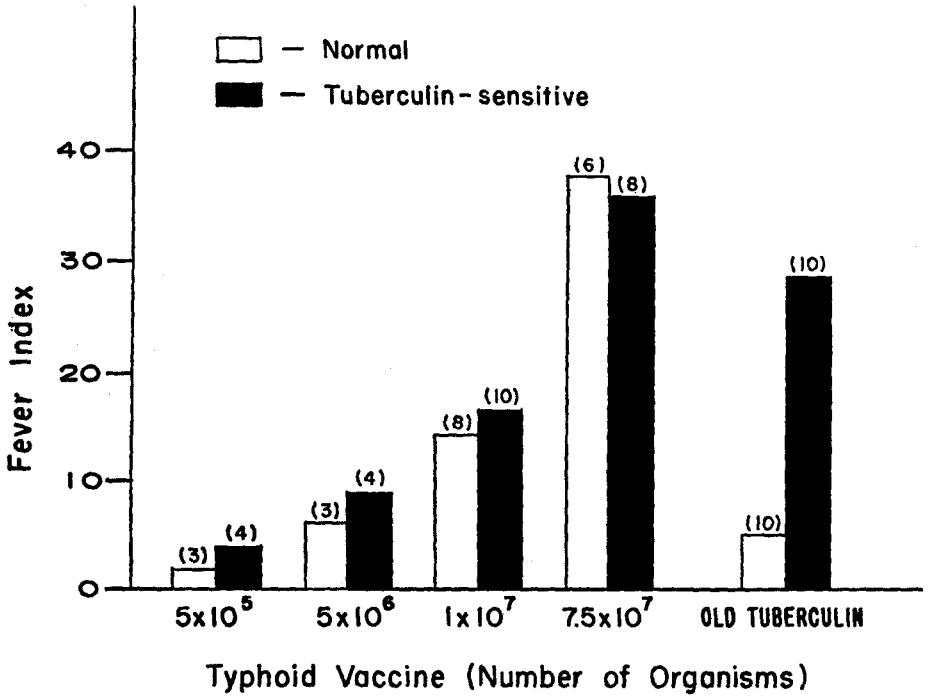

FIG. 2. Mean fever indices of normal and tuberculin-sensitive recipients given increasing doses of typhoid vaccine. Figures above each bar indicate the number of animals tested. The responses of normal and tuberculin-sensitive animals to 100 mg. O.T. are shown for comparison. Fever indices for the two lower doses of vaccine are on the basis of 4 hours; those for the two higher doses represent 5-hour responses.

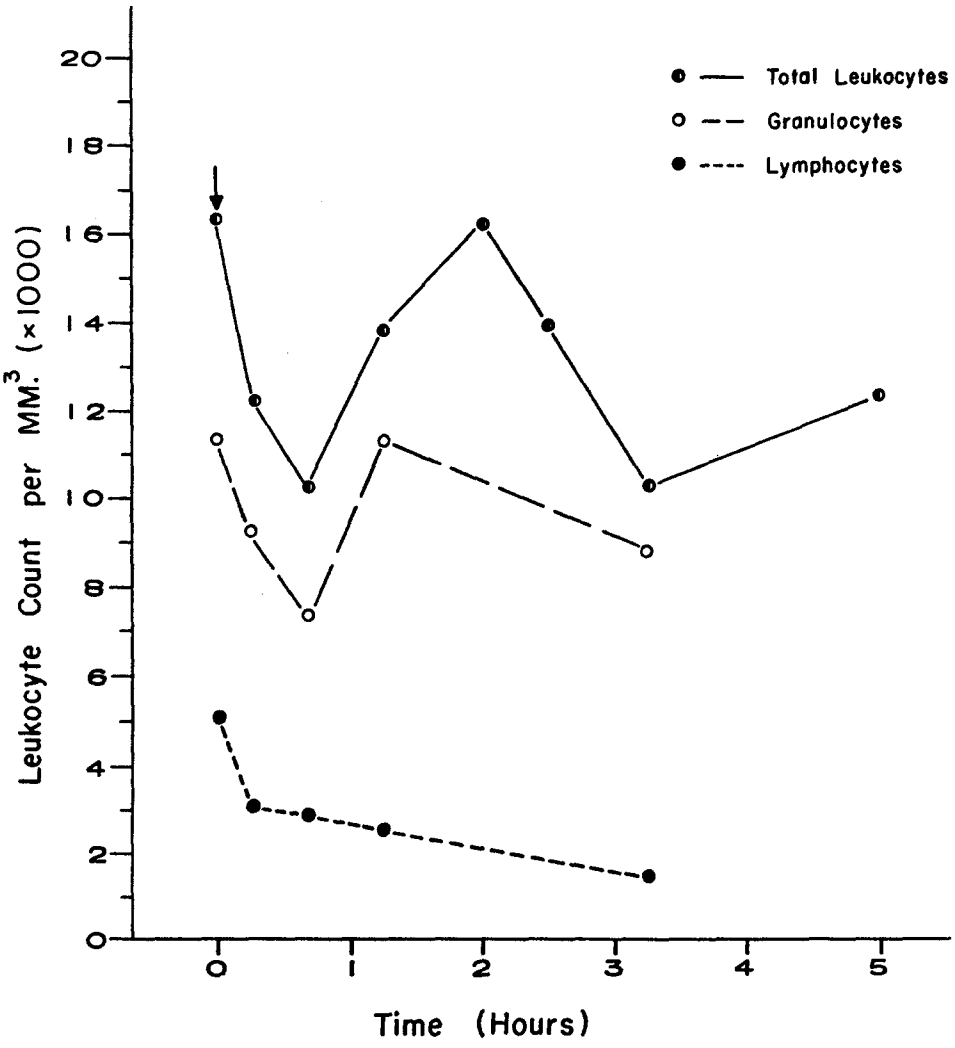

FIG. 3. Mean variation in the total number of leukocytes, granulocytes, and lymphocytes following the injection of 100 mg. O.T. in 4 hypersensitive recipients.

same figure. The following consistent characteristics of the febrile reaction to tuberculin are noteworthy: (*a*) a long latent period of 45 to 90 minutes similar to that seen after the injection of virus (5) which suggests that these agents do not act directly upon the hypothalamus; (*b*) a biphasic response with a maximum temperature elevation of 1.0° to 2.5°C. reached in 4 to 5 hours.

