

## STUDIES ON THE PATHOGENESIS OF FEVER

### VIII. FEVER-PRODUCING SUBSTANCES IN THE SERUM OF DOGS\*

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In man and experimental animals the febrile response to intravenously administered bacterial endotoxin is preceded by a lag period of 30 to 60 minutes. This has been interpreted as indicating that the toxin does not act directly upon the thermoregulatory centers of the brain, and it has been postulated that a second substance, perhaps a product of tissue injury by the toxin, provides the stimulus to nervous centers that results in fever (1). The transient profound leukopenia which follows the intravenous injection of endotoxin has been cited in support of the idea that leukocytes are a source of "endogenous" tissue pyrogen (2). The presence of fever-producing substances in extracts of rabbit leukocytes and in sterile inflammatory exudates of rabbits and dogs strengthens this concept (3-5).

Atkins and Wood found two separate pyrogens in the serum of rabbits given typhoid vaccine intravenously (6, 7). The first appeared within 5 minutes and had virtually disappeared from the serum 30 minutes later; the second was present in maximal concentration 120 minutes after the administration of the vaccine. Injection of 5 minute serum containing the "early" pyrogen into normal recipient rabbits, resulted in fevers typical of those produced by bacterial endotoxin. Animals given the 120 minute serum ("late" pyrogen) responded with short monophasic febrile spikes after a very brief latent period. Furthermore, rabbits made refractory to the thermogenic actions of typhoid vaccine by repeated daily injections, failed to respond to the early serum pyrogen but had brisk fevers after injection of the late serum pyrogen. These results indicate that the early pyrogen is the originally administered typhoid vaccine but that the late pyrogen is a qualitatively different substance, perhaps the postulated product of injured cells. It was therefore designated *endogenous pyrogen*.

Prior to the present studies, endogenous serum pyrogen after injection of

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endotoxin, had been demonstrated only in the rabbit given typhoid vaccine. This paper summarizes experiments in another species, the dog, with reference to the presence of endogenous pyrogen in the serum after injection of several different endotoxin preparations, some biological properties of canine endogenous pyrogens, the role of endogenous pyrogens in fevers produced by other thermogenic substances such as dinitrophenol, lysergic acid diethyl amide, kaolin, and dextran, and the polymorphonuclear leukocyte as a source of endogenous pyrogen.

TABLE I  
*Vaccines and Purified Endotoxins Used in the Production of Endogenous Pyrogen in Serum of Dogs*

Bacterial endotoxin	Method of inactivation	No. of cells per ml.	Designation in text
<i>E. coli</i> vaccine.....	Formalin	$3 \times 10^9$	<i>E. coli</i>
<i>Achromobacter alcaligenes</i> vaccine.....	Formalin	$1 \times 10^9$	<i>Achromobacter</i>
<i>Salmonella typhosa</i> vaccine V-58, NRV-LS # 1*.....	Phenol; heat	$5 \times 10^8$	Ty. V-58
<i>Salmonella typhosa</i> vaccine Str. Ty-2, Felix†.....	Phenol; heat	$1 \times 10^{10}$	Ty. Felix
Purified lipopolysaccharide from <i>Salmonella abortus equi</i> § (AE 1298).....	See reference 8.		Pyrexal
Purified endotoxin from <i>Shigella flexneri</i> type Z  .....	See reference 9.		<i>Shigella</i>

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† Obtained from Dr. Maurice Landy, United States Public Health Service, Bethesda, Maryland.

§ Made available by Dr. Otto Westphal, Dr. A. Wander Forschungsinstitut, Sankingen/Baden, Germany.

|| Obtained from Dr. Leighton E. Cluff.

### Materials and Methods

*Animals.*—Male and female mongrel dogs weighing 8 to 15 kilos were used in all experiments. They were housed in an air-conditioned room.

*Bacterial Endotoxins.*—Several bacterial vaccines and purified endotoxins were employed. These are enumerated in Table I.

*Preparation of Serum Pyrogens.*—Donor dogs were injected intravenously with endotoxin or one of the other pyrogenic substances studied and were bled at various intervals under pentobarbital anesthesia. The blood was allowed to clot at room temperature, stored at 4°C. overnight, and serum was removed after centrifugation at 2,000 R.P.M. for 120 minutes. Sera were stored at 4°C. and were tested within 1 week. All specimens were cultured in thioglycolate broth at 37°C. and at room temperature, and were used only if bacteriologically sterile.

*Recipient Animals.*—Normal recipient dogs for the testing of sera were injected no oftener than once every 2 weeks to avoid the development of tolerance. "Tolerant" recipient animals were given daily injections of endotoxin over a 10 day period; temperatures were recorded on the 1st and 10th day of injection to be sure that tolerance had been achieved.

*Recording of Temperatures.*—Animals were kept in cages unrestrained and food and water were withheld on the day of an experiment. Rectal temperatures were taken with ordinary clinical thermometers after a period of at least 90 minutes had been allowed for baseline recording. Material to be tested was injected intravenously and temperatures were recorded at 30 minute intervals for 4 hours. The readings were plotted on standard graph paper and a "fever index" was determined by planimetry (1).

*Avoidance of Contamination by Bacterial Pyrogens.*—All needles, glassware and instruments were sterilized in hot air ovens at 170°C. for 3 hours. All sera and other injectables were cultured in thioglycollate medium and were discarded unless sterile. Physiologic saline solution (0.85 per cent NaCl) was tested in rabbits and was always free of pyrogenic contaminants.

#### RESULTS

*Thermogenicity of Serum Collected from Normal Dogs 120 Minutes After Injection of Endotoxins.*—Transfer of serum collected from dogs 2 hours after they had received an intravenous injection of endotoxin or bacterial vaccine resulted in fever in recipient animals although serum collected 5 minutes after injection was non-pyrogenic. The presence of transferable pyrogenic material in the serum of dogs after 120 minutes was found to depend upon the dosage of endotoxin. Only when relatively potent preparations were employed in high dosage were demonstrable quantities of endogenous pyrogen found. There was no relationship between the amount of fever observed in donor animals, and the thermogenicity of their sera. In general, the amounts of endotoxin required to elicit the appearance of pyrogen in serum after 120 minutes ranged from 10 to 100 times those required to produce fever in the donor. This is illustrated in Fig. 1, which depicts the febrile responses of normal recipient dogs injected with 20 ml. aliquots of serum from normal febrile donors. Although donor dogs responded to intravenous administration of 5.0  $\mu\text{g}$ . of *Shigella* endotoxin with brisk fevers, this dose was not followed by the appearance of biologically demonstrable serum pyrogen; indeed, 500  $\mu\text{g}$ . was sometimes an inadequate amount. Donor animals given 1.0  $\mu\text{g}$ . of pyrexal, although febrile, did not develop circulating pyrogen in quantities sufficient to produce fever in normal recipients. On the other hand, 120 minute sera from donors given 10  $\mu\text{g}$ . of this toxin, were usually moderately pyrogenic for test animals.

Purified endotoxins, although capable of eliciting high fever in donor animals, were weaker stimuli for the appearance of endogenous fever-promoting substances than vaccines. Administration of typhoid vaccine (Felix) containing  $10^{10}$  cells/ml. consistently elicited the release of endogenous pyrogen into the blood of the donor, whereas, injection of a vaccine containing only  $5 \times 10^8$  organisms/ml. was rarely followed by the finding of pyrogen in donor serum. Similar results were observed with vaccines made from *Aerobacter alcaligenes* and *Escherichia coli*. There appeared to be a marked difference, therefore, in the minimal pyrogenic dose of an endotoxin and the dose required to render the donor serum pyrogenic.

