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STUDIES IN STAPHYLOCOCCAL FEVER
I. Responses to Bacterial Cells†

Despite intensive investigation of the fever caused by endotoxins of Gram-negative bacteria, the pyrogenic properties of Gram-positive bacteria appear to have attracted relatively little attention. There are scattered reports dealing with fever-inducing effects of whole cultures or culture filtrates of several Gram-positive organisms given intravenously.[1-6] Other investigators, however, have been unable to confirm this in the absence of infection[7,8] and in those cases where fever has been obtained, no systematic attempt has been made to determine whether the pyrogenic constituent resides in a cellular component, the culture medium, or both. Only with Group A streptococci has there been recent study of the pyrogens present and, in most instances, these findings do not appear applicable to other Gram-positive organisms.[9-14]

Gram-positive bacterial infections are among the most pyrogenic diseases in man. Since these microorganisms mobilize an exudate rich in polymorphonuclear leukocytes which contain an endogenous pyrogen (EP), fever has been attributed to pyrogenic substances released by host cells at extravascular sites of infection.[8] In confirmation of this concept, substances with properties of leukocytic pyrogen have been isolated at foci of inflammation and in the blood stream following subcutaneous and intraperitoneal infection of rabbits with Group A streptococci and pneumococci, respectively.[15]

On the other hand, there has been conflicting evidence to show that Gram-positive bacteria are capable of causing fever prior to establishment of an infection since, with the possible exception of Group A hemolytic

streptococci,[9] they do not appear to possess endotoxins similar to those present in Gram-negative bacteria.

The following experiments were designed to determine whether Gram-positive microorganisms, other than Group A streptococci, have a pyrogenic action independent of their ability to evoke inflammation.

In the present study, cells of the Giorgio strain of *Staphylococcus aureus* (Phage type VA-4) have been used chiefly, although several other Gram-positive organisms have been investigated for comparative purposes. A preliminary report has already appeared.[16]

METHODS

Male and female rabbits of several breeds were used, weighing 3 to 4 kg. They were housed in air-conditioned quarters and fed a standard diet of pellets. Experiments were performed in an adjacent air-conditioned room in which the temperature was maintained at 65—70° F. The usual precautions were taken to avoid contaminating pyrogens in glassware, needles and biologic materials. Solutions were routinely checked for sterility.

Temperature recording. Procedures used in selecting rabbits[17, 18] and in recording temperatures[19] were similar to those previously reported.

Cultures. Four different strains of *Staphylococcus aureus* were employed. (1) *Staph. aureus,* Giorgio. This organism was obtained from Dr. David Rogers of Vanderbilt University Medical School. It had been originally isolated from a staphylococcal abscess in man. Its biological properties have been characterized in some detail elsewhere[20] and in subsequent studies by the junior author on experimental pyelonephritis in rabbits[21] and rats.[22] It is hemolytic and coagulase-positive, Phage type 83 (VA 4).* A dose of 10[7] or more organisms given intravenously will regularly establish renal infection in rabbits. (2) *Staph. aureus,* Phage type 3A, 3B, 3C. This and the following two organisms were isolated from clinical infections in the bacteriological laboratories of the Grace-New Haven Hospital in New Haven. This organism is nonhemolytic (human blood) and coagulase-positive. (3) *Staph. aureus,* untypable. This strain is hemolytic and coagulase-positive. It produces mucoid colonies on blood agar plates and a distinct capsule is visible with appropriate staining techniques. (4) *Staph. aureus,* Phage type 80-81. This organism is also hemolytic and coagulase-positive.

Phage types of all four strains were checked several times during the study to ensure that no change had taken place. Only the Giorgio strain was used extensively.† Stock cultures of all staphylococci were maintained on agar slants in tubes with screw caps and parafilm covering. The organisms were transferred periodically from the slants to broth. Overnight cultures were streaked on human blood agar plates which were kept at 4° C. and subcultured to broth at intervals of a month or so. Overnight (18-hour) cultures were obtained by inoculating a single colony of organisms from the blood agar plate into either normal rabbit sera or 10—30 ml. beef heart infusion broth which had

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* Complete phage typing on this organism: 6, 7, 47, 53, 54, 75, 77, 81, 83, 84.
† This was the only strain used in the work discussed in this paper.

been previously autoclaved for two hours to inactivate contaminating Gram-negative bacterial pyrogens. Cultures were centrifuged for one hour at 3,000 r.p.m. and the supernates removed. The cell button was usually resuspended in a volume of pyrogen-free saline equal to that of the original culture and washed once or twice. In several instances, a few milliliters of normal sera were used as the suspending medium. For certain experiments one aliquot of resuspended cells was autoclaved at 15 lb. pressure for 10 minutes at 125° C., while the other aliquot served as a control. Whole cultures, supernates and resuspended cells were routinely titered by the pour plate method with serial hundred-fold dilutions from 10^4 to 10^8. Overnight cultures regularly gave titrations of 10^8—10^9 per ml. Supernates usually varied from 10^4—10^6 per ml. All autoclaved material was checked for sterility.