TABLE I

Leukocyte Counts of Tuberculin-Sensitive (S) and Normal (N) Rabbits after Intravenous Injection of 100 mg. O.T.

Recipients	Min. after injection							
	0*	15	40	75	120	150	195	300
S-1	13,600	13,300	11,500	10,250	13,600	9,500	7,200	9,200
S-2	12,500	9,100	10,650	14,100	17,800	22,000	14,050	13,900
S-3	20,600	11,950	8,550	11,400	13,250	12,450	11,200	14,100
S-4	18,800	14,800	10,450	19,400	20,100	12,000	8,550	12,200
Mean.....	16,380	12,290	10,290	13,790	16,190	13,990	10,250	12,350
Per cent Fall..	—	25	37	16	1	15	37	24
N-1	9,450	10,250	7,750	10,400	12,600	13,650	7,200	10,900
N-2	10,650	6,200	6,200	8,050	6,300	7,050	6,300	5,900
Mean.....	10,050	8,230	6,980	9,230	9,450	10,350	6,750	8,400
Per cent Fall..	—	18	31	8	6	0	33	16

* Second of 2 successive counts within range of 3000 cells and immediately before injection.

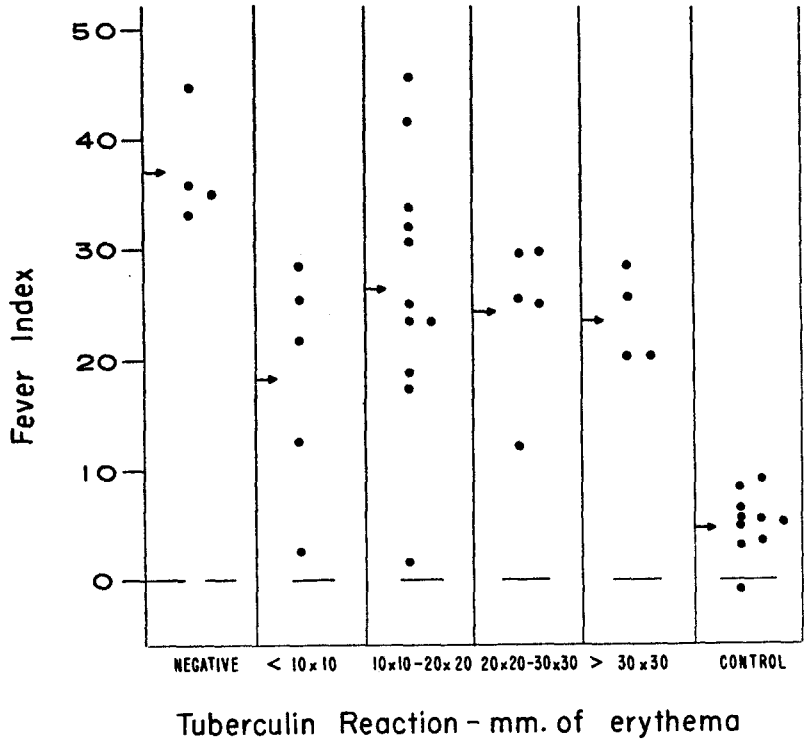

FIG. 4. Correlation of skin reactivity with fevers induced by 100 mg. O.T. in sensitive animals. Arrows indicate the mean value for each group. "Negative" animals were infected with B.C.G. but had negative skin reactions. Responses of uninfected controls to the same dosage of O.T. are shown for comparison.

Comparison of Pyrogen Fever in Normal and Hypersensitive Recipients.—Stetson *et al.* have recently reported that tuberculin-sensitive animals are more reactive than normal animals to the systemic actions of endotoxins (22). Tuberculin fever, therefore, may represent an exaggerated response to doses of contaminating endotoxins too small to elicit fever in a normal animal.

To test this possibility, the pyrogenic effect of small doses of typhoid vaccine was compared in normal and tuberculin-sensitive animals. Fig. 2 shows the average fever indices of groups of normal and sensitive animals given graded doses of typhoid vaccine in saline. It is evident that there is no significant difference between the responses of the two groups to any of the dosages tested.

Leukocyte Response to O.T.—Purified protein derivative (P.P.D), the active fraction of O.T., produces lymphopneia when given intravenously to sensitive animals (26). On the other hand, a granulocytopenia has been reported to follow the injection of O.T. (7). To determine the cellular response to the present lot of O.T., leukocyte counts were obtained in a small group of normal and tuberculin-sensitive rabbits before and at intervals after challenge with the regular 100 mg. dosage.

Free-flowing blood was used from serial razor cuts made distally along the marginal ear vein and counts made in the conventional manner with Trenner (N.B.S.) automatic pipettes and Spencer bright-line hemocytometers using the four corner squares. Differential determinations were performed on samples of blood at selected intervals. The results are presented in Fig. 3 and Table I.

There was a moderate but transient fall in granulocytes and a more sustained lymphopenia which persisted throughout the 3-hour period of observation in the 4 sensitive animals. The 2 controls showed similar changes but of less magnitude in both total and differential counts over the same period.

Relation of Skin Reactivity to Febrile Response.—The lack of correlation between the size of the tuberculin skin reaction and the febrile response to O.T. in sensitized animals is illustrated in Fig. 4. The fever indices of non-vaccinated controls are recorded for comparison.[6] Note that 4 infected animals which were unresponsive to the intradermal injection of O.T. reacted with high fever. Unfortunately, other vaccinated animals which had previously had negative skin tests were not challenged intravenously with O.T. as this was an unexpected finding.

