

STUDIES ON THE PATHOGENESIS OF FEVER

I. THE EFFECT OF INJECTION OF EXTRACTS AND SUSPENSIONS OF UNINFECTED RABBIT TISSUES UPON THE BODY TEMPERATURE OF NORMAL RABBITS*

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(Received for publication, July 18, 1953)

Little is known about the pathogenesis of fever. The physiologic mechanisms controlling heat production and elimination have been studied extensively, but knowledge of the ways in which the equilibrium between these forces is disturbed in disease is scanty.

The endotoxins of Gram-negative bacteria are capable of inducing high fever (1) and there is evidence suggesting that in certain bacterial infections, particularly when there is bacteremia, these endotoxins may play a role in the production of fever (2). A few viruses have also been shown to possess pyrogenic properties (3, 4). On the other hand many common pathogenic microorganisms contain no fever-producing component and even in infections due to pyrogen-producing bacteria there is reason to believe that the fever accompanying active infection is not due to bacterial pyrogen alone (5, 6). Furthermore, many febrile disorders do not involve invasion of the body by any known infectious agent, examples being neoplastic diseases, hemolytic crises, hypersensitivity states, vascular occlusions, and metabolic abnormalities such as thyroid storm, gout, and porphyria. Here it seems obligatory to postulate a mechanism within the host which may be responsible for the disturbance of temperature regulation. The only obvious common denominator in all febrile diseases is cell injury; consequently the hypothesis most commonly offered suggests that fever is caused by the action of products of tissue injury acting upon cerebral regulatory centers. The concept that fever is brought about by absorption of products of tissue injury was proposed as early as 1785 (7) before the role of cerebral heat control centers was recognized. In the last century several investigators showed that fever could be produced in animals by intravenous injection of pus or extracts of necrotic tissue (8-14). More recently, Menkin (15, 16) has reported the isolation of a material—pyrexin—from the euglobulin fraction of exudates of acute sterile inflammations, which produces fever when injected intravenously in animals. All these studies, including Menkin's work on pyrexin, are subject to question because of lack of adequate precautions against contamination by bacterial pyrogens in the course of preparation, collection, or fractionation of the test materials. Bacterial pyrogens are ubiquitous; they resist destruction by ordinary methods of sterilization and can by no means be adjudged absent merely on the

* This study was supported by grants from the Helen Hay Whitney Foundation, the Public Health Service, and Baxter Laboratories.

criterion of bacterial sterility (1). In 1948, Bennett (17), using a technique which avoids pyrogen contamination, confirmed Menkin's finding to the extent that sterile pleural exudates of dogs were found to contain a fever-producing substance. Certain differences were noted between the action of the crude exudate material and that of bacterial pyrogen, one being that the exudate-induced fever is shorter in duration. It was not feasible, however, to carry out the chemical manipulations necessary to isolate the pyrexin fraction while observing precautions required to assure freedom from bacterial pyrogen contamination.

On the assumption that fever is usually caused by the action of some product originating in the tissues of the host, a series of experiments have now been performed, testing various cells and tissues of the rabbit for evidence of capacity to cause temperature elevation in normal rabbits.

General Methods

Healthy male New Zealand white rabbits weighing 2 to 3 kilos were used throughout the study. At various times animals were purchased from four commercial sources, in Atlanta, Philadelphia, New York, and New Haven. Specific methods for obtaining tissues and cell suspensions, preparation of extracts, etc., are described in the protocols of different experiments. Rectal temperatures were recorded in all tests for fever. In experiments lasting more than a few hours, animals remained in their cages and temperatures were taken at appropriate intervals using ordinary clinical thermometers. In short term experiments, the animals were placed in wooden or metal stalls, secured by loose fitting collars, and temperatures were recorded electrically at intervals of 6 to 30 minutes using either micromax copper-constantan thermocouples or foxboro resistance coils which remained in the rectum throughout the test period. In all cases, periods of acclimatization to the stalls preceded actual tests and adequate numbers of base line recordings were obtained before any procedure was carried out.