Other organisms used in the course of the experiments were: (1) *Pneumococcus,* Type IV.* (2) *B. subtilis.* (3) *Listeria monocytogenes,* Type I. (4) *Staphylococcus albus.* This last organism was originally isolated from a human throat. It is hemolytic, coagulase-negative and nonpathogenic to normal rabbits when injected intravenously in dosages of 10^8. (5) *E. coli.* The first three organisms were obtained from the clinical bacteriological laboratories, Grace-New Haven Hospital. The last two came from Dr. L. R. Freedman's laboratory and a number of their biological properties have been described.[23, 24]

The pneumococcus and *Staph. albus* were cultured in normal rabbit sera; the other organisms were grown in beef heart infusion broth. Techniques used for cultures and titrations were identical to those employed with Giorgio strain of *Staph. aureus* except in the case of pneumococcus. Titrations of this organism were carried out in brain-heart infusion agar (Difco) pour plates rather than in plain agar pour plates. For short periods, the pneumococcus was maintained on human blood agar slants or plates before being lyophilized. In later experiments, culture filtrates were obtained by passing supernates of centrifuged whole cultures through either Seitz apparatus or Morton bacteriological filter apparatus with ultrafine fritted disc (Corning). Gentle suction was applied during filtration. All filtrates were cultured to confirm sterility.

Assay of circulating pyrogen. Techniques for passive transfer of sera from febrile donor rabbits to normal recipients have been presented elsewhere.[17, 18]

Endotoxin. Typhoid vaccine (monovalent reference standard NRV-LS No. 1) made from *Salmonella typhosa* V-58, was the Gram-negative bacterial endotoxin used. Rabbits were made pyrogen-tolerant by a course of at least seven daily injections of 1.5 ml. of a 1:10 dilution of the vaccine.[17]

Newcastle disease virus, (NDV). Methods employed in passage and titration of this virus were as previously reported.[19] The hemagglutinin titer of pooled chorioallantoic fluid containing NDV was 1:1280.

Thorotrast. Thorium dioxide (24 to 26 per cent aqueous suspension) manufactured by Testagar Co., Detroit. This material was nonpyrogenic in the 5.0 ml. dosage employed (Lot Nos. 08823 and 11681).

Intravenous infusions. Baxter Plasma-Vac® containers (150 ml.) with Fuso-flo stopper were used with Plexitron Solution Administration Sets (No. R41) and

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* This organism was kindly typed by Mary Ruth Smith and Dr. W. B. Wood, Jr., Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Plexitron Pediatric Scalp Vein Sets with No. 23 needle. Infusions (containing organisms in 100 ml. freshly added saline) generally were given at a rate of two to three drops a minute via the marginal ear vein, with needle lightly taped in place. All rabbits tolerated this without evident discomfort and control rabbits given infusions without staphylococci remained afebrile.

Leukocyte counts. Freely flowing blood was obtained by serial cuts made along the marginal ear vein. Counts were made in conventional manner with Trenner (N.B.S.)
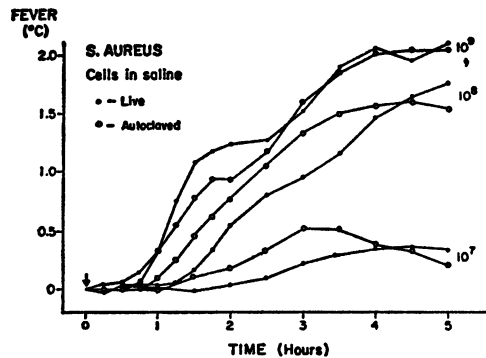

Fig. 1. Average fevers of groups of rabbits in response to three different titrations of cells of *Staph. aureus* (Giorgio strain), washed and resuspended in saline. One aliquot of each concentration of cells was autoclaved; the other served as the live cell control. Each curve represents the average of 5 animals, except at the 10^9 concentration where the average of 4 animals is shown. (In this and following Figures (2-10), suspensions of organisms and filtrates were given in 1 ml. dosages except where specified.)

automatic pipettes and Spencer bright-line hemocytometers. Smears were obtained at intervals to determine differential cell counts which were based on 100 cells.

Fever charts. These were plotted and Fever Indices calculated as in previous reports.[17-19]

RESULTS

Fevers produced by live and autoclaved staphylococci

Figure 1 demonstrates the mean fevers produced in rabbits by intravenous injections of three different concentrations of staphylococcal cells, centrifuged and resuspended in pyrogen-free saline. It is apparent that pyrogenicity of the cells is not related to their capacity to induce infection as similar responses were obtained with the live and autoclaved samples at all tested concentrations. The febrile response produced by intravenous inoculation of this organism is characterized by a prolonged latent period of nearly one hour followed by biphasic fever indistinguishable from fevers produced by a variety of unrelated pyrogenic agents such as Gram-negative

bacterial endotoxin, influenza viruses and antigen in specifically sensitized hosts.

Febrile responses to other Gram-positive bacteria

In order to determine whether this pyrogenic property was generally shared among Gram-positive bacteria, similar suspensions of a number of other organisms within this class were inoculated into groups of rabbits. Figure 2 illustrates the result of intravenous injections of Type IV pneu-
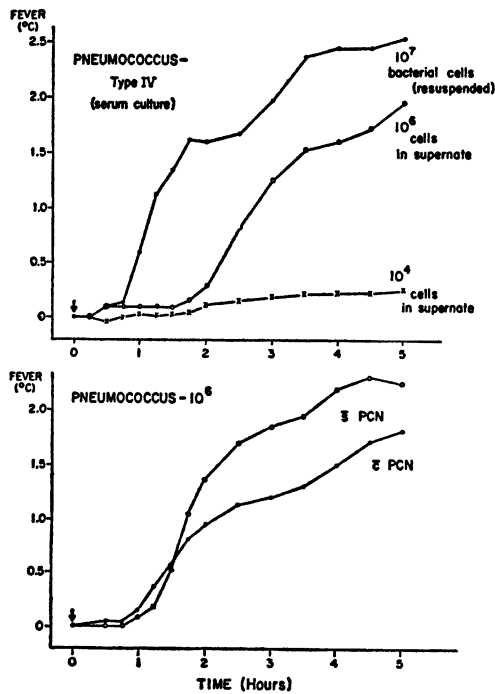

FIG. 2. Top: Mean fevers of rabbits responding to three different concentrations of live pneumococci in serum. Curves are averages of 2 recipients at the 10^7 concentration, and of 3 recipients at the lower two titrations.* Bottom: Average responses of rabbits given 10^8 live pneumococci. One group of 3 animals received a single intravenous inoculation of crystalline penicillin (1 x 10^8 units) 15 minutes before injection of organisms. The other group (2 recipients) served as controls.

