STUDIES ON TUBERCULIN FEVER*

II. Observations on the Role of Endogenous Pyrogen in Tolerance

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A distinctive feature of many experimental fevers is the development of a diminished reactivity, usually defined as "tolerance," to repeated inoculations of the pyrogenic agent.

In the case of Gram-negative endotoxins, tolerance generally appears gradually, reaches its maximum in the 2nd week of daily injections, and persists for several weeks after inoculations are discontinued (1). Since tolerance lapses after a short rest period when antibody titers are still elevated, and may be abolished by blockade of the reticuloendothelial system (2), it clearly does not depend entirely upon specific humoral immunity. Furthermore, tolerance to one endotoxin confers a cross-tolerance to endotoxins of other Gram-negative organisms but there is a degree of specificity since endotoxin-tolerant animals are fully responsive to unrelated pyrogenic agents (3–8).

In contrast, the type of tolerance which characteristically follows one or more injections of myxovirus is transient, and less specific in that the host may be rendered less reactive to entirely different pyrogenic stimuli. This type of tolerance may be seen after a single large inoculation of endotoxin as well as of myxovirus and appears to depend upon a different mechanism (6).

Tuberculin-sensitive rabbits similarly become tolerant to the pyrogenic effect of old tuberculin (OT) after several injections (7). Since abolition of the febrile response to OT is accompanied by loss of skin sensitivity, it has been inferred that both phenomena are due to desensitization.

A characteristic of all these three forms of experimental fever is that the various pyrogenic agents produce their effect either largely or wholly through mobilization of a circulating pyrogen of endogenous origin (9) which presumably acts directly on the thermoregulatory center (10). Conversely, failure to release endogenous pyrogen (EP) appears to be a critical factor in the develop-

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ment of tolerance in both the endotoxin (11) and viral (6) fever models although the mechanism of this failure may be different in the two systems.

In the present study, tolerance to tuberculin was investigated in relation to that seen in the viral and endotoxin systems with particular emphasis on the role of EP in the development of the refractory state.

Materials and Methods

General—Male and female New Zealand rabbits weighing 3.5 to 4.6 kg were used. They were housed in an air-conditioned room, and all experiments were conducted in an adjacent laboratory maintained at 69°F with a daily variation of less than 3°F.

Glassware, needles, and sodium chloride were sterilized by dry heat at 170°C for 2 hours to inactivate pyrogens (12). Physiologic saline was prepared with doubly distilled, autoclaved water and was periodically checked for pyrogenicity by injection of 10 to 20 ml in normal rabbits.

Temperature Determinations.—Rectal temperatures were determined with the rabbits restrained in boxes which had openings for the head and tail. To insure a reliable response, animals used for the first time were boxed for 3 or more days and injected at least once with pyrogen-free saline prior to an experiment. Animals which had been previously trained were placed in boxes 1 or 2 days preceding each experiment. Foxboro rabbit scanning equipment was used in all experiments and recordings obtained at intervals for at least 1 hour before inoculation to establish the base line temperature. Rabbits showing a variation of more than 0.3°C over this period or with initial temperatures greater than 40.5°C were not used. Temperatures were recorded every 15 minutes for the first 2 hours after inoculation and at 15 or 30 minute intervals thereafter.

Details of the methods for plotting fever curves and calculating fever indices have been described elsewhere (7). The area beneath the 5 hour fever curve, the fever index, was determined by planimetry and expressed in units read directly off the vernier. A perpendicular line was drawn from the 5 hour reading to the base line in those instances in which the temperature remained elevated so that the fever index in these studies represents an expression of the height and duration of fever for a period of only 5 hours after injection, as in a number of previous studies (1, 6, 7, 11).

Sensitization of Rabbits.—The Phipps strain of BCG was used exclusively. Cultures were maintained by alternate passage on Lowenstein slants and Dubos medium without tween, or by passage on Middlebrook 7H9 media (Difco). Vaccine was prepared by suspension in pyrogen-free saline of 2 week old colonies which had been scraped from slants and ground with a pyrogen-free mortar and pestle. Animals were sensitized by intradermal or intravenous injection of BCG vaccine. When the intradermal route was used, 5.0 to 7.5 mg (wet weight) of BCG in a final concentration of 5 mg per ml was injected in divided doses in 2 or more sites. Infection by the intravenous route was established by a single injection of 2 mg of BCG suspended in 2 ml of pyrogen-free saline into the marginal vein of the ear. The latter method resulted in nodular infection of the lungs and more consistent and intense hypersensitivity as determined by both skin test and febrile response to OT. Experiments were conducted 18 to 24 days after the BCG had been inoculated.

Old Tuberculin—OT was obtained from the Massachusetts Department of Health, and lot 50 was used exclusively. This material containing 2.0 gm of OT per ml was bottled in pyrogen-free glassware and was diluted in normal saline to a concentration of 20 mg per ml. prior to use. In dosages of 200 mg this lot of OT was found to be consistently non-pyrogenic in normal rabbits. Except when otherwise noted, 100 mg (in 5.0 ml saline) was given intravenously in all experiments.

Test for Tuberculin Sensitivity.—Each rabbit inoculated with BCG was tested with OT and those which developed febrile responses to the 100 mg dosage were considered sensitive. Intradermal tests for sensitivity were not carried out routinely as they have been shown to be an unreliable guide to systemic sensitivity (7, 13). However, all rabbits sensitized by the intravenous route developed positive skin tests to 10 mg of OT when tested. In most instances the area of induration was greater than 10 mm in diameter.

Endotoxin—Typhoid vaccine from *Salmonella typhosa* V-58 (monovalent reference standard NRV LS No. 1)[1] was used as a source of endotoxin. This material contained approximately 500 million killed organisms per ml and had a nitrogen content of 0.03 mg per ml ± 10 per cent (11).

Virus.—Newcastle disease virus (NDV) was grown on the chorioallantoic membrane of embryonated eggs and had an hemagglutination titer of 1:1260. The techniques of harvesting and storing this material have been described elsewhere (14).

Endogenous Pyrogen—Endogenous pyrogen (EP) was obtained from donor rabbits injected with 5 ml of NDV. Donors were exsanguinated by cardiac puncture 4 hours after injection of the virus. The blood was allowed to clot and the serum removed and cleared by centrifugation. Serum thus obtained is free of detectable titers of virus, and the biologic properties of the pyrogenic factor have been described (14, 15). In the present studies the smallest quantity of serum which consistently produced biphasic fever in normal animals was 3 ml. This dose of serum containing endogenous pyrogen was used throughout and is referred to simply as EP.

RESULTS

1. Induction of Tolerance by Intermittent Injections.—A group of tuberculin-sensitive rabbits was injected intravenously with OT every 3rd day over a 13 day period, and temperatures were recorded. The mean fever indices and curves are shown in Fig. 1.