The dose of endotoxin required to elicit the appearance of endogenous pyrogen in detectable amounts was so large that animals given daily injections

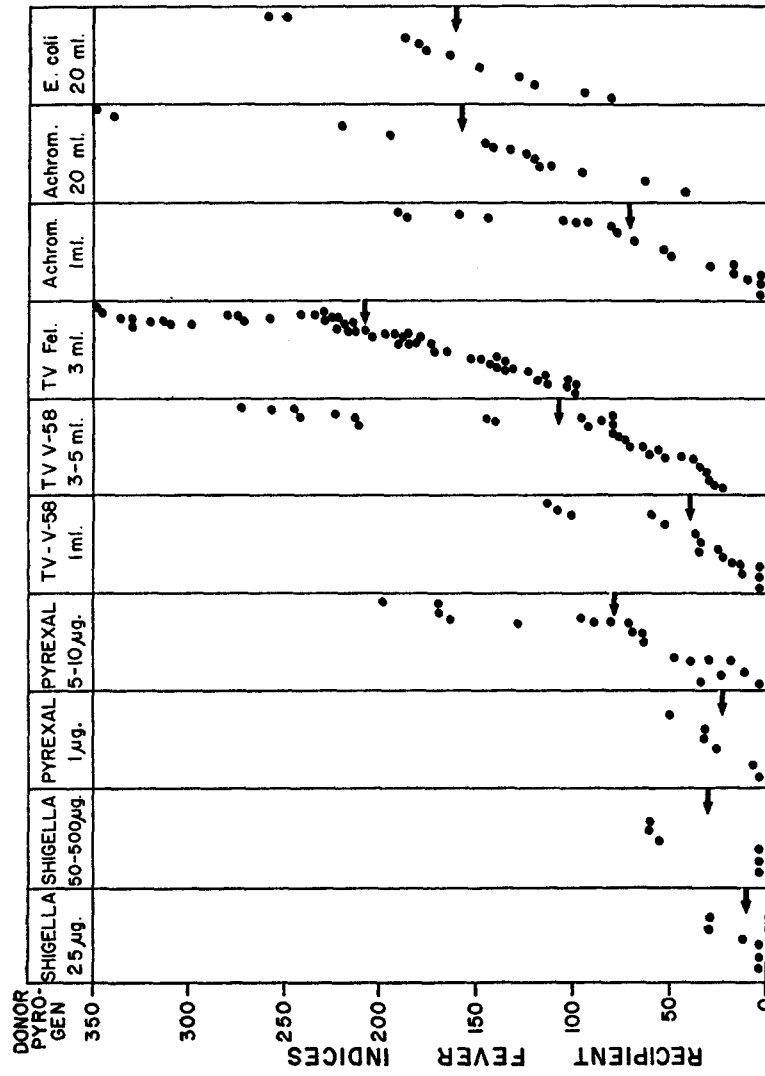


FIG. 1. Fever indices of 181 normal recipient dogs given 20 or 50 ml. of serum collected from donors after intravenous injection of bacterial endotoxins. Arrows indicate the means for each group.

failed to develop tolerance to the pyrogenic action of the toxin. For example, dogs readily became resistant to the fever-promoting effect of 5.0  $\mu\text{g}$ . of *Shigella* or 1.0  $\mu\text{g}$ . of pyrexal and the tolerance was reversed by thorotrast, in accordance with the original findings of Beeson (10) (Fig. 2), but these doses were far too small to result in the appearance of a serum pyrogen (Fig. 1). In contrast,

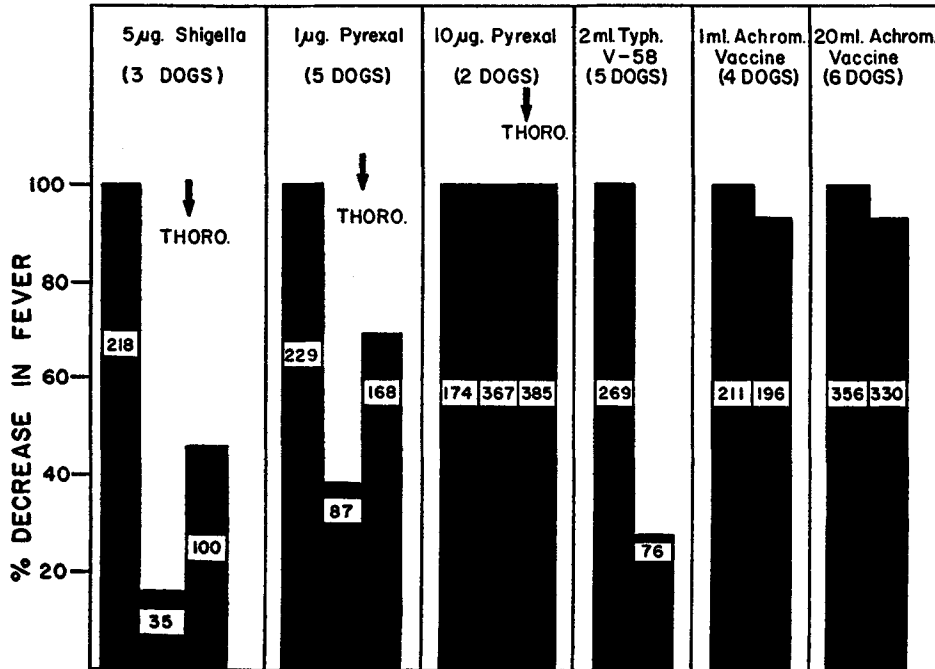


FIG. 2. Change in mean fever indices of groups of dogs given daily injections of bacterial endotoxins. The number in each column is the absolute value of the mean index. The first column in each group is the response after the 1st injection, the second column is the response after the 10th daily injection, and the third columns represent the fevers on the 11th day after treatment with thorotrast. Note the absence of demonstrable tolerance when large doses of endotoxin were employed.

when animals were given daily injections of 10  $\mu\text{g}$ . of pyrexal, or 20 ml. of *Achromobacter* vaccine, refractoriness to the bacterial toxin did not develop (Fig. 2), but endogenous pyrogen was usually detectable in the serum at this dose level.

These results indicate that the amount of endogenous pyrogen in the serum of dogs given bacterial pyrogens is a function of the dose of endotoxin initially administered, and that beyond a certain point, it is not related to the magnitude of fever in the donor animal.

*Absence of Fever-Producing Factor in 5 Minute Serum of Dogs.*—Donors were

injected with several bacterial pyrogens in varying doses and were bled 5 minutes later. Aliquots of 20 or 50 ml. of each donor's serum were administered to 55 normal dogs. Recipient animals remained afebrile except those given serum obtained from donors who had been given  $3 \times 10^{10}$  typhoid organisms (Table II). This material produced modest elevations of temperature in 14 of 25 recipients.

These results differ from those obtained in rabbits by Atkins and Wood, who found that serum collected 5 minutes after injection of typhoid vaccine invariably produced fever in normal recipients but was inactive in tolerant recipients. Failure to observe these changes in dogs was probably a consequence of the dilution of the initially administered endotoxin in a large volume of blood, which resulted in marked diminution or loss of pyrogenicity.

TABLE II  
*Thermogenicity of 20 Ml. Aliquots of 5 Minute Serum in Normal Recipients*

Donor serum	No. of recipients	No. of recipients with fever index $\geq 50$	Mean fever index
5 min. 500 $\mu$ g. <i>Shigella</i> .....	5	2	34
5 min. 5.0 $\mu$ g. <i>Shigella</i> .....	7	1	19
5 min. 1.0 ml. ty. (V-58).....	7	0	15
5 min. 3.0 ml. ty. (V-58).....	7	0	14
5 min. 20 ml. <i>Achromobacter</i> .....	4	1	24
5 min. 3.0 ml. ty. (Felix).....	25	14	90

*Presence of Endogenous Pyrogen in Serum Obtained 60, 120, and 180 Minutes After Administration of Bacterial Endotoxins.*—Normal donors were given 3.0 ml. of typhoid vaccine (V-58) and were bled at 60, 120, and 180 minute intervals after the injection. Although all donors manifested marked elevations in temperature at 60 minutes, their serum in a dose of 20 ml. produced minimal fevers in normal recipients (Fig. 3). By 120 minutes, however, the thermogenicity of donor sera had increased fivefold (Fig. 3). At 180 minutes the amount of transferable serum pyrogen had diminished considerably, and by the fourth hour after administration of vaccine the donor serum was non-pyrogenic.