"Desensitization" to O.T.—After 5 or 6 daily intravenous injections of 100 mg. O.T., hypersensitive animals no longer responded with either significant fever

[6] The low positive fever indices in the control group were due to slight but progressive elevations of temperature above the baseline throughout the 5 hour period. No characteristic febrile responses were observed in any control animals. In addition, since fevers in the sensitive recipients reached their maximum at 5 hours, it should be pointed out that the calculated fever indices of this group represent only approximately one-half of the total fever to this dosage of O.T.

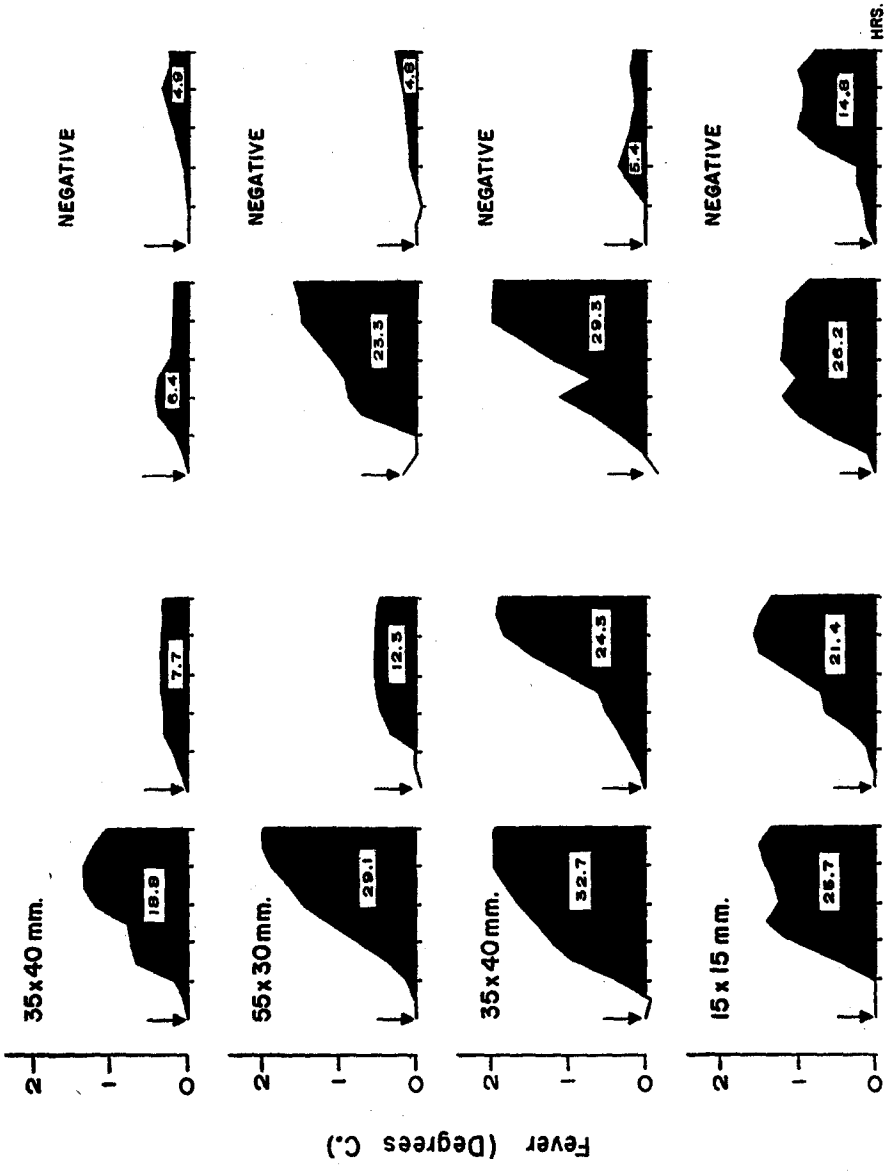

FIG. 5. Febrile responses of 4 representative hypersensitive recipients to daily injections of 100 mg. O.T. Numbers within shadowgraphs indicate fever indices. Note variable but eventual reduction in fever during this period. Tuberculin skin reactions are shown for the 1st and 5th day of injections.

or a positive skin test. The shadowgraphs in Fig. 5 demonstrate the different patterns of response which occurred in a representative group of recipients. It is noteworthy that some animals had a transient return of a biphasic fever after an initial decrease in the febrile response on the 2nd day of injection. However, it is apparent that by the 5th day all animals reacted with markedly diminished fever (see enclosed fever indices). The associated prolongation of the latent period and disappearance of the second fever peak are features which are also characteristic of pyrogen tolerance (25).

Effect of Homologous Serum and Thorotrast on Tuberculin Fever.—Both thorotrast (27) and prior incubation of endotoxin with normal rabbit serum have been shown to potentiate fever (28) and to diminish tolerance (29) to bacterial pyrogens. In view of many reported similarities of host responses elicited by endotoxins and by O.T. in hypersensitive animals (7, 22), a series of experiments was designed to test the effects of serum and thorotrast on both tuberculin fever and tolerance.

In the first of these, tuberculin-sensitive animals were divided into four groups. One group was given O.T. in saline as a control. Each of the other groups was given O.T. incubated for 30 minutes at 37°C. in 5 ml. of rabbit serum from one of the following sources: (*a*) normal donors, (*b*) pyrogen-tolerant donors, (*c*) tuberculin-sensitive donors. The average results of the four groups are plotted in Fig. 6.

Contrary to the findings with incubation of endotoxin in serum, there was no alteration of the febrile response in animals given O.T. incubated *in vitro* with any of the sera.

The capacity of endotoxin preincubated in normal serum to accelerate and augment fever is most evident in the pyrogen-tolerant recipient (29).