The mean results demonstrate a predictable reduction in fever response with successive injections of old tuberculin. However, the response of individual rabbits is erratic early in the course of development of tolerance. The individual responses are shown in Fig. 2.

2. Recovery of Response of Tolerant Rabbits.—The time interval necessary for recovery of the febrile response to OT was determined in different groups of rabbits made tolerant by either daily or intermittent injections. After tolerance had developed, animals were rested for varying periods of time before being challenged again with OT. The results with different schedules are shown in Fig. 3. Some recovery from tolerance is apparent as early as the 4th day. When tolerance is allowed to lapse for 3 weeks (Fig. 3B and C) the fever produced by reinjection of OT is also influenced by the natural loss of hypersensitivity to tuberculin. This feature was not specifically investigated, but the impression was gained from occasional observations that sensitivity generally begins to wane slowly about 5 weeks after vaccination (2 weeks after the period of maximal responsiveness) although significant fevers may be obtained at least as late as 12 weeks after BCG inoculation.

[1] Kindly supplied by Dr. Abram S. Benenson, Immunology Division, Army Medical Service Graduate School, Washington, D. C.

3. Production of Endogenous Pyrogen (EP) by Tolerant Rabbits.—Previous experiments utilizing the technique of passive serum transfer have shown that

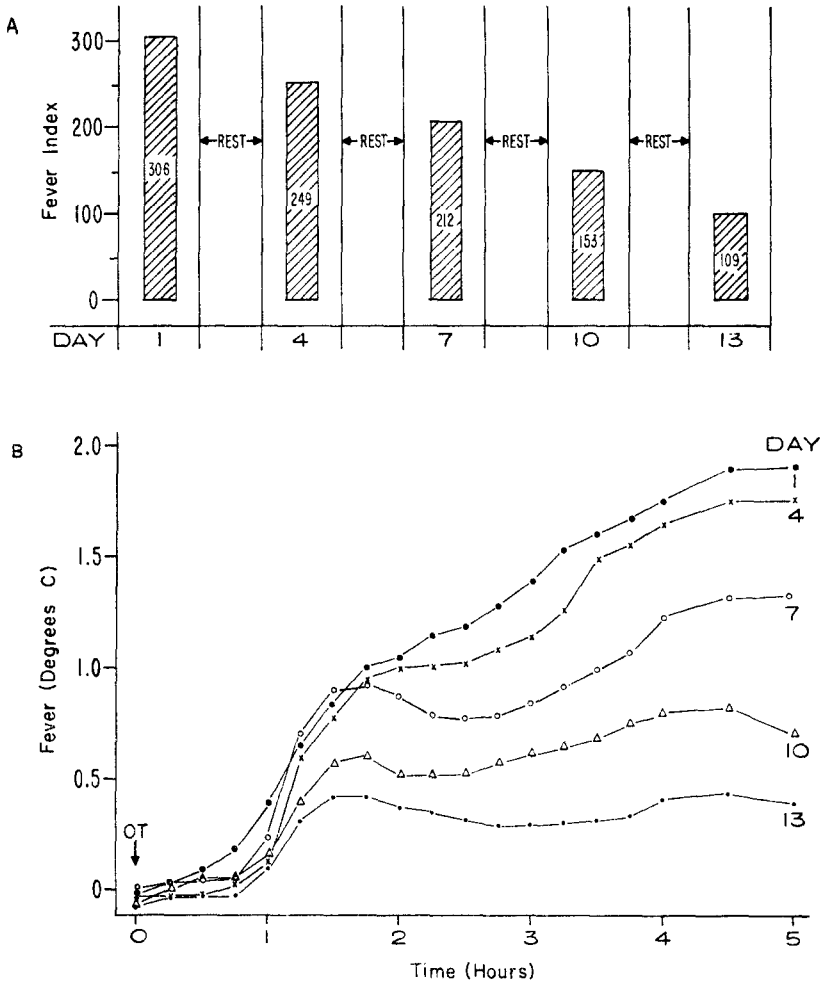

FIG. 1. (A) Mean fever indices of 6 tuberculin-sensitive rabbits injected every 3rd day with 100 mg of OT. On day 13 only 5 rabbits were injected. (B) Mean febrile responses of the same animals to the series of injections.

an endogenous pyrogen (EP) is present in the circulation during tuberculin fever (7). This pyrogen is active in normal recipients and its titer correlates well with the degree of fever produced by OT in sensitized donor rabbits. In order to determine whether tolerance to tuberculin was associated with a failure to mobilize EP, the same technique was adopted.

Two rabbits were rendered tolerant to tuberculin by a series of 5 daily injections and were exsanguinated 3½ hours after the last inoculation. Serum from each donor, in dosages of 25 to 35 ml, was injected into two normal rabbits. The four recipients remained afebrile indicating that tolerant animals given OT do not release amounts of EP ordinarily detectable by passive transfer methods.

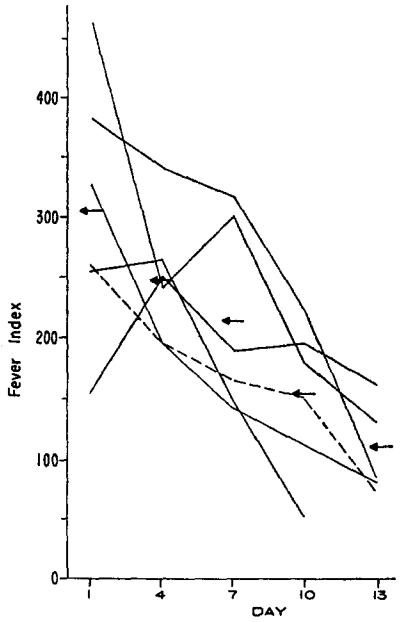

FIG. 2. Individual fever indices of the 6 rabbits shown in Fig. 1. The arrows indicate the mean fever index for the group on each day. Note the variable responses of individual animals to successive injections of OT.

4. Response of Tolerant Rabbits to Endogenous Pyrogen.—The development of biphasic fever following a sufficient dosage of injected EP has been shown to be due to the ability of recipient animals to respond both directly to this stimulus and indirectly by mobilizing additional EP (15). Although tuberculin-tolerant rabbits remain virtually afebrile and do not release detectable amounts of endogenous pyrogen when injected with OT, it seemed appropriate to determine whether such animals were capable of mobilizing EP when given an heterologous pyrogen such as EP from virus-injected donors. In this experiment, the response of tuberculin-sensitive rabbits to the smallest dose of virus-induced EP capable of consistently producing biphasic fever was tested before and after development of tolerance.