*Effect of 120 Minute Serum in Tolerant Recipients.*—Perhaps the most significant way in which endogenous serum pyrogen in rabbits was found to differ from endotoxin (as 5 minute serum pyrogen) in the report of Atkins and Wood (6, 7), was in its ability to produce fever in recipient animals made tolerant to bacterial endotoxin by repeated injections. Similar findings in dogs are illustrated in Fig. 3 which depicts the ability of 60, 120, and 180 minute serum to produce fevers in tolerant animals that were comparable to those elicited in normal recipients.

Donor animals were prepared by administration of 3.0 ml. of typhoid vac-

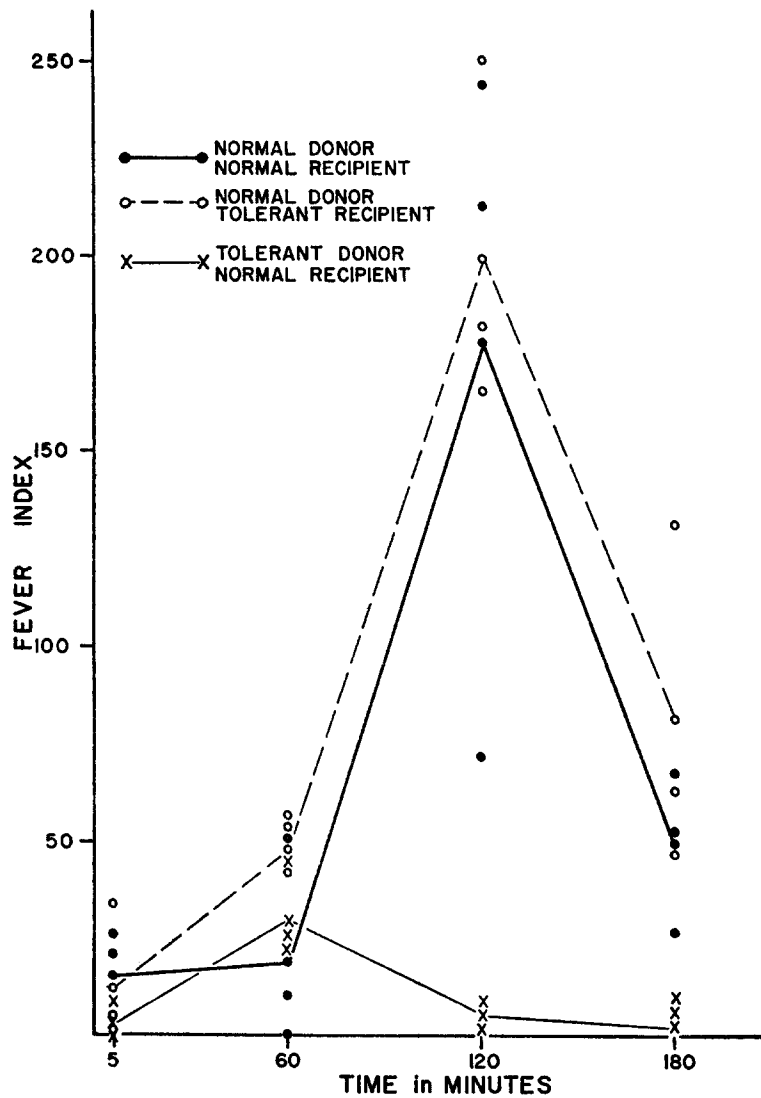


FIG. 3. Febrile responses of normal and tolerant recipients given 5, 60, 120, and 180 minute sera from normal and tolerant donors after intravenous injection of 3.0 ml. of typhoid (V-58). Maximal amounts of endogenous serum pyrogen were present at 120 minutes; detectable quantities were also found in 60 and 180 minute samples. Normal and tolerant dogs responded with similar elevations in temperature. Serum from tolerant donors produced no fever in normal recipients.

cine (V-58) and exsanguinated 5 or 120 minutes afterward. These sera were tested in normal dogs or in dogs made tolerant to 2.0 ml. of typhoid vaccine by a series of daily injections. The results, shown in Fig. 4, illustrate the brief monophasic fevers observed in both normal and tolerant recipients when 120

minute serum was administered. The higher fevers in tolerant recipients observed in this experiment are unexplained and this difference was not noted in several similar studies. As has been mentioned, 5 minute serum produced

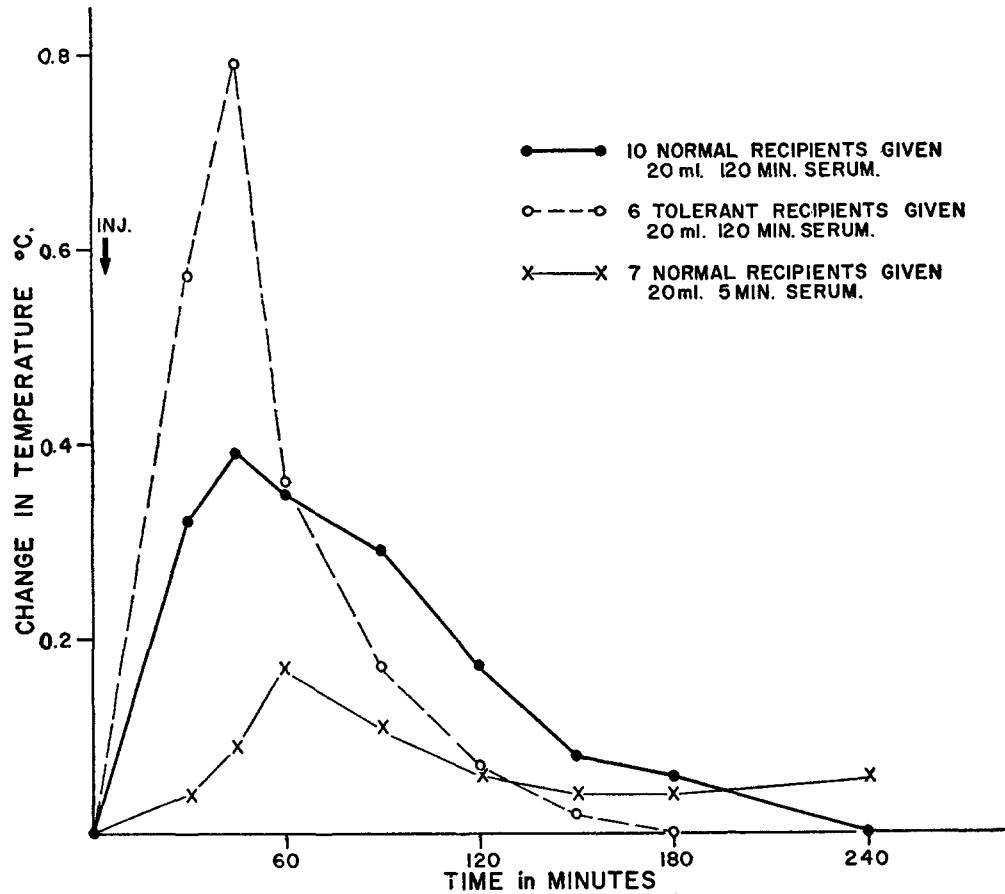


FIG. 4. Mean temperature rises in normal and tolerant recipients given 20 ml. of 120 minute serum from normal donors injected with typhoid vaccine. The febrile responses in both groups of recipients correspond closely. In contrast, 5 minute serum elicited no fever.

fever in neither group of recipients. Similar results were obtained when 120 minute sera from donors given pyrexal or *Achromobacter* vaccine were tested in both types of recipient animal (Fig. 5).