In order to produce an analogous situation in the tuberculin system, a group of tuberculin-sensitive animals was given 5 daily injections of O.T. By the 5th day these animals responded with minimal fever indicating virtually complete "tolerance" to this agent. On day 6, each animal was given the same dosage of O.T. incubated at 37°C. for 30 minutes in 5 ml. of normal rabbit serum. The results are shown in Fig. 7.

No increase in fever or reduction in the latent period was demonstrable, indicating that tolerance to tuberculin, unlike that occurring with endotoxins, was not modified by this procedure.

Thorotrast is more potent than serum in reversing endotoxin tolerance (27).

In a third experiment, another group of animals was similarly desensitized by 6 daily injections of O.T. Each animal was then given thorotrast, 2.5 ml. per kg.[7] together with 3 normal control animals which had received a single prior injection of O.T. Eighteen hours later, both groups were rechallenged with O.T. and skin tests performed. The results are shown in Fig. 8.

[7] A 24 to 26 per cent aqueous suspension of thorium dioxide, manufactured by Testagar Co., Detroit.

In neither the desensitized nor the control group was there any significant change in skin test or increase in febrile response to O.T. after thorotrast.

These experiments indicate the following points of difference between the action of O.T. and endotoxins: (*a*) preincubation of O.T. with serum does not

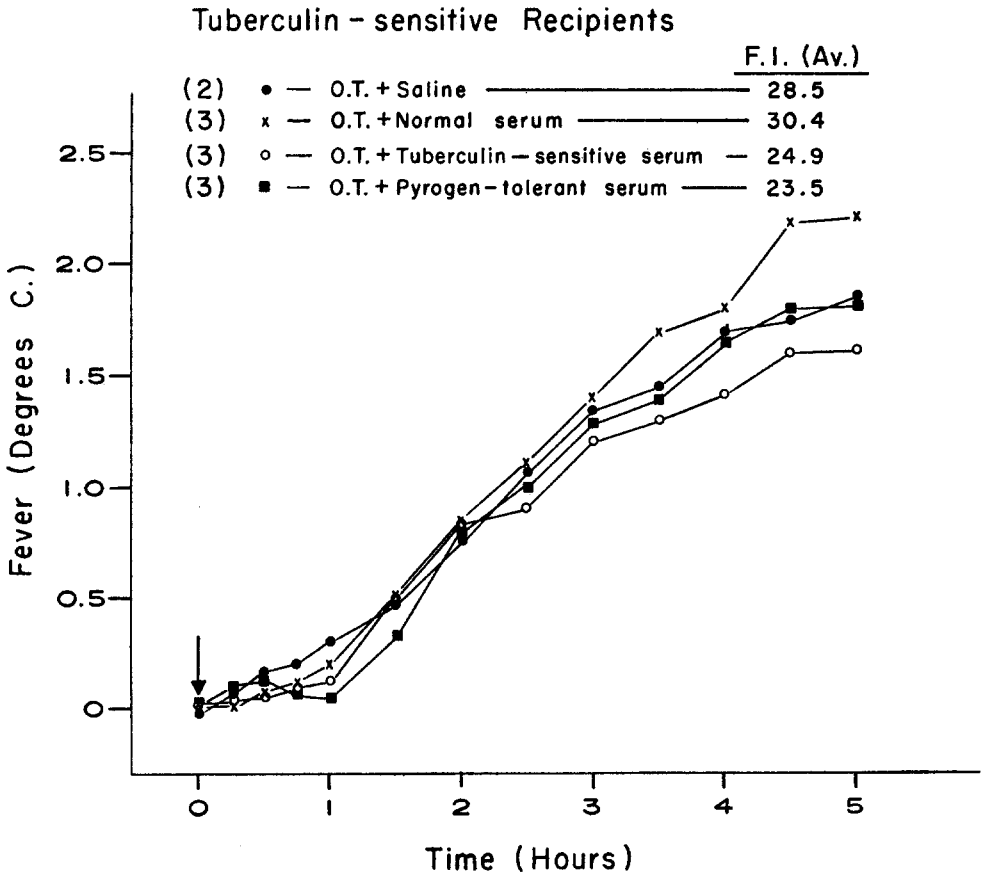

FIG. 6. Febrile responses of hypersensitive recipients to 100 mg. O.T. incubated in various sera. Figures to the left of key represent number of animals in each group; those to right indicate the mean fever indices (F.I.) of each group. Note the lack of change in the latent period induced by incubation of O.T. with either normal or pyrogen-tolerant sera.

accelerate tuberculin fever in sensitized recipients; (*b*) both serum and thorotrast are incapable of reversing the response of desensitized recipients to O.T.; (*c*) thorotrast, which increases the pyrogenicity of endotoxin in normal animals (30), does not appear to make such recipients susceptible to the pyrogenic effects of tuberculin.