A group of BCG-vaccinated rabbits was injected with 3 ml of EP before and

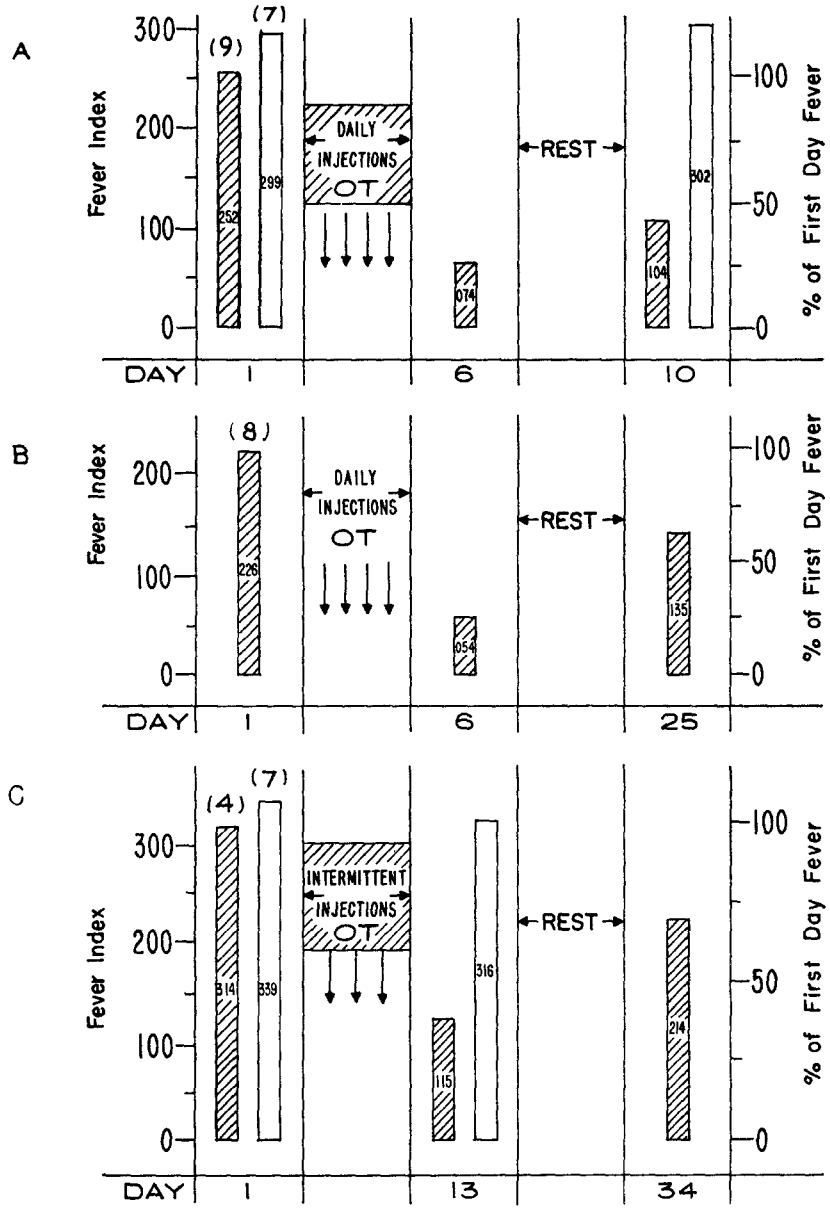

FIG. 3. Mean fever indices of tuberculin-sensitive rabbits injected with OT. Numbers in parentheses indicate number of rabbits represented by each bar. Cross-hatched bars represent rabbits made tolerant by daily injections (A and B) or injections at 3 day intervals (C). Open bars represent control animals rested 8 days (A) or 11 days (C) between injections.

after tolerance was induced by 5 daily injections of OT. The mean fever curves are compared in Fig. 4. Since comparable biphasic fevers were elicited at both times, it seems clear that tolerance to tuberculin does not impair the ability of such animals to respond to either the direct or EP-mobilizing actions of viral-induced EP.

5. Tolerance to Endogenous Pyrogen after Tuberculin Fever.—The foregoing experiment demonstrated that rabbits tolerant to OT develop biphasic fever in response to an injection of viral-induced EP. On the other hand, after a single prior injection of either virus or endotoxin, recipients respond only monophasically to EP apparently because of transient inability of such animals to mobilize EP after these stimuli (6). To determine whether similar suppression of EP release follows tuberculin fever, the response of a single rabbit to EP was observed during the course of several injections of OT.

A tuberculin-sensitive rabbit was injected with 1000 mg of OT on the 1st day and 100 mg of OT on the 4th and 7th days of the experiment. Three ml. of endogenous pyrogen was injected on the day following each dose of OT. Twenty-four hours after the first injection of OT the temperature remained 0.5°C. above the base line, but following the subsequent doses, fever had subsided by the time of the injection of EP.

The results are shown in Fig. 5. After the first 2 injections of OT, the response to EP was monophasic. By the third injection, when significant tolerance to OT had developed, the response to EP became biphasic.

This experiment demonstrates an inverse relationship between the responses to tuberculin and to subsequent injections of EP, indicating that developing tolerance to OT was associated with recovery of the recipient's ability to release EP. Of interest is the fact that although the responses to EP are nearly identical in terms of fever index during the course of injections, there is a significant change in the shapes of the fever curves. The first injection of EP resulted in a high single peak. The second injection produced a modified monophasic fever with a slow return to the base line, and the final response was clearly biphasic with both peaks nearly equal in height but of less magnitude than the peaks of either of the previous responses. EP given after several subsequent injections of OT to maintain tolerance (not shown) continued to produce biphasic fever.

Additional experiments to confirm these differences in fever curves were conducted in 27 tuberculin-sensitive rabbits. Each was injected with 3 ml of EP 24 hours after a single injection of OT.

A spectrum of responses to EP was obtained ranging from brief monophasic to biphasic fevers. However, on further analysis, a consistent relationship between the responses to the two injections could be shown when consideration was given to the magnitude of the antecedent tuberculin fever, which has previously been demonstrated to correlate directly with the titer of circulating EP