*Inability of Tolerant Donors to Produce Endogenous Pyrogen.*—Atkins and Wood found no endogenous pyrogen in the 120 minute serum of rabbits made tolerant to typhoid vaccine (7). Abolition of tolerance by the administration of thorotrast was followed by reappearance of endogenous pyrogen in the blood



after administration of typhoid vaccine. The findings in dogs were entirely similar. Serum collected 120 minutes after injection of endotoxin from 8 donors made tolerant to typhoid vaccine or pyrexal failed to produce fever in normal recipient animals (Fig. 3, and Table III).

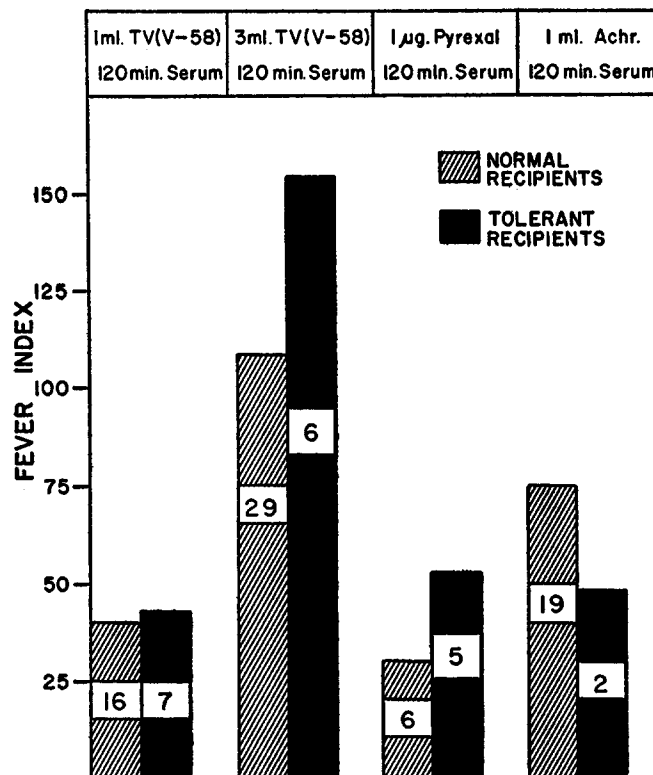


FIG. 5. Fever indices of normal and tolerant animals given endogenous serum pyrogens evoked by administration of several different endotoxins. There was no difference in the response of normal and tolerant recipients. Numbers represent number of recipients.

*Failure of Development of "Tolerance" to Repeated Daily Injection of Endogenous Pyrogen.*—Three dogs were given 3.0 ml. of typhoid vaccine (V-58). Another group of 3 animals received 3.0 ml. of a similar vaccine (Felix). All 6 were exsanguinated 2 hours later, and injections of 20 ml. aliquots of unpooled serum were given daily to each of 6 normal recipients in an attempt to induce a "tolerant" state. As is depicted in Fig. 6, there was no diminution in the febrile responses of the recipients. This contrasts with the rapid development of tolerance in animals given repeated intravenous injections of bacterial endotoxins and adds to the evidence that the late serum pyrogen is not endotoxin.

*The Effect of Temperature on the Thermogenicity of Endogenous and Exogenous Pyrogen.*—It is well known that bacterial endotoxins withstand boiling or autoclaving without loss of potency. Recent studies have shown, however, that moderate amounts of bacterial endotoxin can be inactivated by heating at 90°C. for 30 minutes in the presence of normal serum; endogenous serum pyrogen is also inactivated by exposure to this temperature (11). This heat lability resembles that of tissue pyrogens derived from sterile inflammatory exudates and extracts of polymorphonuclear leukocytes (3). On the basis of this response to heating, however, endogenous pyrogen cannot be differentiated from bacterial endotoxin in serum.

The demonstration by Farr (12) that the ability of bacterial endotoxins to induce fever was potentiated by incubation with normal serum, was challenged

TABLE III  
*120 Minute Sera from Tolerant Donors Tested in Normal Recipients*

Status of donor	Serum tested	No. of recipients	No. of recipients with fever index $\geq 50$	Mean fever index
Tolerant 1.0 ml. ty. (V-58) . . . . .	20 ml. 120 min.	3	0	14
Tolerant 2.0 ml. ty. (V-58) . . . . .	20 ml. 120 min.	4	0	0
Tolerant 2.0 ml. ty. (V-58) . . . . .	20 ml. 120 min.	3	0	0
Tolerant 1.0 $\mu$ g. pyrexal . . . . .	20 ml. 120 min.	6	0	17
Tolerant 1.0 $\mu$ g. pyrexal . . . . .	20 ml. 120 min.	2	0	22
Tolerant 1.0 $\mu$ g. pyrexal . . . . .	20 ml. 120 min.	2	0	0
Tolerant 5.0 $\mu$ g. pyrexal . . . . .	20 ml. 120 min.	2	0	10
Tolerant 10 $\mu$ g. pyrexal . . . . .	20 ml. 120 min.	3	0	17

by Hegemann who noted a diminution of pyrogenicity when serum was brought into contact with endotoxin (13). These apparently conflicting results have been clarified by Cluff who found that the fever-producing effect of endotoxin in rabbits was enhanced by incubation with serum at 37°C. for 30 minutes, but that prolonged incubation at this temperature led to a decrease in the ability of the mixture to produce fever (14).

Employing these observations as a point of reference, the fever-producing action of 120 minute serum pyrogen and serum-endotoxin mixtures, which had been exposed at 37°C. for 30 minutes and 24 hours, and at 4°C. for 24 hours, was studied.

Donor animals received 3.0 ml. of typhoid vaccine and were bled 120 minutes later. The blood was permitted to clot and the serum removed by centrifugation. After incubation at 37°C. for 30 minutes, 20 ml. of serum from each donor was tested in 3 normal recipients. On the following day, the same recipients received similar amounts of serum which had been exposed to 37°C. for 24 hours, and

