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STUDIES IN STAPHYLOCOCCAL FEVER
II. Responses to Culture Filtrates**

In the preceding paper,[1] pyrogenic properties of several Gram-positive microorganisms were described. The bacterial cells of all species investigated, including both cocci and bacilli, when given in sufficient dosage produced vigorous biphasic fevers after a latent period of 45 to 60 minutes. Staphylococcal fevers were associated with a transient granulocytopenia and a circulatory pyrogen of endogenous origin (EP). It was postulated that cells of the Gram-positive group of organisms, like a number of other colloidal substances, produce fever by virtue of some physicochemical property which stimulates host cells (presumably granulocytes) to release an EP which, in turn, acts directly on the thermoregulatory centers of the brain to cause fever.

On the other hand, culture filtrates of all Gram-positive organisms investigated, with one exception, were nonpyrogenic. In several strains of *Staphylococcus aureus,* both broth and serum culture filtrates were found to contain a pyrogen which caused fevers closely resembling those resulting from intravenous inoculation of bacterial cells.

The present paper deals with some of the biological properties of this filterable pyrogen which can be distinguished on a number of grounds from the endotoxins of Gram-negative bacteria. Evidence is presented that a naturally acquired specific hypersensitivity may play a role in the pyrogenic response to culture filtrates of certain strains of *Staphylococcus aureus.*

METHODS

General. All techniques relative to pyrogen-free preparation of materials, use of rabbits, temperature recording, assay of circulating pyrogen, plotting

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of fever curves, etc. were identical to those described in the preceding paper.[1]

Cultures. Four strains of *Staph. aureus* were used exclusively. These belonged to the following phage types:

(a) 83 (VA4), strain Giorgio.
(b) 3A, 3B, 3C.
(c) 80-81.
(d) an untypable, encapsulated strain.

All were coagulase-positive and all but the 3A strain were hemolytic on human blood. Their sources and cultural characteristics have been described earlier.[1]

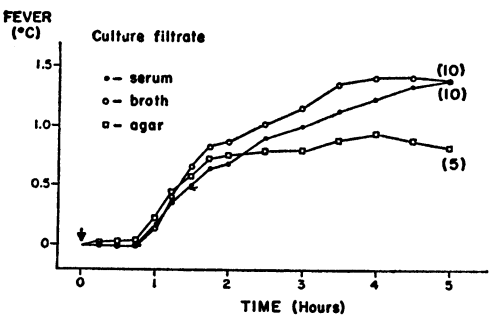

FIG. 1. Febrile responses of three groups of rabbits to culture filtrates of *Staph. aureus* (Giorgio) grown in three different media. In this and following Figures, numbers in parentheses indicate number of animals in each group. Volume of injections in this and in following Figures was 1 or 2 ml. except where indicated.

Eighteen-hour broth culture supernates and filtrates were used largely. While culture titrations at this interval were regularly $10^8 - 10^9$ per ml., titrations of supernates (used in earlier experiments) never exceeded 1 x 10^7 per ml. and were generally $10^4 - 10^6$ per ml. Since this dosage of staphylococci did not cause fever in rabbits,[1] the pyrogenic effects of culture supernates can be attributed to other factors. Filtrates were cultured to confirm sterility. Techniques for obtaining both supernates and filtrates have been described in the preceding paper.[1]

Staphylococci (Giorgio) were grown in one experiment in soft agar, as in techniques for propagating and harvesting bacteriophage.[2,3] Organisms grown on agar slant were emulsified in broth and added (without phage) to semisolid agar which was poured over a base agar layer. After five hours of incubation at 37° C., the plate was kept overnight at room temper-

ature. The semisolid medium was then scraped free, nonpyrogenic broth added, and the mixture centrifuged. The supernatant fluid was filtered and tested for presence of staphylococcal pyrogen.