Rigid precautions against bacterial pyrogen contamination were observed. Glassware, syringes, needles, mortars, pestles, glass beads, abrasives, Waring blender cups, surgical instruments, sutures, and sponges were sterilized in hot air ovens at 170°C. for 2 hours. Materials which could not be treated so drastically, such as sodium chloride solution, buffers, nembutal, heparin, horse serum, sodium citrate, and antibiotics, were tested for pyrogenicity and shown to be free of it before being employed in experiments. Rubber tubing and rubber stoppers were boiled for 30 minutes in 10 per cent sodium bicarbonate, washed many times with pyrogen-free distilled water, and autoclaved for 45 minutes, then tested for pyrogen content before use. At each stage of preparation of extracts and tissue suspensions, samples were cultured in thioglycollate broth, incubated for 48 hours. In the rare instances of contamination, material was discarded.

EXPERIMENTAL RESULTS

1. Tests to Demonstrate a Fever-Producing Factor in Various Body Tissues Saline Extracts of Normal Tissues.—

Extracts were prepared from brain, heart muscle, lung, liver, spleen, pancreas, adrenals, skeletal muscle, bone marrow, and testis. All tissues were obtained from animals under ether anesthesia or immediately after sacrifice by exsanguination. After specimens had been weighed in Petri dishes on a torsion balance, extracts were made by one of two procedures.

Method 1.—A weighed aliquot of tissue was placed in a small Waring blender cup, and, after addition of 10 ml. of 0.85 per cent NaCl solution (hereafter referred to as "physiologic

saline") containing 200 units of penicillin and 0.5 mg. of streptomycin per ml., the blender was run for 5 minutes. The homogenate was decanted into a 40 ml. centrifuge tube, incubated at 37°C. for 6 hours, and centrifuged at 2000 R.P.M. for 30 minutes. The supernatant material was filtered by suction through a Selas number 02 porcelain filter and stored in a 125 ml. flask with rubber stopper at 4°C.

Method 2.—A weighed aliquot of tissue was forced through a monel metal sieve, the mesh of which contained 289 openings per cm.², into a porcelain mortar. Physiologic saline containing the antibiotics was added in a proportion of 2 ml. per gm. of tissue and this mixture was ground with a porcelain pestle until a smooth suspension was obtained (about 10 minutes). No abrasive was added. The tissue suspension was decanted into a 40 ml. centrifuge tube containing glass beads and shaken mechanically for 2 hours at room temperature. After centrifugation at 2000 R.P.M. for 30 minutes, the supernatant material was filtered and stored as in method 1.

TABLE I

Saline Extracts of Normal Rabbit Tissues Tested by Intravenous Injection in Normal Rabbits

Tissue	No. extracts prepared	No. test animals
Adrenals	3	9
Bone marrow*	5	10
Brain	2	6
Heart	2	5
Kidney	5	15
Liver	5	16
Lung	3	8
Muscle	3	5
Pancreas	3	7
Spleen	5	11
Testis	5	10

* Two extracts from bone marrow produced rises of 0.9 and 1.5°C. respectively within 2 hours after injection into a single test animal. All other tissue extracts were non-pyrogenic.

Extracts were tested in rabbits by intravenous injection of amounts containing from 1 to 10 ml. (0.5 to 2.5 gm. of tissue). Rectal temperatures were recorded at intervals of 6 minutes for 3 to 4 hours after injection. In no instance was it possible to demonstrate any consistent effect upon body temperature. With the exception of two extracts of bone marrow which produced rises of 0.9 and 1.5°C. respectively within 2 hours of injection, all extracts were non-pyrogenic. Table I summarizes the types and number of extracts tested.

In the case of brain and lung extracts, sudden death occasionally followed the intravenous injection of 1 ml., presumably due to thromboplastin contained in the extract. This was circumvented into two ways. Animals were pretreated with 50 mg. of heparin intravenously or were "desensitized" by intravenous injection of 0.1 ml. of extract 30 minutes before a larger dose was given, as described by Thomas (18).