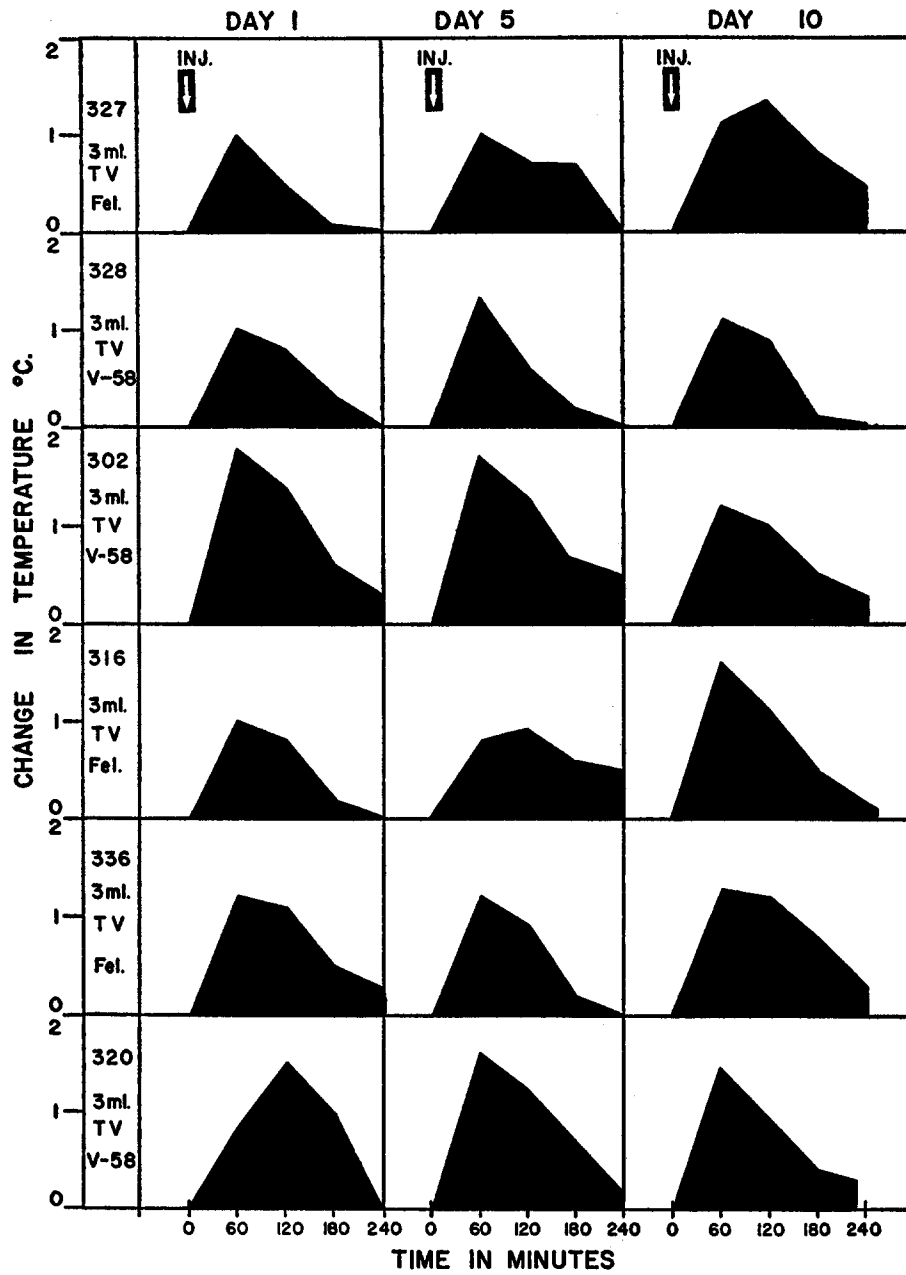


FIG. 6. Failure of daily injections of 20 ml. of endogenous serum pyrogen to result in the appearance of tolerance.

after a rest period of several days, serum which had been stored at 4°C. was tested. The results, shown in Fig. 7, indicated a diminution in the activity of

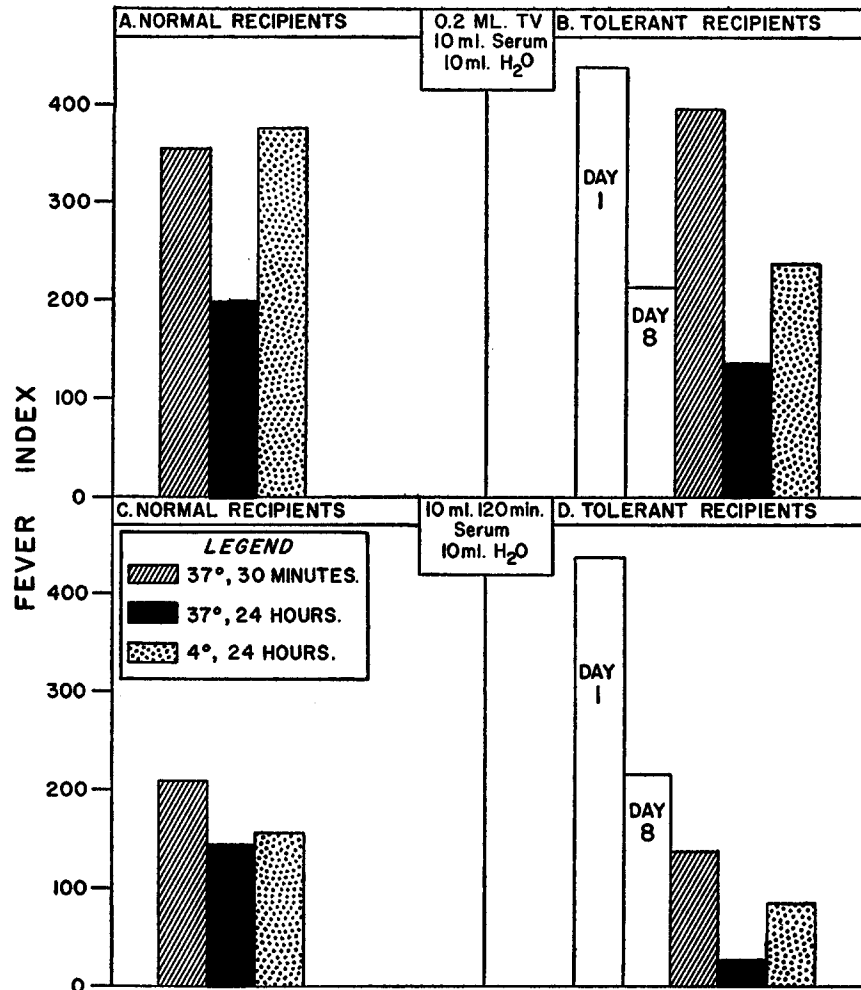


FIG. 7. The effect of incubation at 37°C. for 30 minutes and 24 hours, or 4°C. for 24 hours upon endogenous pyrogen and endotoxin-serum mixtures. The results in normal and tolerant recipients showed diminution in the fever-promoting capacity of both pyrogens after incubation at 37°C. for 24 hours.

endogenous serum pyrogen after incubation at 37°C for 24 hours. The experiment was repeated, using tolerant recipient animals; it was found that endogenous pyrogen which had been exposed to a temperature of 37°C. for 24 hours was almost completely inactive. On the other hand, incubation of 120 minute

serum at 4°C. for 24 hours, or 37°C. for 30 minutes had no discernible effect upon its ability to produce fever in normal and tolerant dogs.

Mixtures of typhoid vaccine and normal canine serum behaved in similar fashion; in both normal and tolerant recipients, serum and endotoxin incubated at 37°C for 30 minutes was approximately twice as potent as the mixture

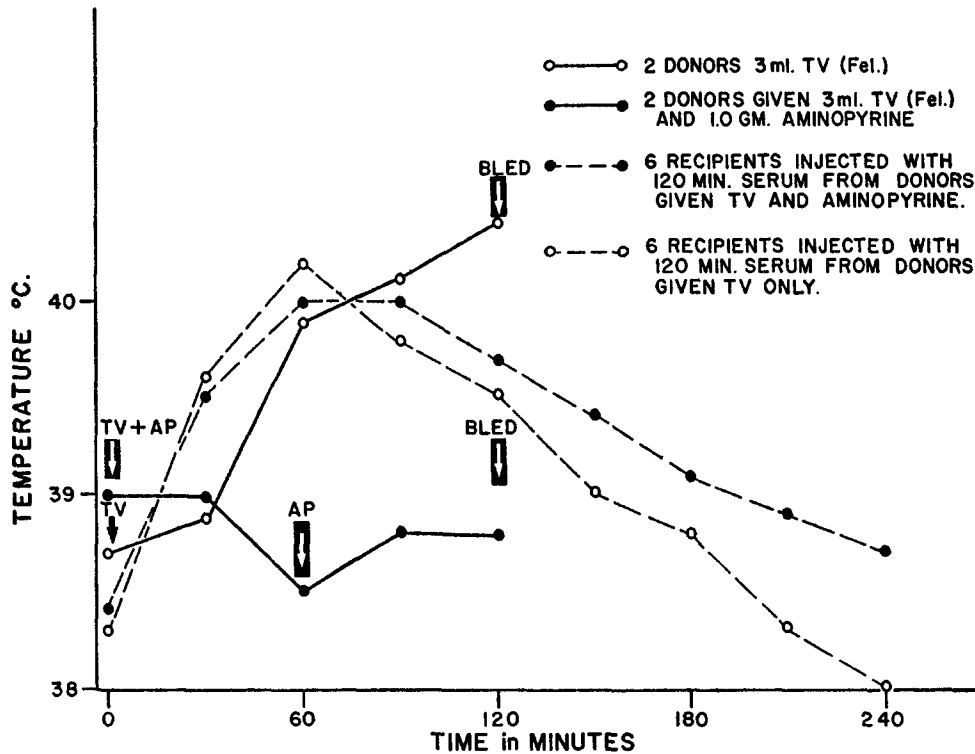


FIG. 8. Failure of suppression of fever by aminopyrine in normal donors to affect the appearance of endogenous pyrogen in the serum.

exposed at 37°C. for 24 hours. Contact of serum with endotoxin at 4°C. for 24 hours failed to diminish the potency of the mixture.