Tuberculin hypersensitivity. In one experiment, a group of rabbits was made hypersensitive to the pyrogenic effects of tuberculin by a single intravenous injection of BCG. Materials used and techniques for culturing BCG and inducing tuberculin hypersensitivity have been presented in other studies.[4,5]

Typhoid vaccine. Source of vaccine and method of producing tolerance to this agent were the same as previously described.[1]

RESULTS

Fevers produced by culture filtrates of staphylococci grown in different media

Figure 1 demonstrates the average febrile responses of three groups of rabbits to intravenous injections of 1 ml. filtrate of 18-hour cultures of staphylococci (Giorgio strain) grown in three different media. It is apparent that the fevers produced by the filterable pyrogen are uninfluenced by the media in which the staphylococci are grown. Two features of the pyrogenic response are noteworthy:

1. There is a regular latent period of 45 to 60 minutes (depending on dose) before the rise in temperature.
2. The response is usually biphasic: the first peak at about the second hour and the second peak (higher when sufficient pyrogen is administered) at 3½ to 4½ hours.

This response closely resembles fevers produced by a variety of Gram-positive bacterial cells and myxoviruses, as well as by antigen in previously sensitized recipients. In contrast to fevers caused by Gram-negative bacterial endotoxin, the latent period is prolonged and the fever cycle is not accelerated or augmented when the staphylococcal pyrogen is liberated in a serum rather than in a broth medium.[6-8]

Responses to culture filtrates of staphylococci of different phage types

Figure 2 shows the average febrile responses of groups of new rabbits to 1 ml. intravenous inoculation of 18-hour broth culture filtrates from four different phage types of *Staphylococcus aureus.* The Giorgio strain produced large biphasic fevers with mean peaks of 0.85° and 1.4° C. at 2 and 4 hours, respectively, whereas filtrates of the untypable (encapsulated) and 80-81 phage types were virtually nonpyrogenic. The average response

to filtrates of the 3A phage type was intermediate with a prolonged latency and more gradual rise in temperature. The resuspended bacterial cells, however, from the cultures of all four phage types produced identical fevers.

Effect of varying dosage of culture filtrate

Responses of groups of new rabbits to three different dosages of Giorgio 18-hour broth culture filtrate are shown in Figure 3. Fevers of about one-half the magnitude of those produced by the undiluted filtrate (by fever index at 5 hours) were obtained with one-fifth the dosage. Dilution of

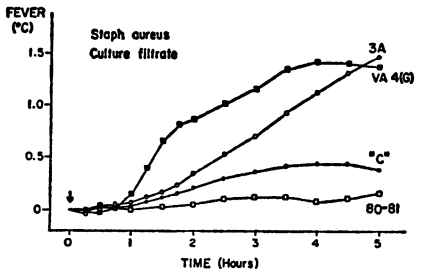

FIG. 2. Fevers produced in new rabbits by 18-hour broth culture filtrates of four different phage types of staphylococci ("C" = a nontypable encapsulated organism). Each curve represents an average of 10 rabbits.

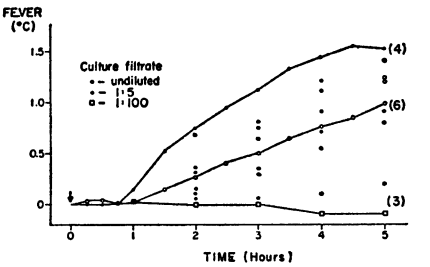

FIG. 3. Mean febrile responses produced by three different dilutions of 18-hour broth culture filtrate (Giorgio). Open circles above and below the mean curve for the 1:5 dilution indicate the individual responses of this group.

filtrate to 1:100 abolished its pyrogenicity. As shown in the Figure, variation in individual responses at the intermediate dilution was considerable.