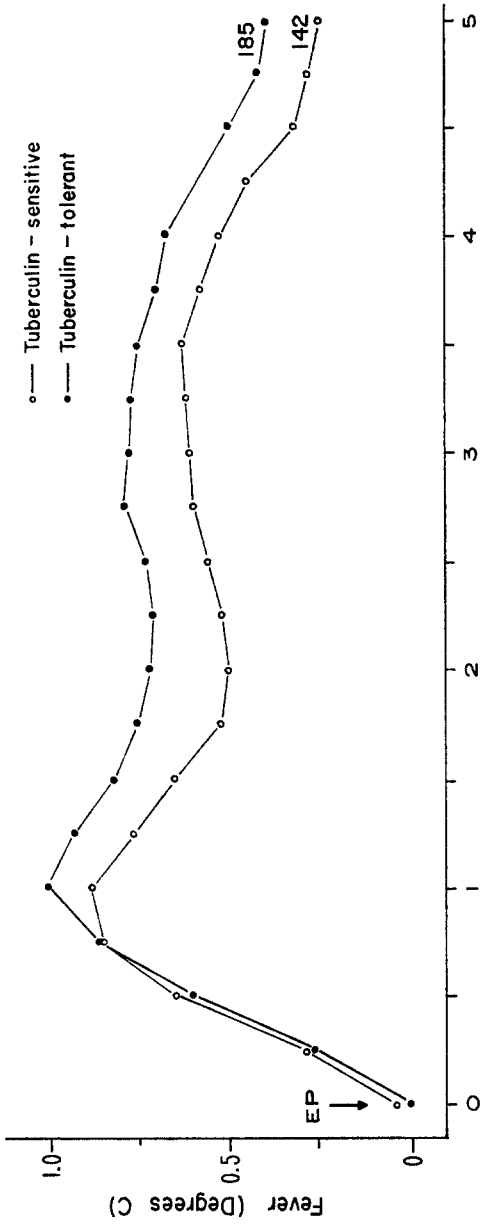

FIG. 4. Mean fever curves of 5 BCG-vaccinated rabbits injected with 3 ml of EP before (open circles) and after (filled circles) induction of tolerance by 5 daily injections of OT. Numbers at ends of curves are mean fever indices.

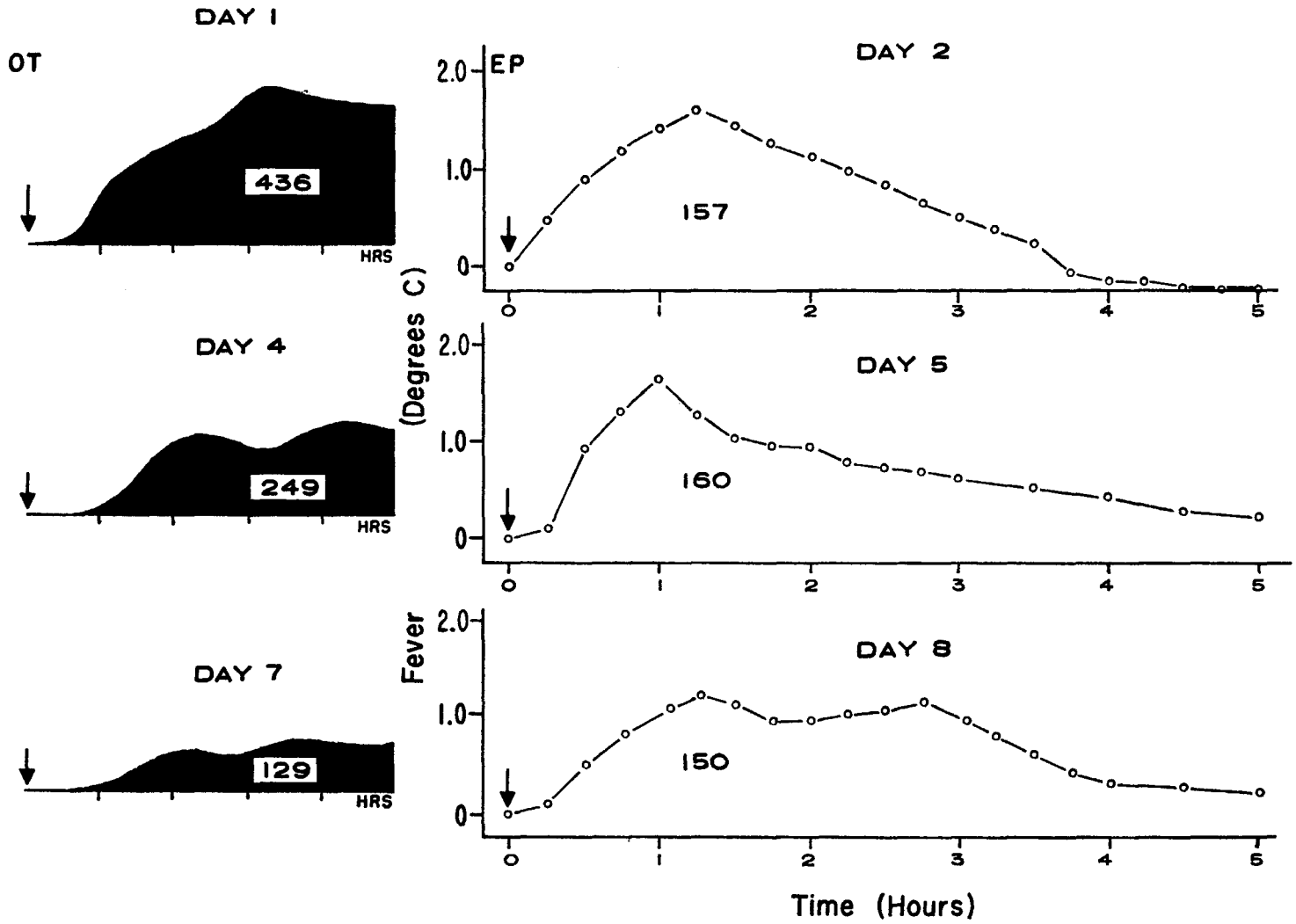

FIG. 5. Mean fever curves of a single tuberculin-sensitive rabbit injected at 3 day intervals with OT and 24 hours later with EP. The shadow-graphs represent tuberculin fever and open graphs, the response to EP. The numbers under the curves are fever indices.

(7). All 15 rabbits with tuberculin fever indices of less than 300 (mean 220) developed biphasic fever in response to the subsequent injection of EP. In contrast, only 1 of the remaining 12 animals with high tuberculin fever (mean fever index 410) developed definite biphasic fever after EP. The mean fever curves of the 2 groups are shown in Fig. 6. The group with maximum fever elicited by OT responded to EP with fever characterized by an exaggerated single peak and a relatively rapid return to the base line. Although the mean fever index for the EP response is similar in both groups, the differences in temperatures at both 1 and 5 hours after injection are significant ($P = <0.001$ and $P = 0.002$, respectively).

6. Tolerance to Tuberculin Fever after Virus.—The previous experiments have demonstrated that release of EP (evidenced by second febrile peak to injected EP) is suppressed following a maximal response to OT. A similarly altered response to EP occurs after a single injection of virus (15). Since both viral and tuberculin fever are produced by circulating EP, it appeared likely that a prior injection of NDV might block release of EP in sensitized recipients given OT and hence modify tuberculin fever.