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* Subsequent studies on the pyrogenicity of pneumococci in this laboratory and elsewhere (Dr. W. B. Wood, Jr., personal communication) indicate that the number of organisms necessary to produce these effects is about two logs higher than those shown here. The lower and apparently inaccurate counts obtained in this experiment may have resulted from failure of the organism to grow maximally in media used for titration.

mococci from serum-grown cultures. It is evident that both the pneumococcus and staphylococcus produced an identical delayed fever directly proportional to the number of cells injected. In the case of the pneumococcus, supernates containing only 10^4 cells were nonpyrogenic as were culture filtrates in dosages of 1 to 5 ml.
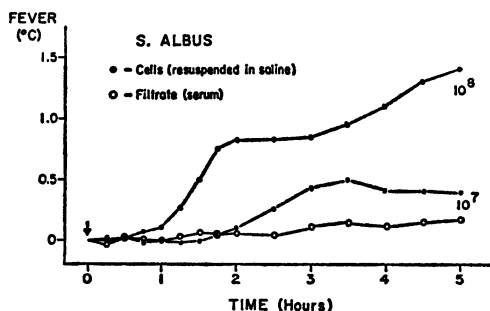

FIG. 3. Average fevers of groups of rabbits responding to two different concentrations of resuspended cells and to 7-8 ml. dosages of culture filtrate of *Staph. albus*. Curves are averages of 3 recipients (10^8 cells), 2 recipients (10^7 cells) and 4 recipients (filtrate). Three recipients (not shown) had no response to 10^6 cells resuspended in saline.
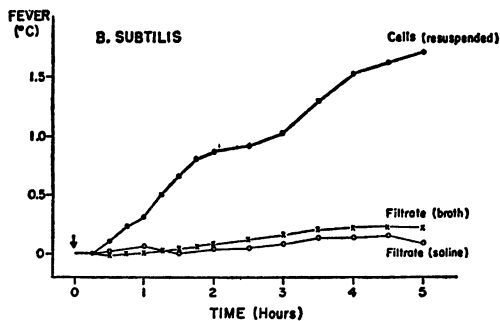

FIG. 4. Mean fevers of 5 recipients to 1×10^8 cells of *B subtilis*, resuspended in saline. Average responses of the same 5 animals to broth culture filtrate and of 3 recipients to a 2½-week-old filtrate of cells resuspended in saline are shown in the lower curves.

As shown in the lower half of the chart, the pyrogenic response to pneumococci was not dependent upon infection since an equivalent fever was produced in recipients given a single large intravenous injection of penicillin 15 minutes prior to inoculation of pneumococci.

Further experiments of the same type were conducted with resuspended cells from 18-hour cultures of a hemolytic *Staph. albus, Listeria mono-*

cytogenes and *B. subtilis.* In each case, as depicted in Figures 3 to 5, the cells regularly evoked the same biphasic pattern of fever. Culture filtrates of all these organisms, with the exception of *Staph. aureus,* (discussed in the following paper), remained nonpyrogenic when injected in dosages of 1 to 10 ml.

Staphylococci, either irradiated or subjected to bacteriostatic doses of streptomycin *in vitro,* similarly retained their abilty to produce fever when tested at titrations of 10^8 to 10^9.

Relation of leukopenia to onset of staphylococcal fever

The long latent period which followed intravenous inoculation of these Gram-positive organisms suggested that they produced fever indirectly by
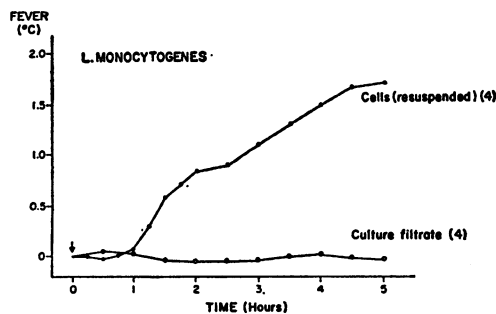

FIG. 5. Mean responses of the same 4 rabbits to 1 x 10^8 cells of *Listeria monocytogenes,* resuspended in saline, and to broth filtrate from the same culture.

releasing a pyrogen from host tissues. Rogers has shown that circulating staphylococci are rapidly incorporated in polymorphonuclear leukocytes.[25, 26] Since granulocytes are the only known source of pyrogen within the body,[27, 28] it seemed probable that injected bacteria, whether live or autoclaved, are phagocyted in the blood stream by these cells which, in turn, release endogenous pyrogen.

In order to ascertain the temporal relation between leukopenia and fever induced by intravenously inoculated bacteria, four rabbits were injected with 10^9 washed and autoclaved staphylococci, and total and differential leukocytic counts were obtained at regular intervals, as shown in Figure 6. Following inoculation, there was an immediate fall in circulating white blood cells to an average level of 50 per cent of the control values. This leukopenia, as has been reported by Rogers after injection of live cells, was almost entirely confined to cells within the granulocyte series and

persisted throughout the latent period before onset of fever shown in the upper half of the Figure.