Factors influencing production of filterable pyrogen

In order to determine whether there was a direct correlation between age of the culture and amount of filterable pyrogen, as in cultures of Gram-negative endotoxin-producing organisms, a series of broth and serum cultures were grown for various periods between 2 and 24 hours. At intervals, the cultures were centrifuged and their supernates (or filtrates) inoculated into groups of rabbits in individual dosages of 1 ml. The results of representative broth and serum cultures grown for 2 to 18 hours are shown in Figure 4. It is apparent that presence of pyrogen in the medium was directly related to the number of microorganisms in the culture rather than to either the age of the culture or type of culture medium per se. In general, when the bacterial count exceeded 5×10^7 per ml. both media were pyrogenic in the dosage employed. Staphylococci cultured in broth

often achieved this titer by 3 hours, whereas none of six serum-grown cultures had reached this level at 6 hours. At 18 and 24 hours, bacterial counts in the two media were equal (10^8 — 10^9) and nearly identical febrile responses were obtained from supernates of both cultures. Unlike cultures in which Gram-negative endotoxin is liberated, older staphylococcal cultures did not produce greater toxic or pyrogenic effects than did those grown for 3 or 4 hours in broth. It appears that delay in appearance of pyrogen in cultures incubated in serum, as compared with broth, may be ascribed

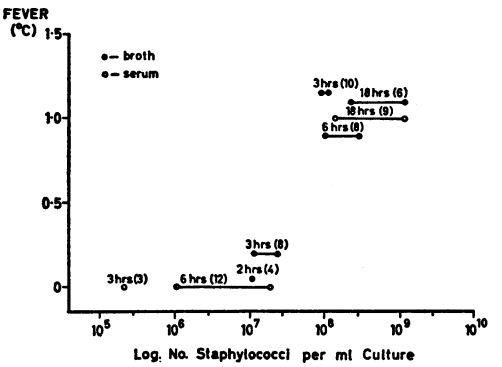

FIG. 4. Average fevers produced by supernates (or filtrates) of staphylococcal cultures grown for various periods in broth or sera. Lines connecting dots indicate range of bacterial counts at those intervals for which more than one culture was used. Note sharp increase in mean fever produced by filtrates of 3-hour broth cultures with higher counts. Responses to either supernates or filtrates of cultures incubated for the other intervals were relatively uniform.

simply to the initial inhibition exerted by serum on growth of these organisms, and hence on production of pyrogen.

To determine whether active multiplication was necessary for release of soluble pyrogen, the following experiment was performed. Cells from an 18-hour broth culture were centrifuged, washed once and resuspended in pyrogen-free broth. The culture was then placed at 4° C. for 18 hours, after which it was recounted and centrifuged in the cold. Although the counts were unchanged after this interval, filtrates of this culture were virtually nonpyrogenic, suggesting that pyrogen is only released by multiplying cells. To confirm this inference, another aliquot of the same 18-hour culture was diluted 1:10 in pyrogen-free broth and placed in the incubator for 6 hours. At the end of this time (when a repeat count revealed growth of approximately 1 log. from 10^7 to 10^8) the culture was centrifuged. The filtrate of this culture was strongly pyrogenic.

In other experiments, cells from both broth and serum-grown 18-hour cultures of staphylococci were resuspended in pyrogen-free saline or water (in a volume equal to that of the original culture) and left for varying periods of time up to one month in the refrigerator. Although viable counts frequently decreased several logs over this period, filtrates of these

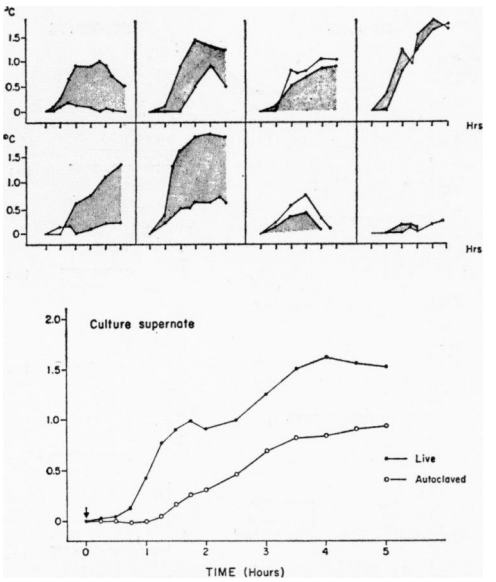

FIG. 5. Top: (shadowgraphs). Fevers of eight individual rabbits given two injections of 1 ml. Giorgio 18-hour culture filtrate. Responses to unautoclaved sample are shown by black silhouette; to autoclaved sample by open fever curve. Bottom: Mean responses of 12 rabbits to autoclaved and of six rabbits to unautoclaved Giorgio 18-hour culture supernates.

resuspended cells were nonpyrogenic in dosages of 1 to 5 ml. Similarly, cells resuspended in saline and then shaken overnight at room temperature or autoclaved failed to release detectable amounts of pyrogen. These observations suggest that under conditions where there is no active growth of organisms, staphylococcal pyrogen is not liberated in amounts detectable by the methods employed in these studies, whether the cells are present in a nutrient medium or in a nonsupportive medium such as saline.