Three injections of OT were administered at 3 day intervals to a group of BCG-vaccinated rabbits. Twenty-four hours prior to the second injection of OT, 2 ml of NDV was given. The initial injection of OT served to establish the control response, and the last injection was intended to define the fever after 2 previous intermittent injections of OT. In this manner, any alteration of response to OT the day after virus fever could be determined by comparison with the first and last injections of OT. The predicted decrement in fever which follows intermittent injection of OT was also established in a control group.

The results were variable. A few animals demonstrated a marked reduction in tuberculin fever after injection of virus, with recovery to the predicted level on the final injection. However, most virus-inoculated animals developed a prolonged monophasic response to the second injection of OT and had fever on recovery which was greater than that produced by the initial injection of OT. The mean fever indices for the 3 injections of OT and the mean fever curves of experimental and control animals after the second injection of OT are shown in Fig. 7. The failure of the virus to modify tuberculin fever significantly is unexplained but may be dose-related since the febrile response to OT is much greater and, therefore, perhaps more difficult to suppress than the small biphasic response to NDV-induced EP which was modified by preceding tuberculin fever. Unfortunately, the variability of the responses produced by smaller amounts of OT in hypersensitive controls made it difficult to ascertain whether virus had any consistent tolerance-inducing effect on these dosages.

7. Tolerance to Tuberculin Fever after Endotoxin.—Since prior injection of a large dose of endotoxin produces tolerance to fevers caused both by viruses and viral-induced EP (6), it seemed appropriate to determine the effect of endotoxin on tuberculin fever.

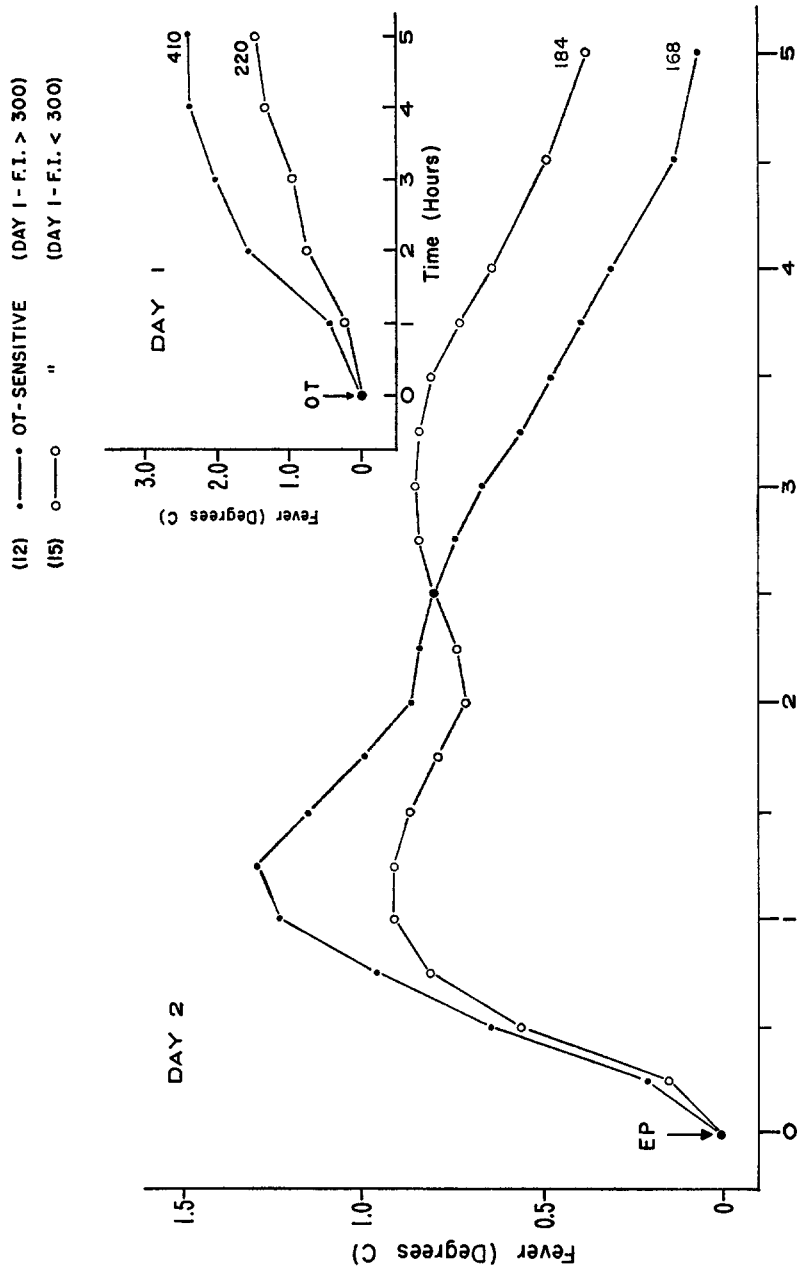

FIG. 6. Mean fever curves of tuberculin-sensitive rabbits injected with 3 ml EP 24 hours after OT. Mean tuberculin fevers shown in insert. Filled circles represent 12 animals with fever indices greater than 300. Open circles represent 15 animals with fever indices less than 300. Numbers at ends of curves are mean fever indices. Differences in fever between the 2 groups at 1st and 5th hour after EP are significant. (FI = fever index.)