Presence of circulating pyrogen after injection of staphylococci

Since granulocytopenia induced by intravenous inoculation of staphylococci preceded the onset of fever, it seemed likely that these organisms, like endotoxins of Gram-negative bacilli, cause fever by stimulating release of pyrogen from leukocytes. A passive transfer technique[18] was utilized to ascertain whether such a circulating pyrogen was present. Two donor rabbits were injected with 10^8 live organisms and bled four hours later (see Fig.
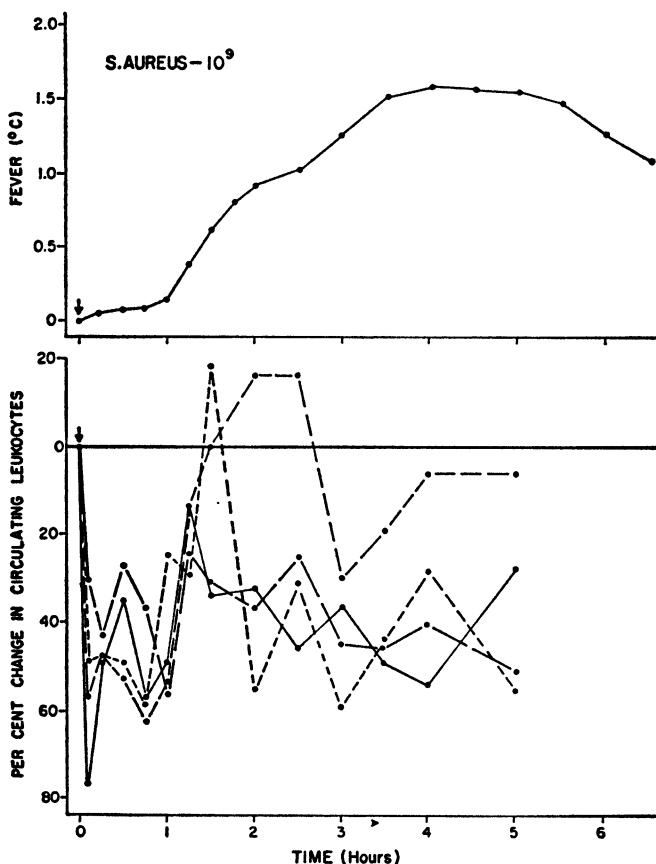

FIG. 6. Top: Mean febrile response of 4 rabbits to 1.4 x 10^9 washed, autoclaved cells of *Staph. aureus* (Giorgio). Bottom: Individual total leukocyte responses of the same 4 rabbits after inoculation.

7). The unpooled serum from the febrile donor rabbits was given to five recipients in 10 or 30 ml. dosages. Serum transferred in this manner produced prompt monophasic fevers which reached a peak approximately one hour after inoculation. The rapid onset of fever produced by the serum in recipients contrasts with the long latent period of one hour which followed injection of staphylococci in the donor animals. A substance (or substances) with the same pyrogenic properties, collectively termed "endogenous pyrogen" (EP), has been demonstrated by similar bioassay tech-
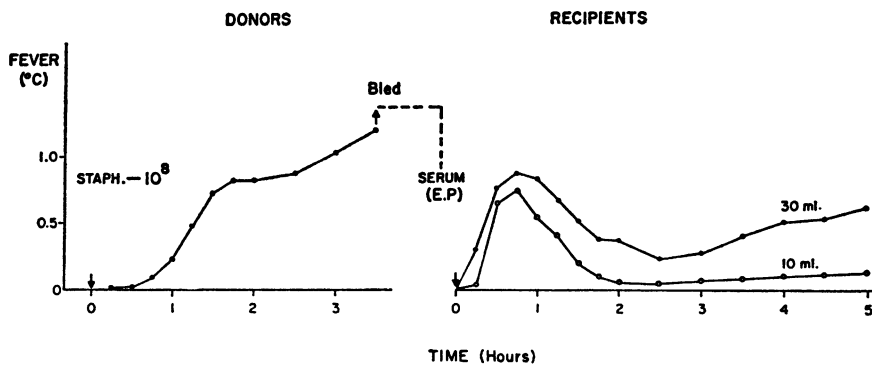

FIG. 7. Left: Average fevers of 2 rabbits given 10^8 live cells of *Staph. aureus* (Giorgio) resuspended in 2 ml. serum. Upright arrow indicates time of exsanguination. Right: Average responses of 3 recipients to 30 ml. unpooled serum and of 2 recipients to 10 ml. unpooled serum from donors bled as indicated by upright arrow at left. E.P. = endogenous pyrogen.

niques in the circulation of rabbits given other fever-inducing stimuli such as Gram-negative endotoxin, myxoviruses and tuberculin in BCG-sensitized hosts.[29]

Responses of endotoxin-tolerant recipients to staphylococci

A characteristic of fevers produced by endotoxins of Gram-negative bacteria is rapidly developing tolerance to daily injections.[30] Tolerance has been shown to be associated with accelerated clearance of endotoxin from the blood stream by cells of the reticuloendothelial system. Cross-tolerance develops to endotoxins from immunologically unrelated Gram-negative bacteria and is, therefore, a sensitive measure of the presence of this substance. After blockade of the RES by colloidal agents such as thorium dioxide (Thorotrast), tolerance to endotoxin is rapidly abolished.[31]