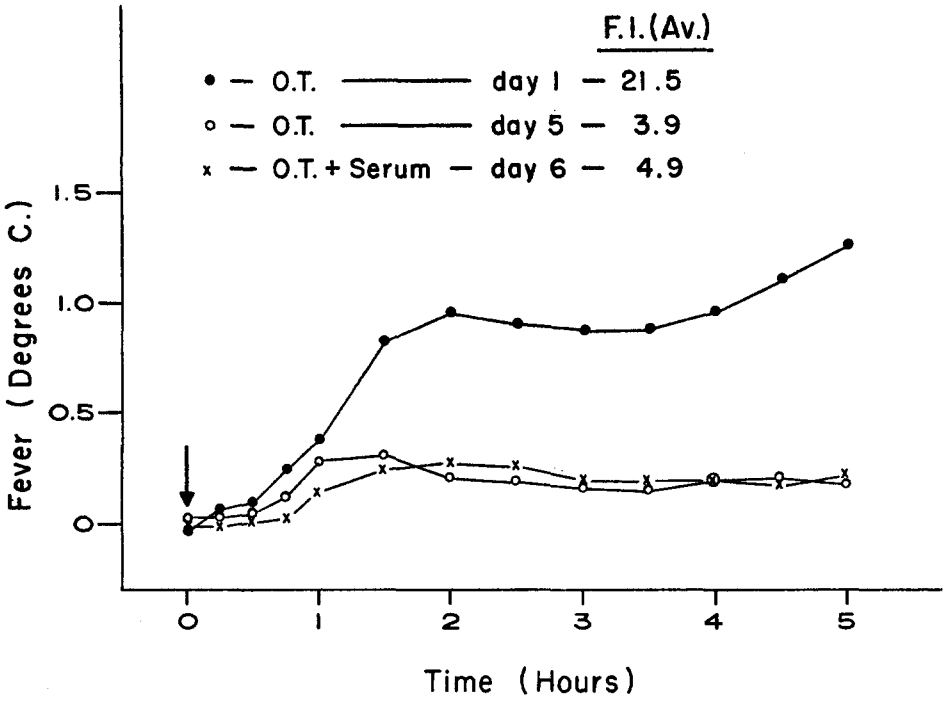

FIG. 7. Mean fevers of 3 desensitized recipients given 100 mg. O.T. incubated in normal serum. Responses of the same group to the 1st and 5th daily injection are shown for comparison. F.I. = fever indices.

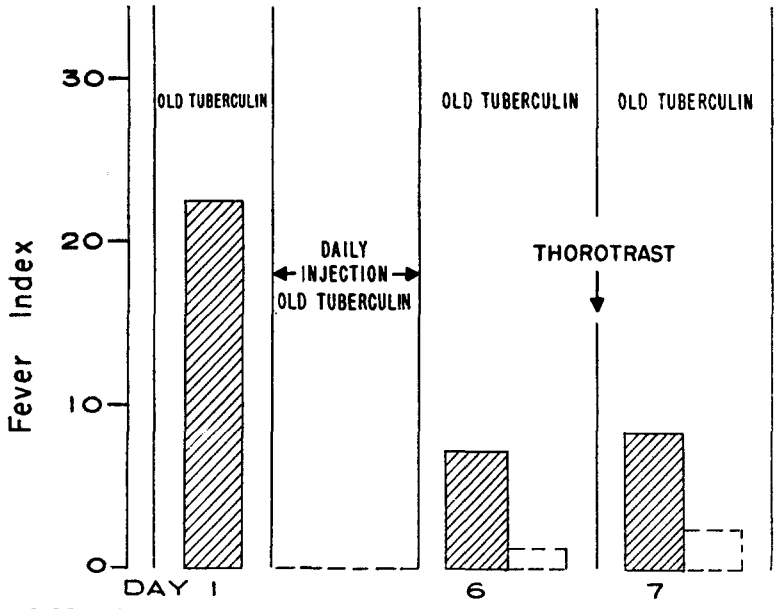

FIG. 8. Mean fever indices of 4 tuberculin-sensitive recipients on the 1st and 6th day of desensitization with 100 mg. O.T. and on the 7th day, 18 hours after blockade of the reticuloendothelial system with thorotrast. Mean responses of 3 controls to the same dosage of O.T. before and after thorotrast are shown in the adjacent open bars.

Absence of Cross-Tolerance between Endotoxins and Tuberculin.—A sensitive biological test for endotoxin is the presence of cross-tolerance to other known endotoxins (25). Accordingly, a test was devised to determine whether cross-tolerance could be demonstrated to O.T. and typhoid vaccine.

In this experiment tuberculin-sensitive animals were divided into three groups, A, B, and C. Each group was challenged on day 1 with 100 mg. O.T. and fevers recorded. Group A was then given 11 daily injections of typhoid vaccine (see Materials and Methods). The fevers were recorded on the 1st and last days of the series to confirm the development of pyrogen tolerance. On day 13 this group was rechallenged with 100 mg. O.T. Group B served as a control. Animals in this group received no treatment for the 11-day period during which group A was made pyrogen-tolerant. On the 13th day these animals were similarly rechallenged with O.T. Group C received O.T. for 5 consecutive days until a minimal response was obtained. On the following day this group was given an injection of the same dosage of typhoid vaccine used to induce tolerance in group A.

The results (Fig. 9) demonstrate that there is no cross-tolerance to the pyrogenic effect of O.T. and typhoid vaccine in tuberculin-sensitive animals. The slight fall in the mean fever index of group A to the reinjection of O.T. after 12 days is evidently unrelated to the establishment of pyrogen tolerance since there was a similar decline in the control group which received no intervening injections of endotoxin. This decrease in reactivity is probably due to a gradual loss of hypersensitivity (see Materials and Methods).

Presence of a Circulating Pyrogen during Tuberculin Fever.—Circulating pyrogens of evidently endogenous origin have been demonstrated in a number of experimentally induced fevers, including those which follow injection of endotoxins (3, 4) and myxoviruses (5) and occur during infections with Gram-positive microorganisms (6). In order to determine whether an endogenous pyrogen is present during tuberculin fever, the method of passive serum transfer was utilized (3, 4).

Tuberculin-sensitive and normal animals were used as donors. Each donor was given 100 mg. O.T. intravenously. The sensitive animals were then divided into two groups: (A) those with high fevers (1.8° to 2.7°C.) were exsanguinated 3 hours after injection; (B) the less responsive donors (0.6° to 1.1°C.) were bled at 4 hours. Serum from each donor in the two sensitive groups was given to 2 normal recipients in individual doses of 15 to 30 ml. (see Materials and Methods).

Two normal animals in a third group (C) injected with tuberculin were bled at 3 hours and their sera transferred to 2 recipients. In most instances, the same individual recipients received serum both from donors with high fever and from one of the other two groups.

The results are shown in Fig. 10 and Tables II and III. A circulating pyrogen was present in the tuberculin-sensitive animals which developed fever after the intravenous injection of O.T.[8] This pyrogen produced an abrupt monophasic

[8] Similar results were obtained in preliminary experiments in which donor rabbits were challenged with a non-pyrogenic lot of O.T. (Eli Lilly and Co.) after sensitization with either BCG or a heat-killed human strain (Jamaica No. 22) kindly furnished by Dr. M. W. Chase, The Rockefeller Institute. Endogenous pyrogen obtained from most of the donors with high fever produced characteristic monophasic fevers of 0.5° to 1.3°C. in normal recipients.