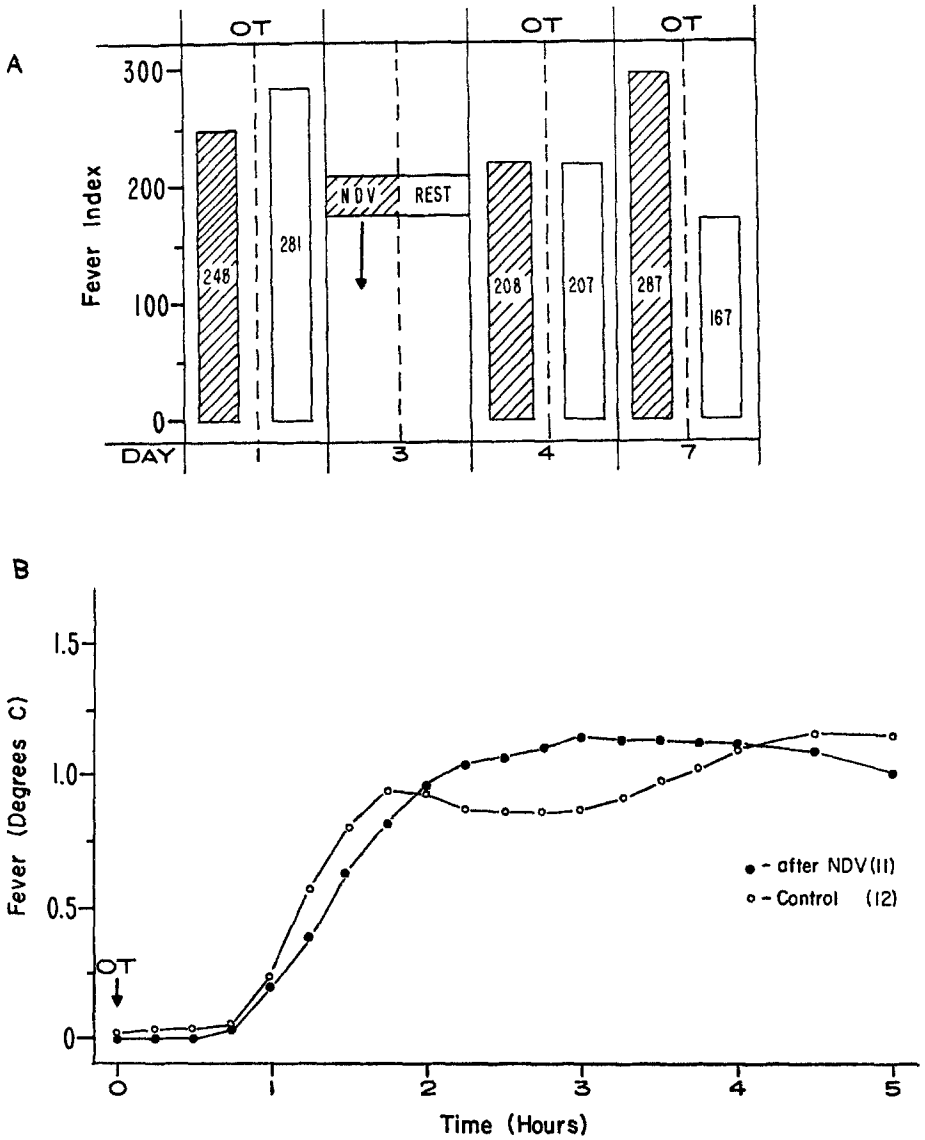

FIG. 7. (A) Mean fever indices of tuberculin-sensitive rabbits injected at 3 day intervals with OT. Cross-hatched bars represent 11 animals injected with Newcastle disease virus on day 3; 12 control rabbits are represented by open bars. (B) Mean febrile responses to OT on day 4 shown in (A). Filled circles represent rabbits injected with virus on preceding day; open circles represent control rabbits.

An experiment was performed which was similar to the previous one except that 2 ml of typhoid vaccine was substituted for virus. Because of the shorter duration of endotoxin fever, the second injection of OT was given 8 hours after typhoid vaccine, at which time the temperature had returned to normal.

The results are summarized in Fig. 8. Four of the endotoxin-inoculated animals responded to OT with small monophasic fevers only, while the fifth rabbit failed completely to develop tuberculin fever after endotoxin.[2] These results

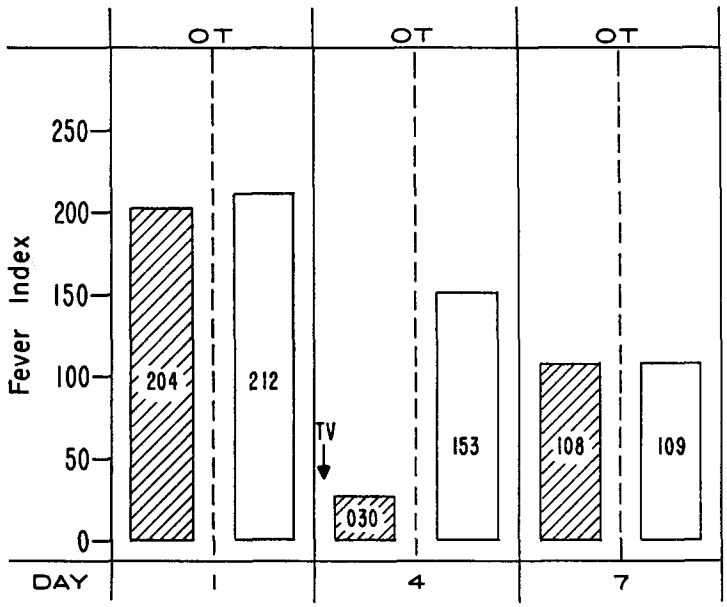

FIG. 8. Mean fever indices of tuberculin-sensitive rabbits given 3 injections of OT at 3 day intervals. Endotoxin was injected 8 hours before OT on day 4 in 5 animals (cross-hatched bars) but not in 6 controls (open bars). (TV = typhoid vaccine.)

contrasted sharply with the biphasic fevers elicited in this group by both initial and recovery injections of OT as well as with the fevers in the control group given the 3 spaced inoculations of OT without intervening typhoid vaccine.

8. The Effect of Intermittent Injections of Endotoxin on Tuberculin Fever.—It was considered that tolerance to OT induced by a single large inoculation of endotoxin might be explained on the basis of either of 2 possibilities.

(*a*) There was cross-tolerance of the usual type which develops to immunologically unrelated endotoxins. Although earlier work had failed to demonstrate tolerance to OT in recipients made pyrogen-tolerant with smaller daily doses

[2] Virtually identical results were obtained with 8 rabbits in an earlier experiment which was of the same design except for omission of recovery responses to OT.

of vaccine (7), it was felt that the increased dosage here might be a critical factor in producing tolerance of the kind described after several injections of endotoxin.

(*b*) A transient tolerance was present similar to that which appears to some agents after the injection of influenzal viruses (6).

The following experiment was designed to test the first possibility by deter-

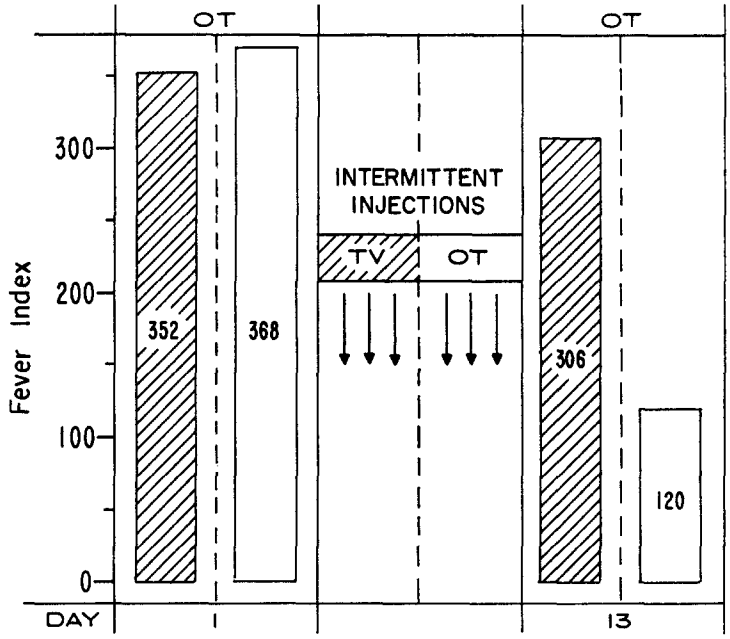

FIG. 9. Mean fever indices of tuberculin-sensitive rabbits injected with OT on days 1 and 13. Cross-hatched bars represent 5 animals, injected 3 times at 3 day intervals with 2 ml of typhoid vaccine, and open bars represent 6 control rabbits given OT at the same 3 day intervals between the first and last injection. (TV = typhoid vaccine.)

mining the effect of repeated, spaced injections of the same large dosage of endotoxin on tuberculin fever. It was reasoned that if tolerance to OT conferred by endotoxin in the preceding experiment were due to one of its nonspecific actions, such as those which modify host resistance, repeated endotoxin injections should be as effective as a single preceding dose in producing tuberculin tolerance.