In an experiment designed to determine whether pre-existing tolerance to endotoxin would modify the pyrogenic response to staphylococcal cells,

a group of rabbits was rendered pyrogen-tolerant by a series of daily inoculations of typhoid vaccine (see METHODS). The day following the last injection of vaccine this group, and a similar control group, were given a minimally pyrogenic inoculum of washed autoclaved staphylococcal cells. As shown in Figure 8, the mean response of the tolerant group was significantly less than that of the controls. Since the febrile response to cells was restored following a blocking dose of Thorotrast, it appears likely that tolerant recipients clear this small dose of staphylococci more rapidly from
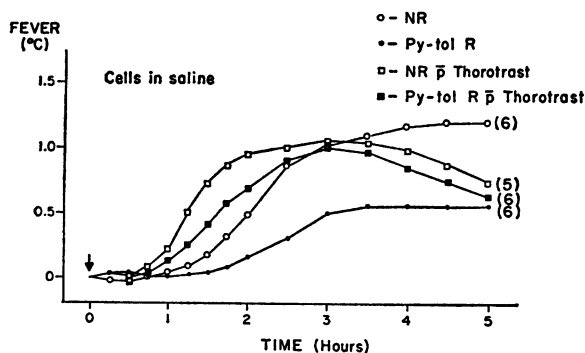

FIG. 8. Responses of groups of normal and endotoxin-tolerant recipients to 7 x 10⁷ autoclaved staphylococci (Giorgio) before and after injection with 5 ml. Thorotrast. The number of animals in each group is in parentheses. (N.R. = normal recipient. Py-tol R. = pyrogen-tolerant recipient.)

the blood stream due to increased activity of the RES, induced by the preceding course of endotoxin. As a consequence, removal of staphylococci by circulating granulocytes, and hence release of EP and fever, would be presumably reduced in such tolerant animals. Conversely, after blockade of RES by Thorotrast, a larger proportion of bacterial cells would be available to be phagocyted by granulocytes, resulting in restoration of the pyrogenic response to normal.

Responses of normal and endotoxin-tolerant animals to washed Gram-negative organisms

The delayed fevers produced by both staphylococci and pneumococci resemble closely those resulting from intravenous inoculation of influenza viruses[19, 32] and colloidal suspensions of other agents such as dextran, Thorotrast, quartz and fat.[33] In addition, the pyrogenic factor in Gram-positive bacteria and in these viruses appear to be closely associated with the bacterial cell and viral particle, respectively. The question, therefore, arose whether

a common physico-chemical property might be responsible for their pyro-genicity. If such were the case, it was reasoned that autoclaved cultures of Gram-negative organisms, repeatedly washed and resuspended in pyrogen-free media to remove endotoxin, might produce delayed biphasic fevers identical to those of staphylococci and virus in normal animals. Accordingly, a six-hour broth culture of *E. coli* was autoclaved and washed four times with saline before injection into groups of normal and tolerant rabbits.
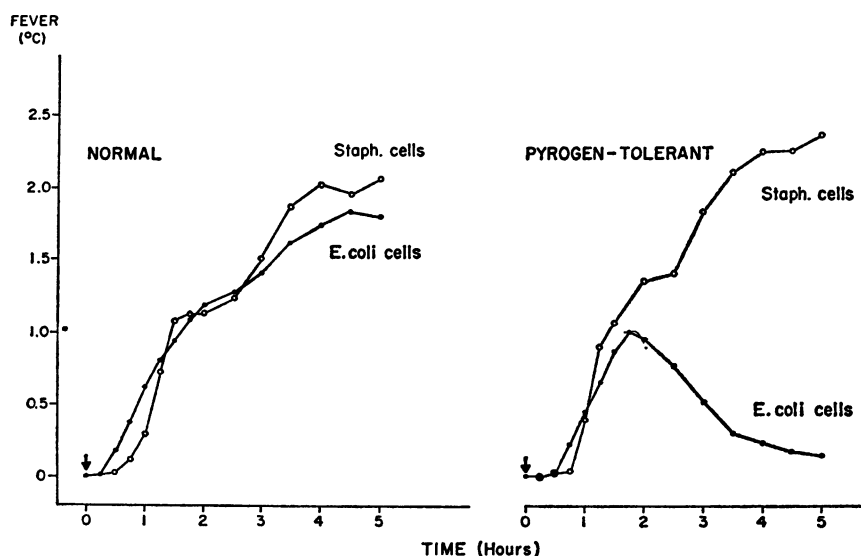

Fig. 9. Mean fevers of 5 normal and 5 endotoxin-tolerant rabbits after receiving *E. coli* 5×10^8 autoclaved cells, washed four times and resuspended in saline. Mean responses of 2 normal and 2 tolerant recipients to 10^9 resuspended cells of *Staph. aureus* (Giorgio) are shown for comparison.

In Figure 9 it can be seen that endotoxin-tolerant recipients had a characteristically diminished response to the washed bacilli suggesting that residual endotoxin, presumably located on the cell surface, had resulted in their more rapid removal from the circulation of tolerant animals. By contrast, approximately the same number of washed staphylococcal cells (an organism about the same size as *E. coli*) produced in this larger dosage fevers which were identical in normal and tolerant animals (compare Figures 8 and 9). These data suggest that properties such as chemical characteristics of the cell surface, where endotoxin is located, may play an important role in the disposition and, hence, pyrogenicity of various intravenously injected bacterial and viral agents.