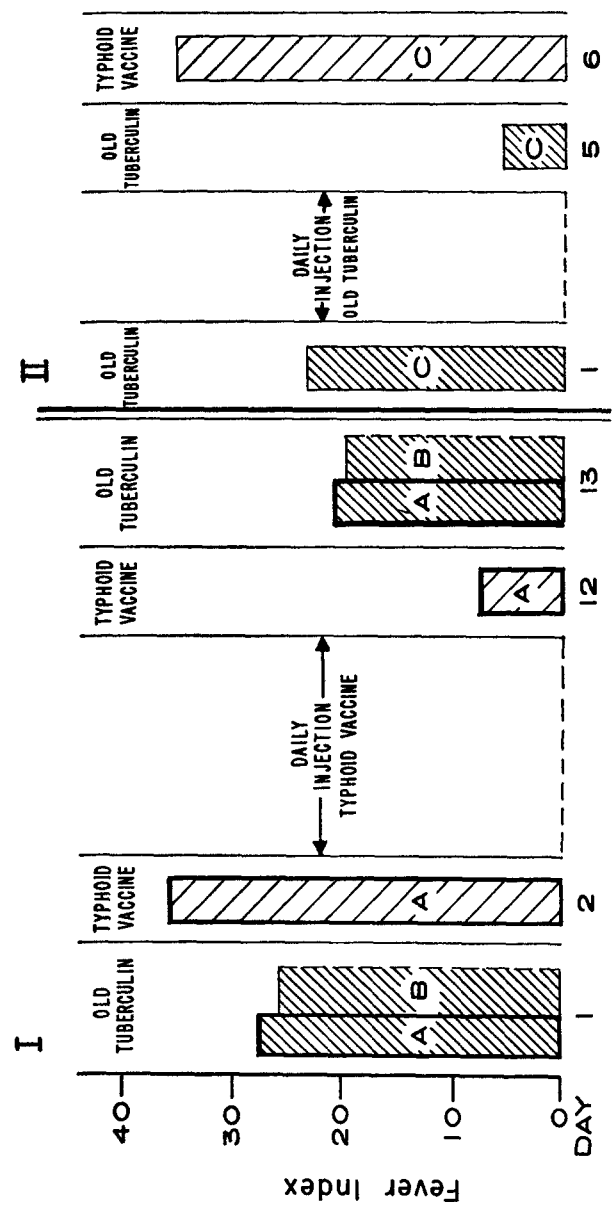

FIG. 9. I. Group A. Mean fever indices of 7 hypersensitive recipients injected with 100 mg. O.T. on day 1 and again on day 13 after the induction of pyrogen tolerance. Group B. Fever indices of 7 hypersensitive controls given the two spaced inoculations of O.T. only. A slight and equal decrease in the fever indices of both pyrogen-tolerant and control groups is apparent on day 13. II. Group C. Fever indices of 6 hypersensitive recipients injected with typhoid vaccine after desensitization to 100 mg. O.T.