In these experiments, the activity of endogenous serum pyrogen did not differ from a serum-endotoxin mixture. This is not surprising in view of the previous finding that both materials can be inactivated by heating at 90°C. for 30 minutes. The mechanisms responsible for the modification of the biological properties of these pyrogens by heat in the presence of serum are poorly understood and merit future study.

*Failure of Aminopyrine to Influence the Elaboration of Endogenous Pyrogen.*—The injection of 1.0 gram of aminopyrine subcutaneously at the time of in-

jection 3.0 ml. of typhoid vaccine (Felix) and again 1 hour later completely suppressed the febrile reaction to the toxin in two normal donors. The animals were bled 120 minutes after injection of the vaccine and were allowed to recover.

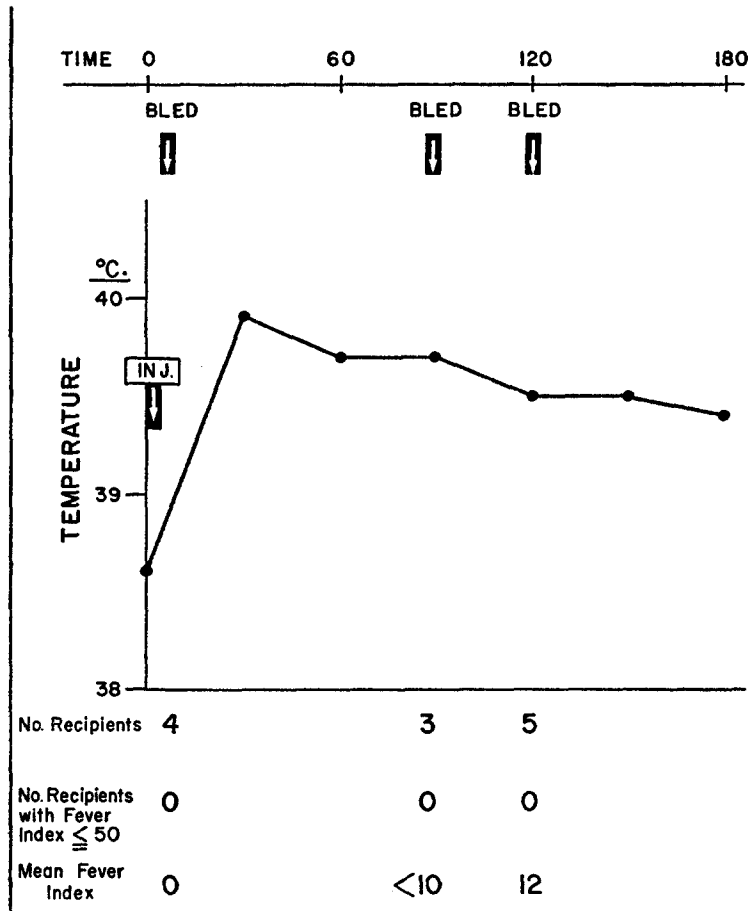


FIG. 9. Mean increase in rectal temperature of 7 dogs given suspensions of 3 to 5 per cent kaolin. No endogenous pyrogen was present in serum collected 5, 90, and 120 minutes after administration of the colloid.

Two weeks later, the experiment was repeated in the same donors, omitting the administration of aminopyrine. Twenty ml. of 120 minute serum from each donor was then tested in 3 normal recipients; there was no difference in the thermogenicity of control serum and that obtained from donors who had been given the antipyretic drug (Fig. 8).

*Ability of Stimuli Other Than Bacterial Endotoxins to Promote Release of Endogenous Pyrogen in Serum.—*

1. *Kaolin.*—The observation that kaolin, a native hydrated aluminum sili-

cate, is capable of producing fever in rabbits when injected intravenously (15) led to the study of the effect of this colloid on the mechanism of fever in dogs.

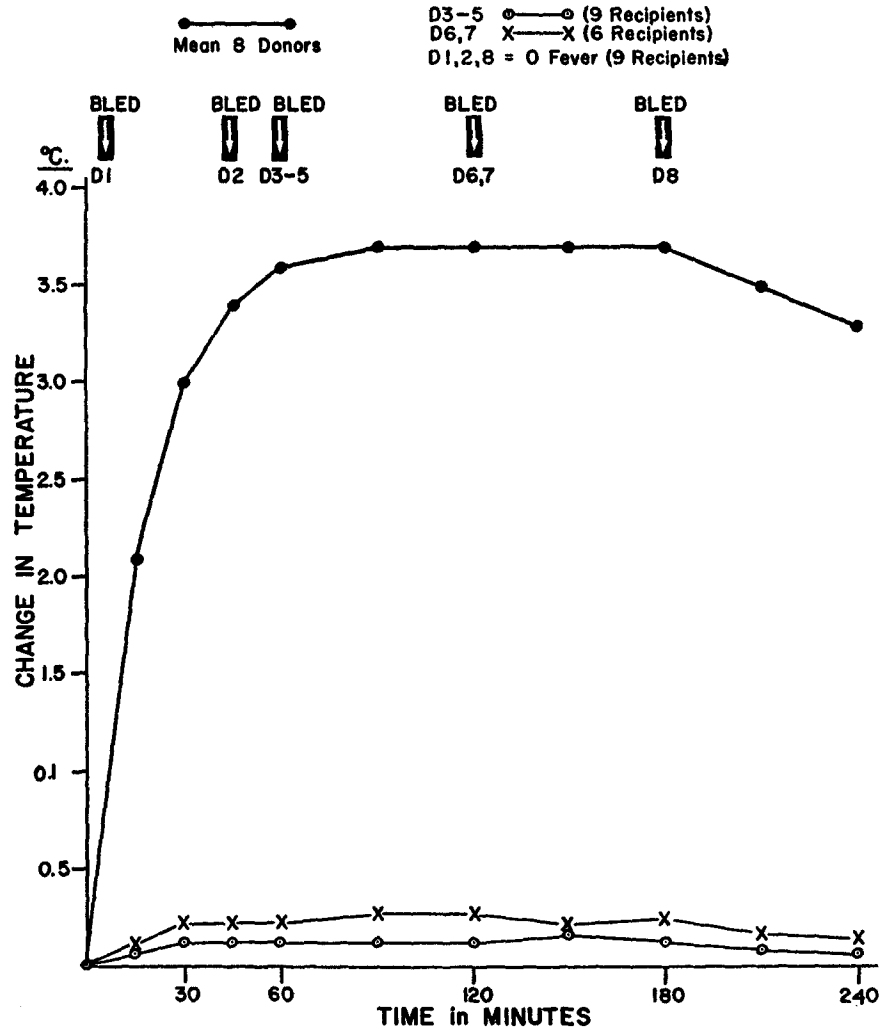


FIG. 10. Febrile responses of 8 dogs to 15 mg./kg. of dinitrophenol. There was no endogenous pyrogen in the serum of these animals.