Fevers produced by daily inoculations of staphylococci

To determine the effect of repeated challenge with staphylococci, four recipients received daily inoculations of 10^8 washed autoclaved organisms. Their fever curves are shown in Figure 10 (shaded figures for the 1st, 2nd, 4th and 6th days). A slight and variable degree of tolerance was manifested by some decrease in the size of the second fever peak by the end
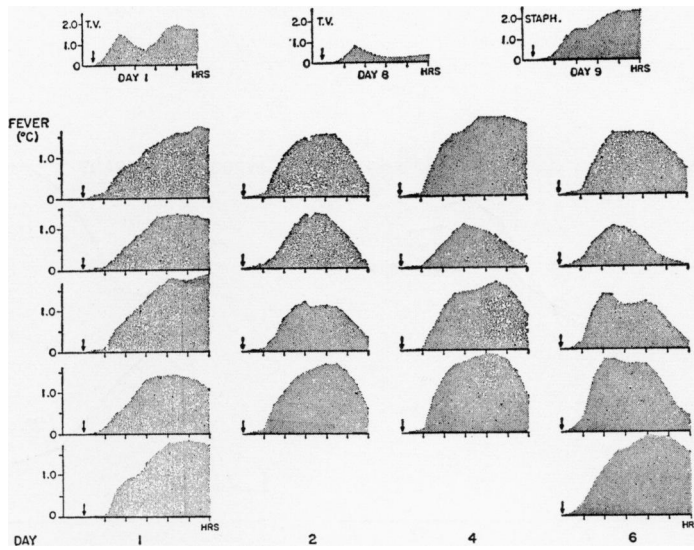

FIG. 10. Fevers produced by 1.7×10^8 washed, autoclaved staphylococci (Giorgio) resuspended in saline and given daily to 4 normal rabbits. Responses on days 1, 2, 4 and 6 are shown in shadowgraphs. Top row of shadowgraphs shows initial and tolerant response (mean of 2 rabbits) to daily inoculation of typhoid vaccine, $(1.5 \times 1:10$ dilution) and the response to staphylococcal cells on the day following last inoculation of vaccine. Bottom row of shadowgraphs shows the mean of 2 controls given injections of autoclaved staphylococcal cells on days 1 and 6 only.

of the series of injections as compared with the average response of two control animals given only two spaced inoculations and shown in the bottom row. This degree of reduction in fever was also seen with daily inoculation of Listeria cells and characteristically appears with repeated injections of a sufficient dosage of EP. Although the mechanism of the altered responsiveness to repeated large injections of EP is still uncertain, this form of tolerance may be clearly distinguished from tolerance to endotoxin shown in the first two figures in the upper row of shadowgraphs. An additional difference shown in tolerance to staphylococci was that the

febrile response was not restored in five rabbits after blockade of the RES by Thorotrast, whereas endotoxin tolerance is rapidly abolished by this procedure.[31]

Effect of continuous infusions of staphylococci

In view of the demonstrated pyrogenicity of cocci injected intravenously, an attempt was made to simulate clinical bacteremia and to produce sustained fevers by prolonging the administration of bacteria. Figure 11
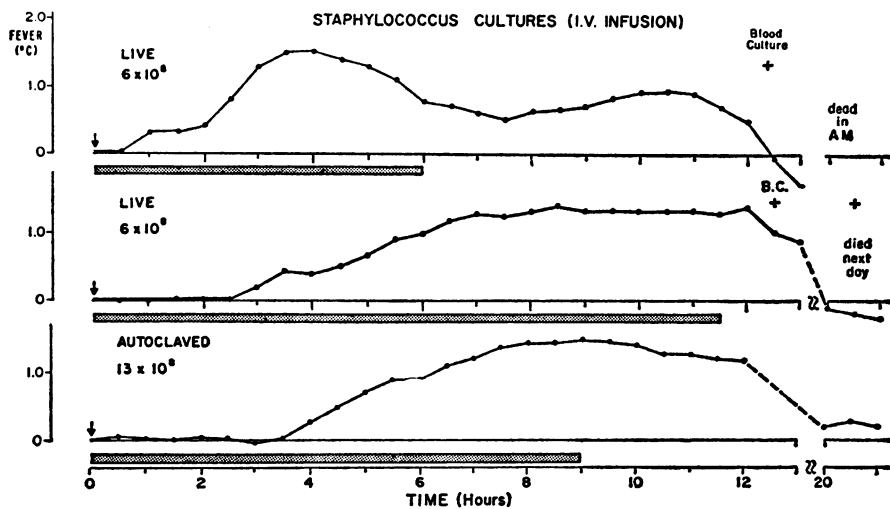

FIG. 11. Responses of 2 rabbits to intravenous infusion of 6 x 10⁸ live staphylococci (Giorgio) and of 1 rabbit to 1.3 x 10⁹ autoclaved organisms, each in 100 ml. saline. Duration of infusions is shown by stippled bars beneath each response.

illustrates three representative fever curves—two in animals injected with 10^8 live organisms, and the third in one injected with 10^9 washed autoclaved cocci, all given by slow intravenous saline infusion, as indicated by the horizontal stippled bars (see METHODS). In the first animal, shown in the top row of the Figure, fever quickly reached a maximum due to rapid infusion of organisms, fell slightly during the infusion and finally decreased abruptly to subnormal values as the animal presumably developed shock before death. When the infusion rate was slowed in the next rabbit, fever remained high throughout the 12-hour period. From the lower curve it is apparent that autoclaved cocci are equally effective in sustaining fever when given by slow intravenous drip. These results indicate that tolerance to pyrogenic stimuli given repeatedly within a period of hours is not