Observations were also made to determine the effect of injection of these extracts upon peripheral leukocyte counts in normal rabbits. With the exception of the irregular production of transient leukopenia due to decrease in polymorphonuclear leukocytes during the first 2 hours after injection of liver extracts, no consistent or significant changes were noted over periods up to 6 hours. Counts were made using blood obtained from small cuts in the ear immediately before injection, at 1, 5, 20, and 60 minutes after injection, and at hourly intervals thereafter.

Suspensions of Normal Tissues.—

Spleen, liver, heart, lungs, and kidneys were excised from normal rabbits under ether or nembutal anesthesia in a cold room at 4°C. Organs were placed in chilled Petri dishes, extraneous fat and connective tissue were trimmed, and the major bronchi stripped from the lung parenchyma. Specimens were ground separately in a Waring blender for 10 minutes in the cold after addition of 20 or 40 ml. of physiologic saline containing no antibiotic. The resulting suspensions were filtered through six layers of gauze into 125 ml. Erlenmeyer flasks. All such suspensions were injected within 2 hours after preparation and were kept at 4°C. during the interval. The volume of suspensions injected into test animals varied from 10 to 25 ml., being adjusted so that each animal received the equivalent for kidney, spleen, heart, and lung of one-half an entire organ and for the liver, 15 gm. of tissue. Tests for fever-producing activity were conducted by injecting suspensions intravenously, intramuscularly, intraperitoneally, or intrapleurally. Intramuscular injections were made into two or more separate sites. Because of sudden death in several animals given intravenous injections of lung, animals receiving intravenous injections were prepared with 50 mg. of heparin given intravenously 30 minutes before injection of the tissue suspensions. Animals were allowed to remain in their cages after injections and temperatures were taken by hand, using ordinary clinical thermometers. Temperatures were recorded at hourly intervals for 24 hours after intravenous inoculations. After injection of suspensions by the other three routes, temperatures were recorded hourly for 12 hours and then three times daily (at 7:00 a.m., 12:00 noon, and 10:00 p.m.) for 21 days.

In additional experiments, normal animals were given 10 ml. of freshly drawn rabbit whole blood intramuscularly, intraperitoneally, and intrapleurally, temperatures being recorded on the above schedule.

Table II summarizes the number and types of suspensions tested in this manner. In no instance was fever observed in any test animal during the period of observation.

Effect of Autolysis.—

To test the effects of sterile autolysis, organs were excised, placed in Petri dishes containing phosphate-buffered saline solution at pH 6.0 or 7.2, and incubated for 6 hours or 24 hours at 37°C. At the conclusion of the incubation period, blocks were taken for histologic examination and suspensions were made by the method described for normal tissues. Suspensions were injected intravenously, intramuscularly, or intraperitoneally into normal animals and temperatures were recorded by the schedule described for normal tissues. Fifty mg. of heparin were given to all animals before intravenous injection of autolysates.

The number and type of preparations from kidney, liver, spleen, lung, and heart which were tested are tabulated in Table III. In no instance was any evidence of fever-producing activity detected.

Effect of Infarction and Other Types of in Vivo Autolysis.—

To obtain a base line for comparison temperatures were recorded on normal rabbits in their cages three times daily (7:00 a.m., 12:00 noon, 10:00 p.m.) for 7 to 10 days. At the

TABLE II

Suspensions of Normal Rabbit Tissues Tested by Injection by Various Routes in Normal Animals

Tissue	No. of animals injected by each route			
	Intravenous	Intramuscular	Intraperitoneal	Intrapleural
Kidney	4	4	7	1
Spleen	4	4	7	1
Heart	4	4	7	1
Lung	4	4	7	1
Liver	4	4	7	1
Blood	—	4	5	5

TABLE III

6 or 24 Hour Autolysates of Rabbit Tissues Injected Intravenously into Normal Animals