Tuberculin-sensitive rabbits were divided into 2 groups and given 3 intermittent injections of either OT or 2 ml of typhoid vaccine. Animals of both groups were given OT on the 1st day to establish the control response and again on the last day to evaluate the effect of the intervening treatment. All injections were given at 3 day intervals to eliminate the possibility of transient tolerance of the virus type (6).

The results are shown in Fig. 9. While the group receiving OT alone developed tolerance to OT, the group which was treated with 3 large intermittent doses of typhoid vaccine had biphasic tuberculin fevers of significant magnitude on the last day. It appears, therefore, that tolerance to OT produced by endotoxin depends critically upon an immediately preceding injection. This time relationship coincides with maximal suppression of RES activity by endotoxin (16, 17). It seems unlikely, therefore, that this form of tolerance can be attributed to the subsequent augmentation of both RES function and non-specific resistance to infection which have been reported to follow 48 to 72 hours after endotoxin inoculation (17–19). Furthermore, additional experiments (data to be published) have indicated that recipients respond normally to the direct action of EP when injected immediately after fever produced by typhoid vaccine. The lack of response to OT demonstrated here, therefore, cannot be attributed to shock or unreactivity of the thermoregulatory center and must be due to failure of this stimulus to mobilize EP.

DISCUSSION

These experiments have defined certain features of tolerance which develops to the pyrogenic effect of tuberculin in sensitized hosts. It has previously been inferred from characteristic changes in the skin test that tuberculin tolerance is due to desensitization (7). Although recent *in vitro* studies have given some support to this concept (20), the precise cellular factors involved in desensitization have not yet been elucidated (21–23). There are a number of aspects in which tolerance to tuberculin superficially resembles tolerance to Gram-negative endotoxins. (*a*) Tolerance develops in both systems after repeated injections. (*b*) The refractory state produced by several injections is associated with failure of the same agent to mobilize a circulating pyrogen of endogenous origin (EP). (*c*) Tolerance has a certain degree of specificity in both instances since there is no impairment of the ability of the tolerant host to mobilize EP, and hence respond normally, when unrelated pyrogenic agents are injected.

Tolerance produced by repeated injections of OT, on the one hand, and by bacterial pyrogens on the other can be differentiated, however, on several grounds. Recovery from tolerance to OT occurs more rapidly and in this respect resembles recovery from tolerance to the pyrogenic effect of bovine serum albumin in specifically sensitized rabbits (24). Tolerance to tuberculin, unlike that to bacterial pyrogens, does not depend upon accelerated clearance of the injected agent by the reticuloendothelial system (RES) since tuberculin tolerance is not abolished by RES-blockading agents such as thorotrast (7). In addition, other experiments (unpublished data) have shown that clearance of injected endotoxin is not accelerated in rabbits made tolerant to tuberculin. Finally, the mechanisms of tolerance in these two systems can be distinguished by the failure of the repeated injections of one agent to confer tolerance to the

other and by the inability of certain serum factors which alter both fever and tolerance produced by endotoxins to affect tuberculin fever (7).

In addition to the characteristic tolerance which develops to daily injections of OT or endotoxin, a transient refractory state may be induced to either agent by unrelated pyrogenic stimuli. This latter type of tolerance follows both tuberculin and endotoxin fevers of sufficient magnitude. It can be readily differentiated from tolerance produced by repeated injections of either agent by the response of such refractory animals to an injection of EP.

The use of NDV-induced EP as a test substance under the various conditions of this study is based on evidence that EP normally has two actions when injected in adequate amounts.[3] The first appears to be a direct action on the thermoregulatory center of the brain; the other is believed to be on a peripheral tissue, perhaps the leukocyte, resulting in release of additional EP (15). These two activities are thought to be responsible for the sequence of the first and second temperature peaks, respectively, in normal animals. Hence, in these experiments, when biphasic fever was elicited following the injection of EP, it was considered an indication that EP was "available" in the recipient's tissues. On the other hand, a monophasic response to the test dose of EP was interpreted as evidence that there was interference with the normal release of EP in the host, permitting only the initial, or direct, action of the injected EP to occur.

Rabbits made tolerant by repeated injections of OT responded to EP with biphasic fevers as do endotoxin-tolerant animals (6) indicating that in both forms of tolerance there is no impairment of EP mobilization to heterologous agents. However, following a single injection of OT resulting in sufficient degree of fever, the response to EP was monophasic. A similar modification of the response to EP occurs the day following a single injection of either virus or endotoxin (6). Since all these agents mobilize considerable amounts of EP when given initially but not in recipients made specifically tolerant by a course of injections, it may be hypothesized that EP released by the first injection temporarily prevents additional EP mobilization and hence induces tolerance to the small stimulus of EP, as manifested by absence of the second fever peak. In conformity with this concept is the observation that large doses of viral-induced EP itself produce a similar response to a second smaller injection (6).

Characteristic changes in the shape of the fever curves with development of tolerance to EP also support this hypothesis. When the response produced by injected EP was modified in such a way as to suppress the second peak of fever,

[3] Although as little as 3 ml sera containing NDV-induced EP regularly causes biphasic fever when injected in normal recipients, dosages of 50 ml or more are usually required to produce the same effect in the case of EP evoked by Gram-positive infections (5), typhoid vaccine (6), or OT in sensitized rabbits (unpublished data).

the first peak became exaggerated (see Figs. 5 and 6). This finding, as depicted diagrammatically in Fig. 10, suggests that the activity of EP is conserved so that when its effect on peripheral tissues is blocked, more of the injected material becomes available to stimulate the thermoregulatory center directly, an action to which there appears to be no tolerance (9). The importance of the fever contour rather than fever index in establishing certain tolerant states