due primarily to refractoriness of the thermoregulatory centers of the brain. It also appears that persistent bacteremia was sufficient to cause prolonged fever independently of the ability of these organisms to establish an extravascular focus of infection. Similar persistent fevers over a period of 14 hours have been produced by slow saline infusions containing small amounts of EP, further evidence that continued release of small amounts of this material by staphylococci is directly responsible for the observed fever.
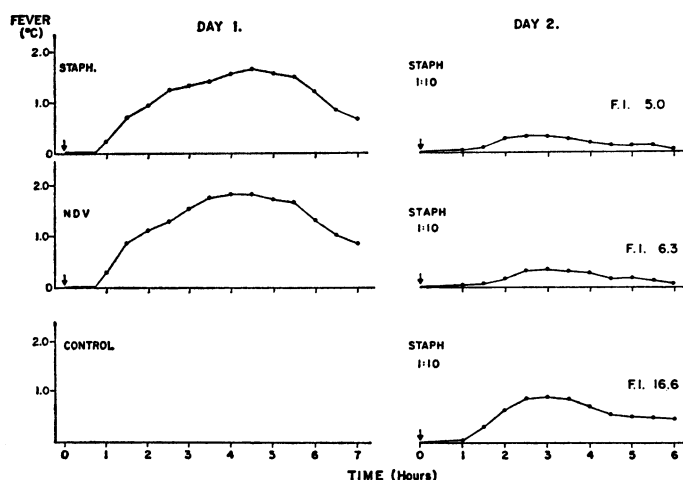

FIG. 12. *Day 1.* (left) : Mean responses of 4 rabbits to 2 x 10⁸ autoclaved staphylococci (top graph) ; and of 4 rabbits to 3 ml. Newcastle disease virus (NDV) (middle graph). The control group (6 rabbits) was not injected. *Day 2.* (right): Mean febrile responses of the same groups to 5 x 10⁷ washed autoclaved staphylococci (2.5 ml. of a 1 : 10 dilution of original injection).

Tolerance to staphylococcal fever induced by large inoculation of staphylococci or Newcastle disease virus

When two different pyrogens produce fever by mobilizing an EP, but do not modify each other's clearance from the blood stream, cross-tolerance would seem indirect evidence that they compete for a common source of EP. Since the thermoregulatory center responds to injected EP, except during shock or pre-existing fever, it seems likely that this form of tolerance involves mechanisms which block the release of EP. To determine if cross-tolerance could be demonstrated between Newcastle disease virus (NDV) and staphylococci, both of which produce a circulating EP, the following experiment was performed. Two groups of rabbits were given

a large injection of either staphylococci or NDV. The following day both groups received a smaller dosage of staphylococci, as did a similar group of controls. As can be seen in Figure 12, the mean febrile responses of the two experimental groups were suppressed on the second day. In this instance, tolerance appears to be due to the inability of the recipient, which has recently been stimulated to mobilize a large amount of EP, to release a normal additional supply in response to a second smaller stimulus. Since NDV is capable of inducing as much tolerance to staphylococcal cells as a large preceding dosage of staphylococci, there may be a common source of EP available to a variety of heterologous stimuli, including endotoxin, certain viruses and Gram-posititve organisms.[29]

DISCUSSION

Since the classic researches of Hort and Penfold[7, 34, 35] and later those of Seibert[36] and Co Tui[2, 3] on the relation of microorganisms to fever, a great deal of work has been devoted to the role of Gram-negative bacteria and their endotoxins in the pathogenesis of a variety of biological phenomena, including fever.[37]

In contrast to the immediate but transient fevers which follow intravenous injections of both living and dead Gram-negative bacteria, most Gram-positive organisms were reported by Hort and Penfold to cause fever only a number of hours after inoculation when an infection had presumably been established in the tissues of the host.[7] Furthermore, heat-killed micro-organisms of the Gram-positive group were said to be nonpyrogenic, both on initial and subsequent injection. Similarly, Bennett has stated that pneumococci given intravenously produced fevers which appeared late after inoculation and were felt to be due to infection since they could be prevented by prior treatment of the recipient rabbit with penicillin.[8]

The results reported here are at variance with these observations. In sufficient dosage, all Gram-positive organisms tested, whether live or dead, produced vigorous biphasic pyrogenic responses after a characteristic latent period of 45 — 60 minutes. Other investigators, using whole cultures of several of these bacteria, have reported similar findings.[1, 4-6]

These responses to Gram-positive cells may be differentiated from those produced by Gram-negative endotoxin in a number of respects.

1. Except in the case of some types of staphylococci (discussed in the following paper) culture filtrates of Gram-positive bacteria were nonpyrogenic in the dosage injected, whereas filtrates of virtually all Gram-negative bacterial cultures are a potent source of endotoxin.

2. There was a latent period of nearly one hour before onset of fever, at least twice that seen with comparably pyrogenic doses of Gram-negative endotoxin.

3. The rapidly-developing leukopenia after inoculation of cells was limited almost entirely to polymorphonuclear forms, as previously reported, and was more transient than that seen after injection of Gram-negative endotoxin which also affects cells of the mononuclear series.

4. Although recipients with pre-established tolerance to endotoxin (contained in typhoid vaccine) had moderately diminished responses to a low dose of staphylococcal cells, a larger inoculum of cells produced equal fevers in normal and tolerant animals. By contrast, there was marked reduction in the response of pyrogen-tolerant rabbits to a similar large dose of washed heterologous Gram-negative cells, presumably due to endotoxin still contained on their surface.

5. Daily inoculations of washed autoclaved staphylococcal cells resulted only in slight tolerance which was unmodified after blockade of the RES with thorium dioxide (Thorotrast). The greater degree of tolerance which develops to moderate doses of endotoxins, on the other hand, is abolished by Thorotrast.