Heat stability of culture filtrate pyrogen

To test the heat stability of staphylococcal pyrogen, a group of four rabbits was given an injection of broth culture filtrate and temperatures recorded. Four days later, the same animals received a second injection

of the same material which had been autoclaved (at 125° C. for 15 minutes). In a second group of animals, the order of injections was reversed, the autoclaved filtrate being given four days before the unautoclaved sample. The results are shown in Figure 5. The responses were variable, although uninfluenced by the order of the injections: four recipients (two in each

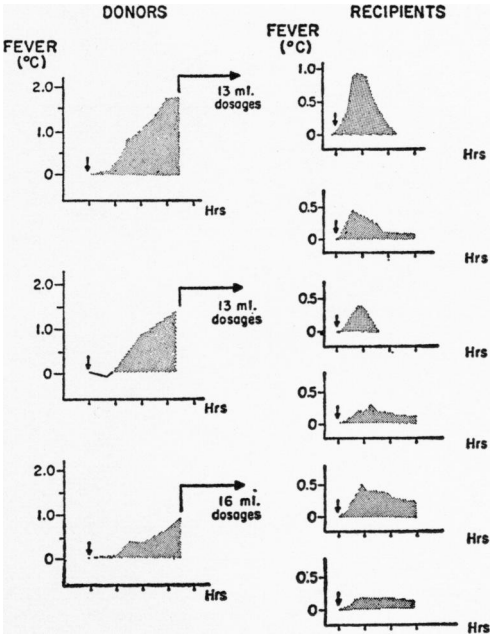

FIG. 6. Transfer of circulating endogenous pyrogen from three febrile donor rabbits given 2 ml. Giorgio 18-hour broth filtrate (silhouettes on left) to six recipients (silhouettes on right).

group) had markedly diminshed responses to the autoclaved filtrate; in two others the fevers were nearly equivalent, whereas the last two animals had minimal responses to both autoclaved and unautoclaved material. The results show the marked individual variability which makes interpretation difficult in a small group of animals. However, when the mean response of a larger group of rabbits given autoclaved supernate is compared with the average fever induced by unautoclaved material, as shown in the lower half of Figure 5, it is apparent that the pyrogenicity of autoclaved culture filtrate was significantly reduced (to about one-fifth to one-tenth that of the unautoclaved sample, cf. Fig. 3).

Appearance of a circulating pyrogen after intravenous injection of culture filtrate

After intravenous inoculation, a variety of microbial agents, including Gram-negative endotoxins, myxoviruses, and tuberculin in sensitized recipients, release one or more tissue pyrogens which are believed to be directly responsible for the ensuing fever.[9] A substance with similar properties appears in the sera of rabbits inoculated with staphylococcal cells.[1] To determine whether fevers following intravenous injection of staphylococcal

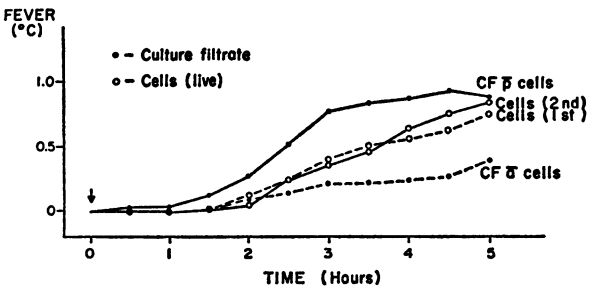

FIG. 7. Average fevers induced in six rabbits to 1 ml. 1:10 Giorgio culture filtrate before and two weeks after an infecting dose of washed cells from same culture. Responses of the same animals to the first (infecting) and second, similarly spaced injections of washed live cells are shown for comparison. There was no change in response to the same two injections of culture filtrate in five uninfected controls (not shown).

filtrate were likewise due to a circulating endogenous pyrogen (EP), the following experiment was devised.