Intravenous administration of 10 ml. of a 3 to 5 per cent suspension produced fever in a majority of dogs injected; smaller doses were usually inactive and larger amounts were frequently lethal. Duration and magnitude of fever varied widely in different animals and repeated daily administration of kaolin did not result in tolerance. The mean febrile response of seven animals to an injection

of kaolin is shown in Fig. 9. Serum collected 5, 90, and 120 minutes after injection was tested in 12 normal dogs, none of which responded with elevation in body temperature. The mechanism by which kaolin produces fever is obscure,

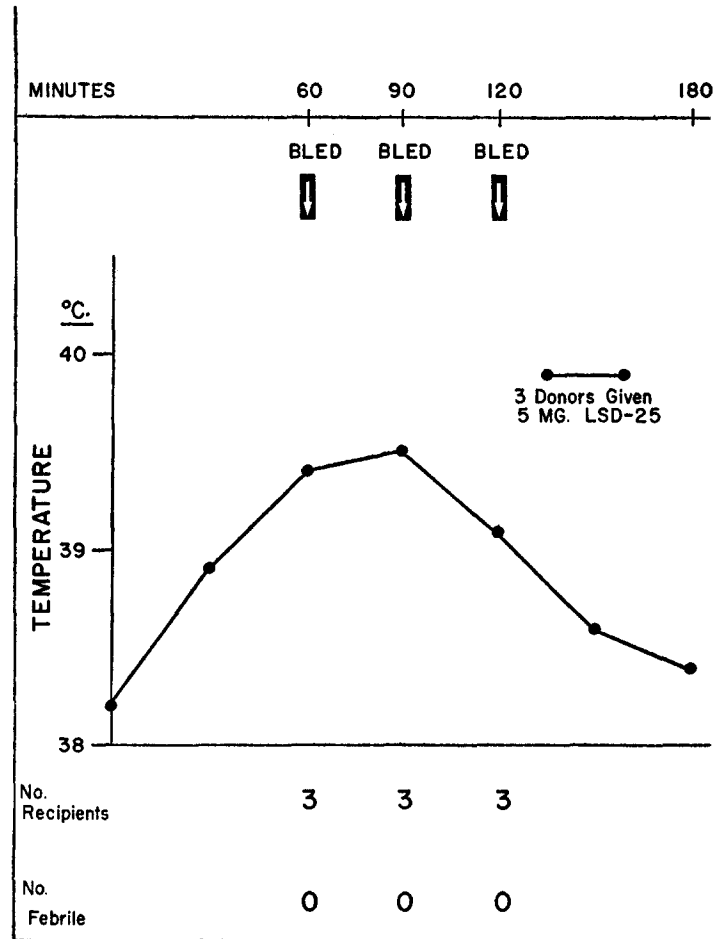


FIG. 11. Febrile responses of 3 dogs to intravenous administration of 5.0 mg. of LSD-25. The serum of these animals contained no endogenous pyrogen.

but it is clear that its administration in maximally tolerated amounts is not followed by the appearance of detectable amounts of endogenous serum pyrogen.

*2. Dinitrophenol.*—It is well established that 2,4 alpha dinitrophenol, a potent metabolic accelerator which exerts profound effects on many organ systems, can produce fever in man and experimental animals (16). Despite



rises in body temperature as high as 5°C. in dogs given this drug in doses of 15 mg./kg., sera collected 5, 45, 60, 120, and 180 minutes after its administration produced no fever in 24 normal recipients (Fig. 10). Repeated injections of dinitrophenol did not result in tolerance to its fever-producing effect and there was no diminution of its activity in animals tolerant to bacterial endotoxins.

3. *Lysergic Acid Diethyl Amide*.—This compound, an inhibitor of serotonin (17), has been reported to be pyrogenic for rabbits when administered intravenously (18), and tolerance to the thermogenic effect has also been described (19). In large doses (5 milligrams), lysergic acid diethyl amide<sup>1</sup> (hereafter referred to as LSD-25) resulted in an increase in body temperature of 3 dogs that averaged slightly more than 1°C. Serum collected 60, 90, and 120 minutes after administration of LSD-25 was non-pyrogenic (Fig. 11). LSD-25 presumably produces fever by a direct action on the thermoregulatory centers. Further studies of the mechanism of action of this compound are needed.

4. *Dextran*. Previous investigations have indicated that native dextrans possess properties similar to those of bacterial pyrogen including ability to elicit fever and leukopenia after intravenous administration in rabbits (20).

Six normal dogs were injected intravenously with native dextran<sup>2</sup> (lots N-316 and N-112, previously shown to be free of contaminating bacterial pyrogens) in amounts varying from 1.0 to 10 gm. All showed elevations of body temperature ranging from 1° to 3°C. Serum collected from each animal 2 hours after injection was tested in 3 normal dogs. Fig. 12 depicts the mean temperature records for all 6 donors and for each group of 3 recipients. The 120 minute sera elicited significant rises in temperature in every recipient. As was the case with bacterial endotoxins, the pyrogenic activity of 120 minute serum was greatest when the amount of dextran administered to the donor was large (5 to 10 gm.).

5. *Sterile Inflammatory Reactions*.—Two dogs were inoculated intramuscularly with 2.0 ml. of turpentine suspended in 3.0 ml. of 95 per cent ethanol. Within 48 hours large fluctuant, deep seated abscesses were present at the injection sites. The body temperature of 1 dog rose from 38.3°C. to 40°C. and that of the other from 38.2°C. to 39.2°C. Blood collected from both animals at intervals during the next 2 days, failed to produce fever when tested in 8 normal recipients in doses of 20 and 50 ml.

*The Effect of Leukopenia Produced by Nitrogen Mustard upon Serum Pyrogen*.—Blood leukocyte counts were performed on 6 normal dogs. They were then given 3.0 ml. typhoid vaccine (Felix) intravenously and bled by femoral artery puncture 120 minutes later. Serum from each donor was then tested in 2 to 5 normal recipients. Two weeks later, the 6 donor animals were given intravenous injections of 5 to 10 mgm. of nitrogen mustard (hereafter referred to as HN<sub>2</sub>)

<sup>1</sup> Sandoz Laboratories, Hanover, New Jersey.

<sup>2</sup> Commercial Solvents Corporation, Terre Haute, Indiana.

on alternate days until the peripheral leukocyte count had fallen to less than 1500/mm<sup>8</sup>. The injection of 3.0 ml. of typhoid vaccine was repeated and the

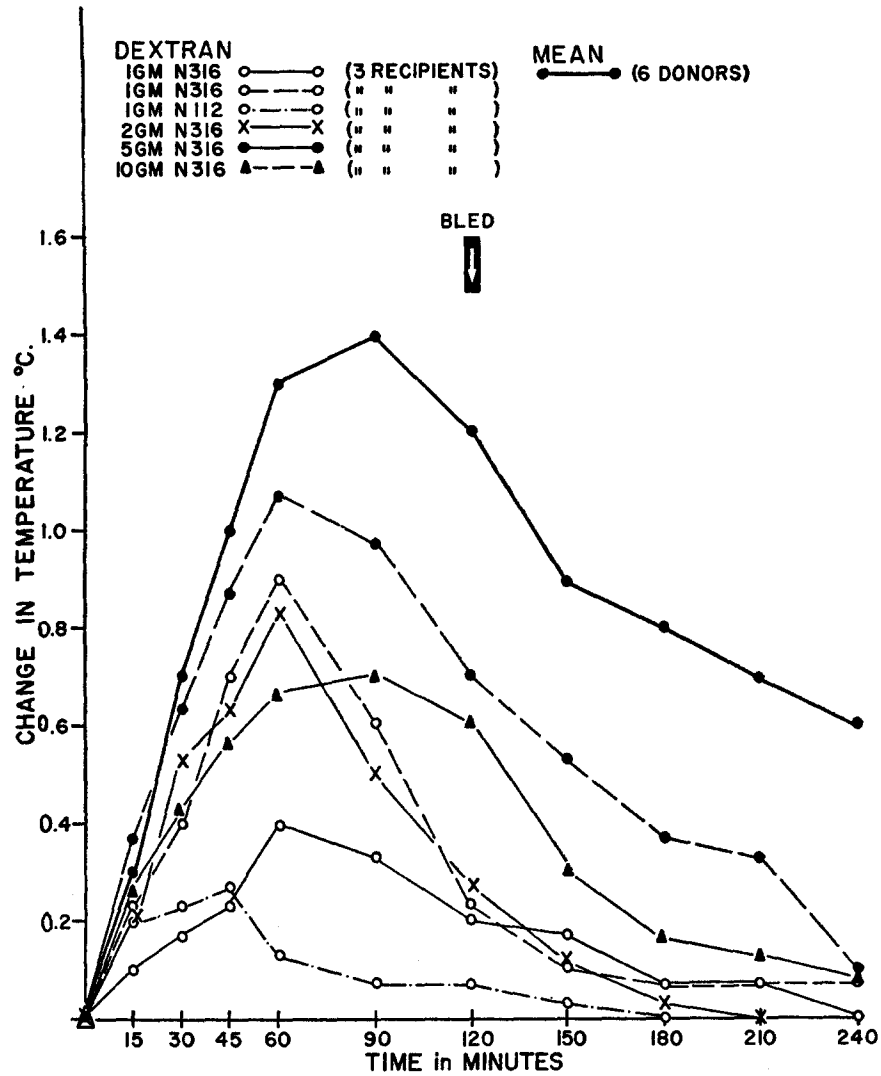


FIG. 12. Mean febrile response of 6 dogs injected intravenously with native dextran (heavy solid line). Serum from each animal produced fever in 3 recipients.

animals were bled 2 hours later. The serum from each donor was again tested in the same recipient animals that had received the serum obtained prior to HN<sub>2</sub> administration. The results of this experiment, recorded in Fig. 13, indicate clearly that there was a consistent and striking decrease in the pyrogenic

activity of serum collected from the animals at a time when they were granulocytopenic as a result of receiving  $\text{HN}_2$ . Despite this difference in circulating pyrogen, the febrile responses of the *donors* to the typhoid vaccine were of comparable magnitude on both occasions.