The similarity of febrile responses produced by these various Gram-positive bacteria and the direct correlation between degree of fever and number of injected microorganisms suggest that a similar physical or chemical property may be responsible for the pyrogenic effect. It is possible that particle size and/or surface charge is of importance. The data presented do not provide information on this point. It seems significant, however, that nearly identical febrile responses, which usually appear after a prolonged latent period of 45 — 60 minutes, may be produced by a diverse group of substances of colloidal or particulate nature, including fat, glycogen, starch, dextran and zymosan, as well as certain chemical preparations in the colloidal state, such as iron, gold, sulfur, calcium phosphate, thorium dioxide and particles of silica.[35] Earlier reports of particle fever[36] have been unconfirmed and have in any case been subject to suspicion because of lack of precautions taken to prevent contamination of the materials with Gram-negative bacterial endotoxin.[37] This possibility appears to have been adequately excluded in the present experiments and the hypothesis should be reconsidered that this diverse group of substances causes fever by virtue of a common physicochemical factor. Since most of the agents studied in this group produce leukopenia soon after intravenous inoculation, it seems plausible that fever is due to

release of an EP from circulating leukocytes. Intravenously injected staphylococci and pneumococci are rapidly phagocyted by granulocytes,[25, 26] an activity which in these cases may provide the stimulus to EP mobilization.

Despite the heterogeneity of agents capable of causing "particle fever," the specific chemical structure of the surface of the injected agents may be important in certain instances, since washed autoclaved Gram-negative bacterial cells (*E. coli*) produced only abortive fevers in recipients made tolerant to heterologous endotoxin contained in typhoid vaccine. These cells, although approximately equal in size to staphylococci, apparently were much more rapidly cleared by the RES in pyrogen-tolerant recipients (shown also by other techniques) so that their ability to mobilize EP was reduced. In this case, it appears that residual endotoxin on the surface of Gram-negative cells determines their different fate in endotoxin-tolerant rabbits.

Fevers with Gram-positive infections are often sustained or remittent for many hours or days. This is particularly true of bacterial endocarditis, where inflammation is minimal, as well as of pneumonia or diseases with localized suppuration in which EP, produced by granulocytes at the infected site, eventually enters the circulation. Since live or autoclaved Gram-positive bacterial cells cause persistently elevated temperatures when given by slow intravenous drip, an explanation may be provided for the continuous fevers present in clinical states where low grade bacteremia persists without a significant extra-vascular inflammatory focus.

Worthy of comment are certain similarities in the response produced by Gram-positive cells, on the one hand, and by endotoxins of Gram-negative bacteria, on the other. In both instances, there is a well-defined though different latent period before the onset of fever, suggesting that the pyrogenic action of each agent is indirect. Second, during the latent period there is a marked fall in circulating leukocytes due to sequestration of cells in various viscera and in the periphery (the latter visible *in vivo*). Third, fever in both systems is associated with release of an EP (presumably from leukocytes). Finally, since EP itself, when given in sufficient dosage, causes biphasic fevers[15, 39] it seems likely that the biphasic febrile responses produced by both endotoxins and Gram-positive cocci are due entirely to mobilization of this substance[39] rather than in part to any direct action of either injected agent on the thermoregulatory center.[40-42]

An unsolved question in the pathogenesis of both experimental and clinical fevers is whether there is a single tissue source of EP.[39] With Gram-negative endotoxins and infections with Gram-positive organisms,

there is increasing evidence that polymorphonuclear leukocytes are the only cells involved. However, in fevers produced by influenzal viruses and by tuberculin in specifically sensitized recipients, the cellular source of circulating pyrogen is unknown. Since a large dose of an unrelated pyrogen such as NDV has been shown to suppress the febrile response to a small injection of staphylococcal cells, it seems reasonable to infer that these agents compete for release of EP from a common tissue source. Large doses of endotoxin similarly produce a nonspecific tolerance to the pyrogenic action of either myxovirus[43] or tuberculin in specifically sensitized recipients.[44] Such tolerant animals respond normally to a small injected dose of EP but appear transiently unable to release a normal additional amount of this substance following its mobilization by another agent.

Studies of febrile responses to these various pyrogens in severely neutropenic rabbits may help to determine whether there is a single tissue source of EP. Markedly suppressed or absent fevers under these conditions would provide indirect evidence that the polymorphonuclear leukocyte is the source of EP, as seems to be the case in endotoxin fever.[45]

SUMMARY

1. Cells of a variety of Gram-positive organisms, given intravenously to rabbits, produced brisk, biphasic fevers after a latent period of one hour.

2. The minimal pyrogenic dose appeared to vary with different bacterial species but was generally about 10^7.

3. Since equivalent fevers were also produced by dead organisms, this response is independent of infection.

4. In the case of staphylococcus, fever was preceded by a transient, profound granulocytopenia. The mechanism of fever involved release of an endogenous pyrogen (EP), presumably from circulating granulocytes, which was similar in its activity to that mobilized by endotoxin and influenzal viruses in normal animals and by antigen in specifically sensitized hosts.

5. Fever was continuous or intermittent depending on the method of administration of bacterial cells.

6. Fever produced by these Gram-positive microorganisms differed in a number of respects from that caused by endotoxins of Gram-negative bacteria. Unlike the febrile tolerance which develops to daily inoculations of Gram-negative bacteria or their endotoxins, no significant tolerance was evident to repeated injections of autoclaved Gram-positive cocci or bacilli. Endotoxin-tolerant animals exhibited only a slight degree of tolerance

when given minimal doses of staphylococci. Such animals were not, however, tolerant to a larger dose of staphylococci, though they had much smaller pyrogenic responses to the same number of Gram-negative bacterial cells, presumably due to residual endotoxin on their surface.

7. Since responses similar to those evoked by Gram-positive bacteria may be produced by colloidal suspensions of a variety of non-bacterial agents, it seems possible that these diverse substances share some common physicochemical property (such as molecular size or charge) which causes fever via release of an EP from granulocytes.

8. The possible relevance of these findings to clinical fevers associated with persistent bacteremia is evident.

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