Three rabbits were inoculated with 1 ml. doses of 18-hour broth culture filtrate and their temperatures recorded. Three and a half hours later, at about the time of maximal fever, each donor was bled 60 ml. by cardiac puncture. The following day, serum from each donor was injected in 13 — 16 ml. dosages into two recipients. The results are shown in Figure 6. Prompt, brief monophasic fevers were induced by the donor sera in four of the six recipients and weaker responses in the remaining two. These responses resemble those obtained by similar passive transfer methods for demonstrating circulating EP in other experimental fevers.

Modification of response to culture filtrate after staphylococcal infection

The types of fever and lymphopenia[12] produced in normal rabbits by staphylococcal culture filtrate were noted to resemble closely the reactions of BCG-sensitized rabbits to intravenous inoculation of tuberculin.[4, 10, 11] The

possibility was therefore considered that responses to the staphylococcal product might be due to a naturally occurring hypersensitivity arising from inapparent infection with this ubiquitous organism. To test this hypothesis, subpyrogenic doses of filtrate were given to rabbits infected several weeks previously with washed cells of the same organism.

Two groups of six rabbits each were injected with a minimally pyrogenic dose of filtrate and their temperatures recorded. The experimental group was then given 5 x 10^7 washed live cells intravenously, a dose which has regularly produced renal infection in rabbits.[18] Two weeks later, both experimental and control groups were injected with the same dose of filtrate as given initially. The results are shown in Figure 7. The groups which had been infected with cells had a mean fever which was significantly higher than on first inoculation and higher than the average responses of the controls to the two spaced injections. Since the febrile response to staphylococcal filtrate may be augmented by infection, it appears likely that the reactions of normal rabbits to this material are due to a previously existing state of hypersensitivity to one or more antigens in culture filtrates of this organism.

In contrast to the increased response to filtrate after infection, the same group of rabbits showed no change in response when given the same low dose of cells after infection (Fig. 7). This result would seem to support indirect evidence presented earlier[1] that staphylococcal cells produce fever largely because of some physicochemical property, rather than by virtue of an immunological reaction due to antigen on the cell surface.

Failure of injection with BCG to modify response to staphylococcal culture filtrate

To determine whether specific hypersensitivity induced to other agents might modify the response to staphylococcal culture filtrate, a group of rabbits was rendered tuberculin-sensitive by infection with BCG (see METHODS). Three weeks later their hypersensitivity to a standard dose of tuberculin was confirmed. After several days, this group and a similar group of new rabbits that had received no previous injections were given a small inoculation of staphylococcal filtrate. Although there was considerable variation in individual responses to this dosage, the average fevers of the two groups were comparable, as demonstrated in Figure 8. Specific hypersensitivity to other agents, therefore, does not apparently modify the febrile response of rabbits to staphylococcal culture filtrate.

Attempt to produce an intermediate tissue pyrogen by incubation of culture filtrate with sensitized spleen cells in vitro

Johanovský has reported[14-16] that when antigens such as tuberculin and diphtheria toxoid were added *in vitro* to cells or cell extracts from spleens or lymph nodes of specificially sensitized animals, a pyrogenic substance was released. This material produced immediate brief fevers in normal animals and has been called "hypersensitivity pyrogen." In view of the accumulated evidence that the pyrogen appearing in sera of animals given

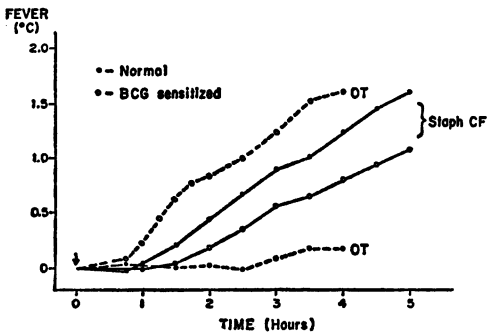

FIG. 8. Mean responses of six new and six BCG-sensitized rabbits to 1 ml. 1:10 18-hour broth culture filtrate (Giorgio). Average fevers of the BCG group and of four normal animals to 100 mg. old tuberculin (OT) are shown for comparison.

staphylococcal culture filtrate was due to a specific immunological reaction, attempts were made to produce a pyrogen with similar properties by *in vitro* incubation of staphylococcal culture filtrate with whole or disrupted spleen cells from reactive donors.