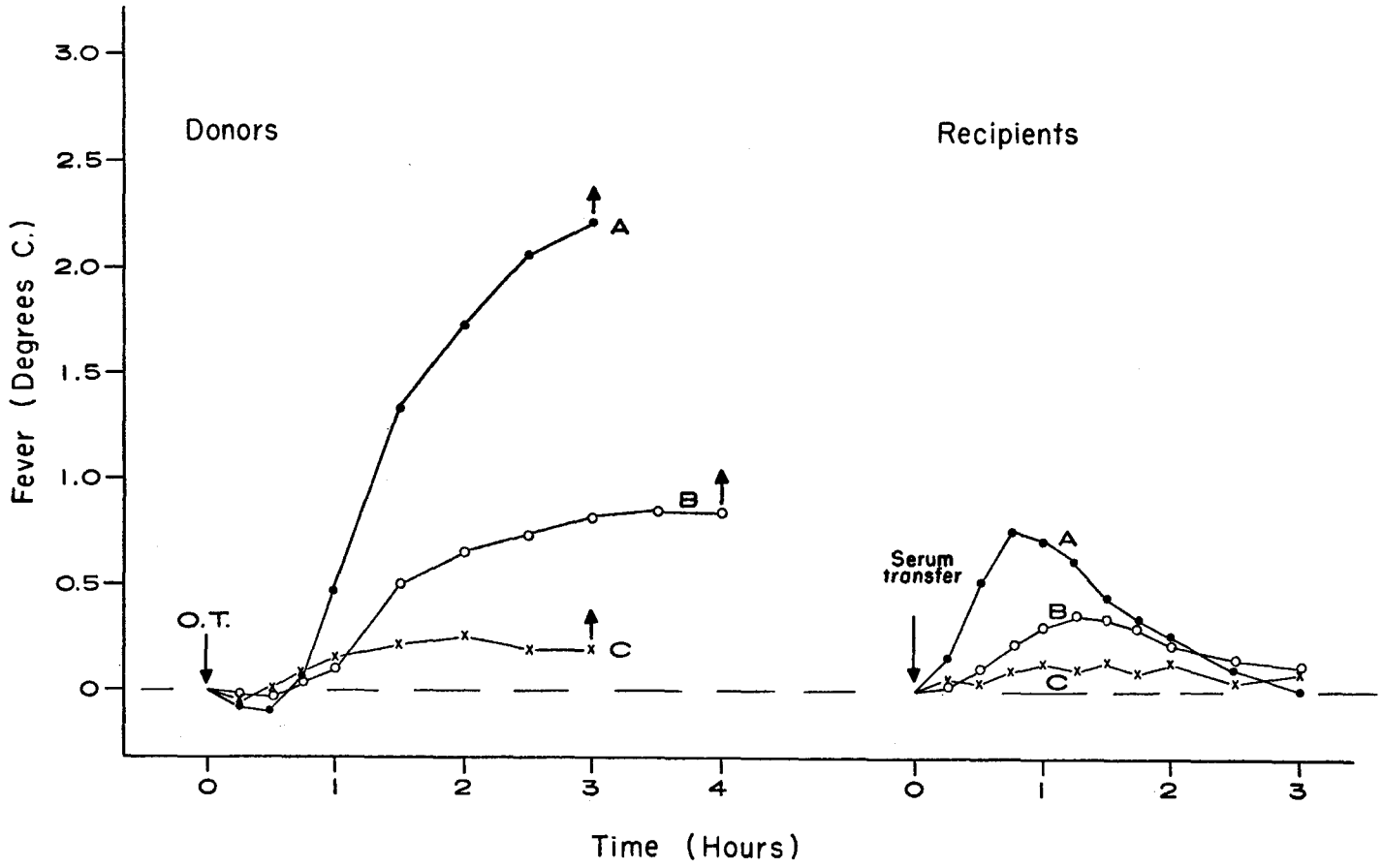

FIG. 10. Left half: Mean febrile responses of controls (C) and sensitized donors developing high fever (A) or moderate fever (B) after intravenous injection of 100 mg. O.T. Upright arrows indicate the time of bleeding. Results of serum transfer from each donor group to normal recipients are indicated in right half of chart.

TABLE II

Mean Fever Indices of 2 Hypersensitive (A and B) and 1 Control Group (C) of Donor Rabbits Injected with O.T.

Results of serum transfer from each group to the same normal recipients are shown in the right half of table. F.I. = fever index (3 hours).

Group	Donors		Recipients		
	No.	F.I.	No.	Vol. serum	F.I.
A	D-1	18.3	R-1	20	4.9
			R-2	18	4.7
	D-2	20.0	R-3	18	4.6
			R-4	18	6.3
	D-3	15.8	R-5	30	8.1
			R-6	30	6.7
Average		18.0	Average		5.9
B	D-4	7.2	R-4	18	0.6
			R-5	18	1.5
	D-5	7.0	R-6	15	6.9
			R-7	15	0.9
	D-6	6.3	R-7	20	2.7
			R-8	21	4.8
Average		6.8	Average		2.9
C	D-7	4.4	R-1	30	0.0
	D-8	3.0	R-2	20	1.7
	D-9	1.2		No transfer	
Average		2.9	Average		0.9

TABLE III

Comparison of Mean Febrile Responses to O.T. in 2 Hypersensitive (A and B) and 1 Control (C) Group of Rabbits with Degree of Fever Induced by Their Sera in Normal Recipients

Group	Donors		Recipients		P*
	No.	Fever—3 hrs.	No.	Fever—45 min.	
		°C.		°C.	
A	3	2.2 ± 0.26	6	0.8 ± 0.05	<0.01
B	3	0.8 ± 0.14	6	0.2 ± 0.08	
C	3	0.2 ± 0.02	2	0.1	

* Normal recipients, sera from group A as compared to sera from group B.

fever of brief duration similar to that seen with endogenous pyrogen obtained in other types of fever (3–6). The smaller fevers elicited by sera from the less responsive donors suggest in addition that the amount of circulating pyrogen was closely correlated with the degree of fever induced by O.T. in the donors. Finally, it seems evident that the transferable pyrogen in the sera of hypersensitive donors cannot be circulating tuberculin since neither O.T. itself nor sera of control animals injected with O.T. were pyrogenic to normal recipients.

DISCUSSION

The apparent presence of bacterial endotoxins in many commercially available samples of old tuberculin has raised the question whether such pyrogens may play a hitherto unsuspected role in the systemic manifestations of tuberculin hypersensitivity (22, 31). Several lines of evidence indicate that the O.T. used in the experiments reported here was not significantly contaminated with endotoxins.

1. Doses of O.T. twenty times larger than the dose causing high fever in hypersensitive recipients failed to produce fever in normal animals.[9]

2. *In vitro* incubation of O.T. with serum did not accelerate fever in hypersensitive recipients nor did it augment the minimal febrile response of desensitized animals.

3. Thorotrast similarly failed to potentiate the response of normal animals to O.T. or to reverse the "tolerance" of desensitized recipients.

4. Hypersensitive animals rendered pyrogen-tolerant showed no significant diminution in their response to O.T. Conversely, repeated daily injections of O.T. did not confer tolerance to a small dose of typhoid vaccine.

The evidence, therefore, as summarized in Table IV, indicates that the pyrogenic activity of O.T. in sensitized recipients was due to the presence of specific antigen rather than to contaminating endotoxins. Further support for the concept of the specificity of the febrile response to O.T. is the demonstration that hypersensitive recipients were not significantly more reactive than normal animals to graded doses of endotoxin.

A possibility which is difficult to exclude entirely on the basis of the present data is that tuberculin fever results from hyperreactivity to contaminating endotoxins which occurs *only when the sensitive recipient is simultaneously challenged with the specific antigen contained in O.T.* Both the absence of a characteristic endotoxin-induced leukopenia and the long duration of the latent period after injection of O.T., nearly twice that occurring with endotoxins, argue

[9] Occasional irregular fevers observed in both normal and pyrogen-tolerant recipients given 1000 mg. O.T. were believed to be unrelated to specific contaminating pyrogens as larger doses (2000 mg.) produced no fever (see Fig. 1).

against this hypothesis, particularly in view of the marked pyrexia. Furthermore, the lack of cross-tolerance to tuberculin in a sensitized animal made pyrogen-tolerant constitutes an additional point against this possibility. Tolerant recipients clear injected endotoxin so rapidly (27) that it seems unlikely any trace originally present in O.T. could remain in the circulation throughout

TABLE IV

Comparison of the Effects of Endotoxin with the Response of Sensitized Rabbits to Old Tuberculin