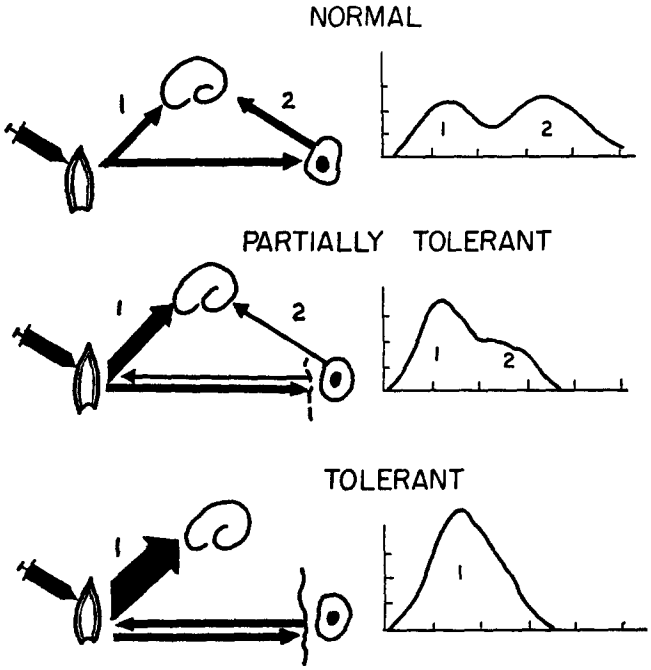

FIG. 10. Diagram of postulated mechanism of action of injected endogenous pyrogen. Symbols to left of graphs represent: (a) rabbit ear (left); (*b*) leukocyte (right); and (*c*) brain (center, upper). 1 = direct action (first peak on graph); 2 = indirect action (second peak on graph). See text for details.

should be emphasized, for under these conditions the area beneath the fever curve may remain unchanged despite fundamental alterations in the reactivity of the host.

The concept that prior circulation of EP produces transient tolerance was also tested by attempting to alter the pyrogenic response to OT injected after fever produced by 2 other exogenous pyrogens. In the present study, a preceding injection of Newcastle disease virus (NDV), unlike typhoid vaccine, failed to suppress tuberculin fever, although there was an alteration in the shape of the fever curve (absence of a second peak) which may indicate a degree of tolerance (Fig. 7). Since NDV (14) produces much higher titers of EP than

endotoxin (11) in the dosages used, it is apparent that the more effective tolerance-inducing capacity of endotoxin must be largely related to some intrinsic factor of the injected agent rather than to its capacity to release EP. In this connection, it is perhaps significant that endotoxins similarly inhibit certain systemic and local reactions of immediate hypersensitivity if given a short time before the challenge injection of antigen (25). The transience of the effect of endotoxin in suppressing tuberculin fever is evidenced by the fact that several spaced injections of the same dosage failed to modify the response to OT appreciably (Fig. 9). In the case of virus, the significance of the prolonged monophasic response to tuberculin on the day following virus inoculation and apparent enhancement of tuberculin fever 3 days later remains to be elucidated.

Although events which mobilize circulating EP may produce transitory tolerance, it is evident in certain cases either that release of EP *per se* is not the chief factor in developing this state or that endogenous pyrogens evoked by various stimuli may differ qualitatively and hence vary in their tolerance-inducing capacity. On the other hand, the biologically similar effects of the pyrogen obtained from polymorphonuclear (PMN) leukocytes (4) and that which is present in the serum after Gram-positive infections (5) or injection of a variety of exogenous pyrogens (7, 8, 11, 14), suggest that this circulating substance may be derived from a single cell source. At present, only leukocytic pyrogen has been partially characterized biochemically (26), but it has been demonstrated that at least endotoxin is capable of releasing a pyrogen from granulocytes *in vitro* (27, 28). Such information in support of a single cellular origin of EP suggests that a pyrogenic agent may produce tolerance nonspecifically by modifying this common cellular target either directly, or indirectly *via* release of EP, in such a way that a different stimulus given a short time later is unable to mobilize normal amounts of additional EP from the same source.

It is not known, however, whether EP which appears in tuberculin fever is derived from PMN leukocytes of the sensitized rabbits. Although such cells are said to be damaged by OT *in vitro* (29, 30), efforts to date have been unsuccessful in demonstrating release of EP when OT is added *in vitro* to granulocytes from peritoneal exudates of BCG-vaccinated rabbits (unpublished data). Furthermore, there is evidence that lymphocytes from sensitized animals release a pyrogenic material when exposed to OT *in vitro* (31, 32) though such a substance has not been obtained from normal lymphocytes (33).

In spite of present uncertainty about the cellular origin of EP in hypersensitivity reactions, it is clear that tolerance to agents which mobilize EP must be defined in terms of factors which limit the subsequent release of EP since all tolerant animals remain responsive to the direct action of this substance (5, 6, 9, 11). The role of cellular antibody in mediating some of the reactions of tuberculin and other forms of delayed hypersensitivity has been well

established and is summarized in several recent reviews (22, 34–36). It may be hypothesized that in tolerance to tuberculin, repeated injections of specific antigen alter this antibody in such a way that it no longer reacts with antigen to activate EP. In addition, however, these experiments have shown that under certain conditions an unrelated agent such as endotoxin may induce a non-specific tolerance to tuberculin. From the data presented here, it seems likely that heterologous substances which are capable of producing this transient refractory state either compete directly or through intermediary products, such as EP, for common cellular "sites" involved in mobilization of EP and by such means may temporarily block the pyrogenic effects of antigen in specifically sensitized hosts.

SUMMARY

Certain characteristics of tolerance which develops to the pyrogenic effects of old tuberculin (OT) in BCG-vaccinated rabbits have been described.

Rabbits made tolerant by several injections of OT lost their ability to produce detectable amounts of endogenous pyrogen (EP) in response to the specific agent (OT) but mobilized normal amounts of EP when given a small unrelated stimulus. On the other hand, when this stimulus followed shortly after an initial tuberculin fever of sufficient magnitude, release of additional EP was suppressed, presumably due to an inhibitory effect of the EP previously mobilized by tuberculin.

Similarly, a single large dose of endotoxin almost completely suppressed the response of sensitized rabbits to OT given several hours later. Since several spaced injections of the same dosage were ineffective, this phenomenon does not appear to be attributable to the known mechanisms by which endotoxins promote non-specific resistance to toxicity and infection. Tolerance to tuberculin could not be definitely shown following an injection of Newcastle disease virus which also produces a circulating EP, and it has been inferred that endotoxin blocks the pyrogenic action of antigen on host tissues directly rather than through mobilizing EP.

On the basis of these observations, the relationship of specific to non-specific tolerance to tuberculin fever has been compared in terms of the ability of such tolerant animals to mobilize EP to heterologous stimuli and it is concluded that the two forms of tolerance are different. Furthermore, the fact that a number of unrelated agents produce tolerance non-specifically supports the concept that there may be a common source of EP released by a number of stimuli, including endotoxins and myxoviruses, as well as antigen in specifically sensitized hosts.

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