Spleens were aseptically removed from infected (hypersensitive) donor rabbits, cut in fine pieces and sieved with addition of cold saline through a 40 mesh wire screen. Counts revealed 2-4 x 10^9 cells per spleen, virtually all mononuclear.

Suspensions of whole cells in saline were then incubated for two hours in aliquots of 0.5-1.0 x 10^9 cells with 1.0 ml. staphylococcal culture filtrate. At the end of this period, the mixture was centrifuged and the supernatent injected into normal rabbits. In other experiments, spleen cells were initially ground up with a mortar and pestle in saline. Supernates from disrupted cells (confirmed by microscopic examination) were then incubated for two hours with staphylococcal culture filtrate before injection into recipients. In both types of experiment spleens were processed in an ice bath and pyrogen-free precautions observed throughout.

In no instance was a rapidly acting pyrogen produced with properties similar to pyrogen appearing in serum of rabbits given staphylococcal culture filtrate or to Johanovský's hypersensitivity pyrogen.

Fevers produced by staphylococcal lipopolysaccharide

Sultzer and Freedman[17] have reported reactions of rabbits to an agent isolated commercially from staphylococci by methods used for the preparation of Gram-negative endotoxin (lipopolysaccharide). Upon intravenous

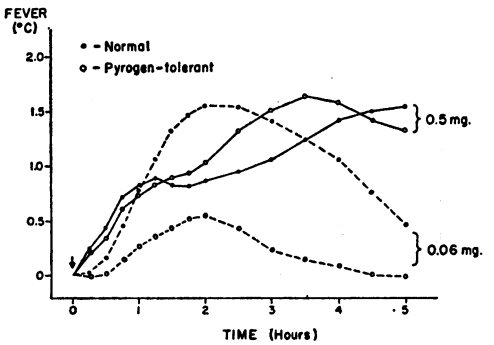

FIG. 9. Mean fevers induced by two dosages of staphylococcal lipopolysaccharide (Difco) in groups of normal and pyrogen-tolerant recipients. Each curve represents five animals, except normals given lower dosage (4 animals).

inoculation, this material produced high biphasic fevers after a short latent period similar to fevers induced by Gram-negative endotoxin. It was felt, however, that there were certain differences in circulating leukocytes by which the effects of staphylococcal "endotoxin" could be distinguished.

To provide a comparison of the febrile responses reported by Freedman to this agent with the fevers obtained with staphylococcal culture filtrate reported here, various dosages of staphylococcal lipopolysaccharide* were injected into groups of normal and pyrogen-tolerant rabbits (see METHODS). The mean fevers produced by two different doses are shown in Figure 9.

Two features of the fevers produced by this agent are of interest and suggest that staphylococcal lipoplysaccharide is similar or identical to Gram-negative endotoxin. First, the latent period before onset of fever was brief

*Kindly supplied by Dr. C. W. Christensen, Difco Laboratories Inc., Detroit, Michigan. This material was stated to have an LD 50 for mice of 6.285 mg. Nitrogen content was 3.94%, phosphorus 4.38%. It gave positive Molisch and anthrone reactions, a weak biuret reaction and a negative ninhydrin reaction.

(15 minutes or less) as is characteristically seen in fever produced by Gram-negative endotoxins, and contrasts sharply with the prolonged latent period of 45 — 60 minutes which occurs after inoculation of culture filtrates of staphylococci reported here.