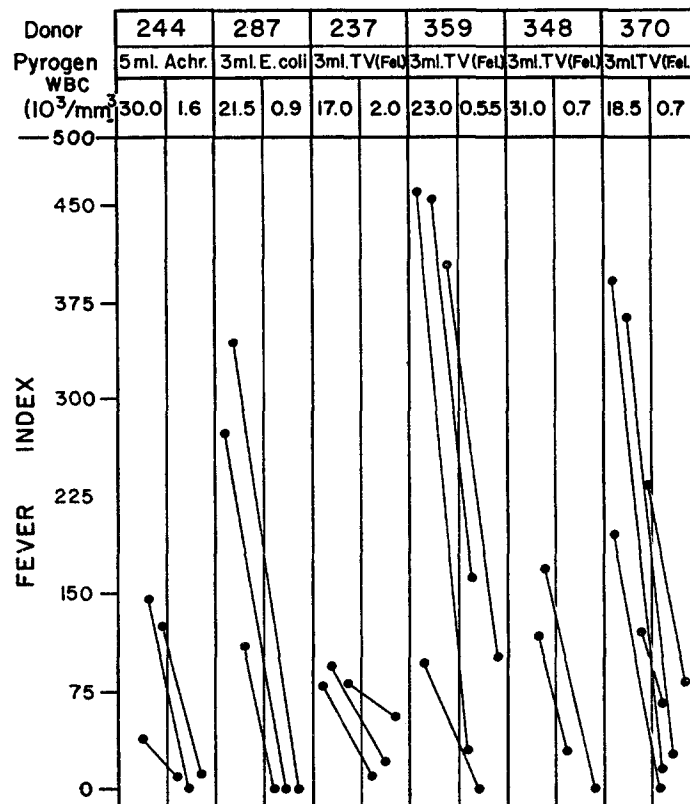


FIG. 13. Comparison of fever-producing ability of serum collected from normal and granulocytopenic donor dogs given typhoid vaccine.

#### DISCUSSION

It is evident from the present studies that a fever-producing substance appears in the serum of febrile dogs 60 to 120 minutes after intravenous administration of various preparations of bacterial pyrogen and that the activity of this secondary "endogenous" pyrogen is proportional to the amount of endotoxin originally injected. Failure to detect a pyrogen in the serum of dogs 5 minutes after injection of toxin and for almost an hour thereafter, supports strongly the concept that endogenous serum pyrogen is a new substance, distinctly different from bacterial endotoxin. The present experiments in dogs also confirm the important observation of Atkins and Wood that endogenous serum

pyrogen is as active in tolerant as in normal animals, and they extend these observations in showing that daily injection of serum containing endogenous serum pyrogen results in no resistance to its fever-producing action.

Atkins and Wood have suggested that polymorphonuclear leukocytes are a source of the endogenous fever-promoting factor. This is based upon the fact that the appearance of this substance in the serum is regularly preceded by a transient, profound granulocytopenia, as well as observations indicating that a pyrogen can be extracted from these cells (3). The hypothesis that bacterial endotoxins injure cells, predominantly polymorphonuclear leukocytes, and that products of these injured cells constitute the endogenous pyrogen is supported by the finding of great reduction of endogenous pyrogen in the serum of dogs made granulocytopenic with nitrogen mustard.

Although leukocytes are the only cells from which "endogenous" pyrogen has been extracted, acellular exudates obtained from leukopenic rabbits are pyrogenic, and Atkins has found large amounts of serum pyrogen in rabbits given Newcastle disease virus (21), in spite of the fact that injection of virus produces no alteration in circulating granulocytes. The probability of tissue sources of pyrogen other than the leukocyte must be recognized, although the results of the present study in dogs given  $\text{HN}_2$  certainly favor the granulocyte as an important source of serum pyrogen after injection of endotoxin. The fevers produced by agents such as dinitrophenol, kaolin, and lysergic acid diethylamide were not associated with the release of endogenous pyrogen although animals reacted to these agents with extreme elevations in body temperature. The mechanism governing the fever-promoting ability of these substances is obscure; it certainly cannot be stated that their ability to produce fever is independent of tissue injury. It is clear, however, that the febrile response to these agents involves mechanisms different from those of the response to Gram-negative bacteria or viruses.

Dextran differs from the other agents tested in that the temperature response to its administration is typically biphasic, and is associated with striking leukopenia. Like endotoxins, dextran is also capable of eliciting the local Shwartzman reaction (although not in preparing for it). Its chemical structure resembles that of bacterial endotoxins, and it would not be surprising if it acted in a similar fashion.

Although serum pyrogens are capable of causing fever when injected into other animals, their essential role in the febrile response of the donor or host animal remains to be shown. It is well known, for example, that granulocytopenic animals respond with fever to endotoxins. The present studies have demonstrated that leukopenic dogs whose serum is non-pyrogenic for normal recipients, manifest marked elevations in body temperature after administration of bacterial endotoxins. Rabbits and dogs with sterile peritonitis may elaborate exudates that are highly pyrogenic for other animals but do not

become febrile themselves (22). The data at hand do not permit the conclusion that fever appearing consequent to administration of bacterial endotoxins is *caused* by the action of endogenous pyrogen on the cerebral centers. Recent studies in this laboratory have demonstrated that bacterial pyrogens administered intrathecally produce high fevers that are not associated with release of endogenous serum pyrogen, suggesting that they act directly on the central nervous system (23). It is conceivable that fever in the host results from direct action of exogenous pyrogen on the brain, and that the endogenous factor is a by-product of the reaction to endotoxins. It is reasonable to suggest on the basis of present evidence, that both mechanisms are operative, and that endotoxin fever is the end-product of both direct and indirect acting (endogenous) pyrogen. Studies are now in progress to assess the relative importance of the mechanisms in precise terms.

#### SUMMARY

Intravenous administration of bacterial endotoxins in dogs is followed within 2 hours by the appearance of a fever-producing substance in the blood. This endogenous pyrogen differs from the endotoxins originally administered by its ability to produce fever in tolerant recipients and failure to promote tolerance after repeated daily injections. Endogenous serum pyrogen is destroyed by heating at 90°C. for 30 minutes, and is also inactivated to some degree by incubation at 37°C. for 24 hours. Suppression of fever by aminopyrine does not affect appearance of the endogenous factor. Animals made febrile with dinitrophenol, kaolin, or lysergic acid do not elaborate a fever-promoting substance in the blood. Sterile abscesses, accompanied by elevations in body temperature of the host, are unassociated with detectable amounts of secondary pyrogen in the serum. The absence of endogenous pyrogen in the blood of febrile dogs made leukopenic with nitrogen mustard favors the idea that polymorphonuclear leukocytes injured by endotoxins release the endogenous factor. On the other hand, the finding that the granulocytopenic animals are febrile when no circulating endogenous pyrogen is present, casts doubt upon the essential role of this substance in endotoxin fever.

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