	Typhoid vaccine (1.5 ml. of 1:10 dilution)	Tuberculin (100 mg.)
1. Fever:		
(*a*) Onset	15 to 30 min. after injection	45 to 90 min. after injection
(*b*) Curve	Biphasic	Biphasic
(*c*) Tolerance	After 7 to 10 daily injections	After 5 to 6 daily injections; associated regularly with desensitization to intradermal injection of O.T.
(*d*) Incubation with serum	Accelerates onset of fever; partially reverses tolerance	No acceleration of fever; no effect on tolerance
(*e*) Injection of thorotrast	Potentiates fever in normal recipients; reverses tolerance	Fails to modify afebrile response in normal animals; does not reverse tolerance
(*f*) Endogenous pyrogen	Present	Present
2. Leukocyte response	Marked leukopenia followed usually by leukocytosis	Slight, transient leukopenia
3. Febrile response—recipients:		
(*a*) Normal	Marked	None
(*b*) Tuberculin-sensitive	Marked	Marked
(*c*) Tuberculin-sensitive made pyrogen-tolerant	Minimal	Marked
(*d*) Tuberculin-tolerant ("desensitized")	Marked	Minimal

the latent period of nearly 1 hour before hypersensitivity to tuberculin becomes evident.

The development of tolerance to O.T. superficially resembles the acquisition of tolerance to endotoxins (25). Moreover, changes in the febrile response occurred so rapidly that it is questionable whether they are due to desensitization in the classical sense (32). The relatively large dose of O.T. used to elicit fever indicates, however, that these animals were not markedly hypersensitive. Since the skin reaction became negative in conjunction with the diminution in

fever, this phenomenon has been termed "desensitization," although there is no direct evidence at the present time that either fever or tolerance in this system is due to the presence or absence, respectively, of reacting antibody.

These studies have demonstrated the appearance of a circulating pyrogen of presumably endogenous origin during tuberculin fever. Suggestive evidence that the febrile response is directly due to the liberated endogenous pyrogen is the correlation which exists between the degree of fever induced by O.T. and the amount of endogenous pyrogen in the circulation. The pyrogenic properties of this material resemble closely those of circulating pyrogens in fevers produced by endotoxins, viruses, and bacterial infections. The presence of an endogenous material with similar characteristics in these fevers of diverse etiology suggests a common pathogenetic mechanism. In the present system it should be emphasized that the injection of an agent pyrogenic only to specifically sensitized animals resulted in the appearance of a circulating substance which produced fever in normal animals as well. This finding along with those findings previously mentioned would appear to cast doubt upon the hypothesis that serum pyrogen may be simply a modified moiety of the injected pyrogen (20, 21).

The origin of this endogenous material is unknown. Since tolerance to a bacterial endotoxin confers tolerance to the action of other similar agents, the absence of cross-tolerance to tuberculin fever makes it appear unlikely that endogenous pyrogen liberated by O.T. is identical with the endotoxin-like tissue polysaccharides recently isolated by Landy and Shear (33). There is considerable evidence implicating the granulocyte as a source of endogenous pyrogen in other experimental fevers (1, 4, 6, 34).

The significance of the transient but consistent granulocytopenia during the latent period before the onset of tuberculin fever is at present uncertain as a similar change occurred to some degree in the controls. The leukocyte response to O.T. was considerably less than that which follows comparably pyrogenic doses of endotoxin (23, 28, 33, 35) and constitutes additional evidence for the distinctiveness of tuberculin fever. Nevertheless, the absence of a marked fall in circulating polymorphonuclear leukocytes does not exclude this cell as a source of endogenous pyrogen since this substance is released *in vitro* by granulocytes which show no evidence of cellular damage (36). It seems possible, therefore, that release of endogenous pyrogen from such cells *in vivo* may not be necessarily associated with changes in the peripheral leukocyte count.

The lack of correlation between the intensity of the tuberculin skin reaction and the magnitude of tuberculin fever appears paradoxical in view of the fact that desensitization to the pyrogenic effect of O.T. is regularly associated with loss of a positive skin test. However, other studies have shown that *in vitro* cytolysis of tuberculin-sensitive leukocytes by O.T. is not correlated with the degree of skin reactivity of the donor animals (12). The precise factors re-

sponsible for such *in vitro* cytotoxicity have not been fully defined. There is evidence that the tuberculin-sensitive lymphocyte may release a substance which is capable of sensitizing other cells including granulocytes to the toxic effects of O.T. (37).

The relation of this *in vitro* evidence for the cytotoxic action of O.T. to the *in vivo* elaboration of endogenous pyrogen in the hypersensitive recipient given O.T. remains to be determined. Studies to investigate this possibility are in progress. However, the findings reported here are consistent with the hypothesis that tuberculin fever may be due to the release of an endogenous pyrogen from cells specifically sensitized to an antigen contained in O.T.

SUMMARY

Evidence has been presented that the fever elicited by intravenous administration of old tuberculin (O.T.) in BCG-infected rabbits is a specific property of this hypersensitivity system and is probably not due to contamination of tuberculin with bacterial endotoxins. Daily injections of O.T. in sensitized animals resulted in a rapid tolerance to its pyrogenic effect. Tuberculin tolerance can be differentiated from that occurring with endotoxins and was invariably associated with the development of a negative skin test. The mechanism of this tolerance would thus appear to be desensitization.

A circulating pyrogen found during tuberculin fever was indistinguishable in its biologic effects from endogenous pyrogens obtained in several other types of experimental fever. This material produced fevers in normal recipients and therefore may be clearly differentiated from O.T. itself which was pyrogenic only to sensitized animals. Since the titer of serum pyrogen was directly proportional to the degree of fever induced by injection of O.T. in the donor animals, a causal relation is suggested.

On the basis of these findings, it is postulated that tuberculin fever is due to a circulating endogenous pyrogen released by a specific action of O.T. on sensitized cells of the host.

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