Second, at the lowest dose tested (0.06 mg.), the febrile responses of rabbits rendered tolerant by typhoid vaccine were markedly less than those of controls. Cross-tolerance of this type has only been demonstrated with certainty to endotoxins of other Gram-negative bacteria where it has been repeatedly confirmed.[18] With large doses of Gram-negative endotoxin, as in the case of the highest dosage of staphylococcal endotoxin tested (0.5 mg.) in these experiments, the responses of normal and tolerant recipients may be indistinguishable, presumably due to "swamping" of the mechanism of tolerance.[19]

DISCUSSION

The results reported here indicate that two phage types of coagulase-positive *Staphylococcus aureus* are capable of producing a potent filterable pyrogen. Since filtrates of all media in which staphylococci were grown, including broth, serum and soft agar, were similarly active, it seems likely that the pyrogen is a product of bacterial cells rather than, as inferred by Hort and Penfold, a modified constituent of the nutrient medium.[20]

There are a number of features by which the staphylococcal pyrogen described here may be differentiated from endotoxins of Gram-negative bacteria.

1. Its distribution among various bacteria was variable; a minimal amount or none was detectable in the same dosage of culture filtrates of two other types of *Staphylococcus aureus* or in those of other Gram-positive microorganisms previously described.[1] By contrast, culture filtrates of virtually all Gram-negative bacteria contain large amounts of pyrogenic endotoxin.

2. Fever produced by staphylococcal filtrates had a delayed onset, 45—60 minutes after inoculation, a response distinctly different from that seen in normal rabbits given an equally pyrogenic dose of Gram-negative endotoxin where the latent period is characteristically 15—30 minutes.

3. Filtrates of serum-grown cultures of staphylococci did not cause the accelerated and augmented febrile responses regularly produced by Gram-negative organisms grown in normal rabbit serum.

4. Only actively multiplying staphylococci seemed capable of liberating pyrogen. Counts of 5 x 10^7 per ml. or more were required to produce

enough of this material to be detectable in 1 ml. of the culture medium. With Gram-negative bacteria, solutions containing as few as $10^4 - 10^5$ microorganisms, resuspended in a nonsupportive medium such as saline and autoclaved, are comparably pyrogenic.[21]

5. In contrast to the marked heat stability of Gram-negative endotoxin, the pyrogenicity of staphylococcal filtrate was significantly reduced in most instances by a brief period of autoclaving.

The sample of commercial staphylococcal A lipopolysaccharide tested (Difco Labs.) had distinctly different pyrogenic properties. It produced biphasic fevers after a brief latent period in normal rabbits, and in appropriate dosage was also much less active in recipients tolerant to typhoid vaccine. These two criteria suggest that this material is similar if not identical to Gram-negative endotoxin. Whether this is due to incidental contamination during chemical fractionation is unclear, but the arguments advanced by Sultzer and Freedman against this possibility seem unconvincing,[17] since only minute amounts of Gram-negative endotoxin would be necessary (1 to 10 thousandths the dosage of so-called staphylococcal endotoxin used in these studies) in order to produce typical endotoxin fevers.[22, 23] Furthermore, no details are supplied as to precautions taken to ensure freedom from pyrogen contamination during the commercial processing of this material.

Rabbits infected with washed staphylococci (Giorgio) had significantly greater febrile responses to a small dose of staphylococcal culture filtrate than did either normal rabbits or those infected with an unrelated agent, BCG. It appears likely, therefore, that responses of normal rabbits to larger doses of the culture filtrate are a manifestation of a naturally acquired hypersensitivity to a staphylococcal antigen. This need not imply that normal animals are infected with the same strain. Further studies will be required to determine the distribution of the antigen among other staphylococci as well as possibly among other bacteria.

On the other hand, febrile responses to staphylococcal cells and to small doses of Gram-negative bacterial endotoxin (unpublished observations) were not modified by staphylococcal infection—further evidence that the augmented response to staphylococcal culture filtrate is immunologically specific and not attributable to a general hyper-reactivity conferred by infection with this organism.

In support of this concept is the finding of other studies that antibodies to staphylococci are present in low titer in uninfected "normal" rabbits.[24, 25] Moreover, dermal hypersensitivity of the delayed type has been

produced to staphylococci in rabbits by various techniques, including repeated intravenous and intradermal infections, or injection of heat-killed vaccine,[26-30] although such hypersensitivity could not be unequivocally demonstrated to either autoclaved whole culture or filtrate in normal or infected rabbits in these experiments.[12] Since, in BCG-immunized rabbits febrile responses to intravenous tuberculin can be obtained with greater regularity than positive skin tests,[4] it appears possible that fever is a more sensitive indication of staphylococcal hypersensitivity than is the delayed type of cutaneous reaction.

In several instances of both immediate and delayed hypersensitivity, antigen given intravenously produces fevers after a latent period similar to that seen here.[4, 31-33] In most of these, recipients react to the injected antigen only after specific experimental sensitization. However, Braude has recently shown that "normal" rabbits react to intravenous inoculations of various pathogenic fungi with fever and that these responses may be greatly augmented after specific infection.[34]

In view of the prevalence of Gram-negative bacteria in the gastrointestinal tract, the question should be reconsidered whether naturally acquired sensitivity plays a role in fevers produced by endotoxin,[35] although there are evident differences between reactions produced by these agents and by known systems of hypersensitivity. This possibility has not yet been investigated by studies with germ-free animals which have had no previous exposure to endotoxins. Since there are considerably more problems in achieving an environment free of endotoxins than one simply germ-free, it would be difficult to perform such experiments. However, animals raised in an environment relatively free of ordinary Gram-negative pathogens show markedly increased resistance to the lethality of endotoxin,[36] a finding consistent with the hypothesis that prior sensitization with these ubiquitous agents may be an important factor in determining their toxicity and hence, perhaps, their pyrogenicity.

Fever caused by injection of staphylococcal culture filtrate appeared to be mediated by a circulating pyrogen of endogenous origin. Passive transfer of sera from febrile donor rabbits produced prompt monophasic fevers in normal recipients. In view of evidence pointing to the immunological basis of staphylococcal culture filtrate-induced fever, an attempt was made to produce a similarly rapid-acting pyrogen by adding the antigen to specifically sensitized cells *in vitro*. Culture filtrate was incubated with either whole cells or cell extracts from spleens of rabbits rendered hypersensitive by recent staphylococcal infection, according to techniques

used by Johanovský for demonstrating so-called "hypersensitivity pyrogen" in fevers induced by tuberculin or diphtheria toxoid.[14-16] No such pyrogen was obtained in these experiments and the tissue source of the pyrogen which appears in sera of rabbits given staphylococcal culture filtrate remains unknown.

SUMMARY

1. Pyrogen present in cell-free filtrates of both broth and serum cultures of Giorgio strain of *Staphylococcus aureus* (phage type VA 4) caused biphasic fevers in rabbits after a characteristic latent period of 45-60 minutes.

2. Culture filtrates of two other types of *Staphylococcus aureus* were nonpyrogenic or only weakly so. Those of a fourth type (3A) were intermediate in potency. Cells of all four organisms, however, produced identical fevers.

3. In certain of its physical and biological properties, staphylococcal pyrogen differed from Gram-negative bacterial endotoxin. In contrast, a sample of staphylococcal lipopolysaccharide obtained commercially (Difco) produced pyrogenic reactions indistinguishable from those of Gram-negative endotoxin and cross-tolerance was demonstrable between them.

4. Most recipients given an intravenous infecting dose of washed staphylococcal cells (Giorgio) had brisk pyrogenic responses 14 days later to a dosage of filtrate previously nonpyrogenic to the same animals.

5. These observations indicate that the febrile response of normal rabbits to culture filtrates of certain strains of *Staphylococcus aureus* may be due to prior, naturally acquired hypersensitivity to one or more products of these microorganisms.

6. Fever produced by staphylococcal culture filtrates appeared to be mediated by a rapidly acting, circulating pyrogen. The tissue origin of this pyrogen in unknown. *In vitro* studies failed to implicate the spleen cell as a source of this material, as has been reported in some other hypersensitivity fevers.

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