Tissue	No. of animals injected			
	6 hours at 37°C.		24 hours at 37°C.	
	(pH 6.0)	(pH 7.2)	(pH 6.0)	(pH 7.2)
Kidney	4	4	4	4
Spleen	4	4	4	4
Heart	4	4	4	4
Lung	4	4	4	4
Liver	4	4	4	4
Blood	4	4	4	4

end of this time they were subjected, under ether anesthesia, to various operative procedures as noted below and temperatures were observed during the succeeding 14 days. Following operations, including sham procedures, procaine penicillin in a dose of 150,000 units was administered daily for 3 days by intramuscular injection. At the end of 14 days, animals were sacrificed, and blocks of tissue were taken for histologic study.

In four animals the entire renal pedicle was ligated, in four the renal artery was ligated, and in seven the posterior main branch of the main renal artery was ligated. In four additional animals, nephrectomy was performed and the kidney was implanted free in the peritoneal cavity. In ten animals branches of the splenic artery were ligated and in four, the spleen was left free in the abdominal cavity after severing its pedicle. In twenty animals

the left chest was opened and an attempt was made to ligate the posterior coronary vessels. Seven animals survived the test period after successful completion of this procedure. In ten animals, ligation of a large pulmonary vein was attempted at thoracotomy. Five rabbits survived this procedure. Sham operation in the form of thoracotomy, laparotomy, or delivery of the kidney through a lumbar incision was carried out in four animals for each type of procedure.

The operative procedures designed to cause infarction of an organ regularly resulted in the production of areas of hemorrhagic necrosis involving one-third to nine-tenths of the kidney, about one-half of the spleen, one lobe of the lung, and about one-fourth of the myocardium. In no instance did fever result from

TABLE IV
Procedures Used in Normal Rabbits to Produce Infarction and in Vivo Autolysis

Procedure	No. of animals
<i>Kidney</i>	
Ligation of pedicle.....	4
Ligation of artery.....	4
Ligation posterior branch of artery.....	7
Peritoneal implantation.....	4
<i>Spleen</i>	
Ligation branch of artery.....	10
Peritoneal implantation.....	4
<i>Heart</i>	
Ligation posterior coronary vessels.....	7
<i>Lung</i>	
Ligation branch pulmonary vein.....	5

one of these operative procedures. Table IV summarizes the results of all experiments of this type.

2. Attempts to Demonstrate a Fever-Producing Factor in Areas of Acute Inflammation

The Shwartzman Reaction.—

Healthy rabbits were shaved and the skin of the trunk was prepared in multiple sites (usually 30) by intracutaneous injection of P-35 polysaccharide of *Serratia marcescens*¹ or a filtrate of agar washings from a culture of *S. marcescens*. The preparatory dose of P-35 polysaccharide was 0.1 mg. in 0.5 ml. of physiologic saline and of the filtrate 0.5 ml. of a 1:10 dilution. Twenty-four hours later, animals were anesthetized with nembutal and five of the prepared sites were excised. The skin defects were closed with clips and bleeding was

¹ Obtained from Dr. M. J. Shear.

controlled by ligation and the topical application of thrombin. An intravenous "provocative" dose of 0.2 mg. of P-35 or 1 ml. of a 1:25 dilution of filtrate was then injected. The animals were maintained under anesthesia and at intervals of $\frac{1}{2}$, 1, 2 $\frac{1}{2}$, 4, and 6 hours after the provocative injection, groups of five lesions were excised. There was no indication that the operative procedure or anesthesia interfered with the development of typical Shwartzman lesions; by the 4th hour, the remaining lesions showed extensive hemorrhagic necrosis.

One lesion from each group was taken for histologic study. The changes found were similar to those which have been described previously (19). From mild inflammatory edema with infiltration of polymorphonuclear (heterophile) leukocytes in the prepared skin, there was progression of inflammatory change with heavier infiltration of leukocytes, the appearance of white cell "thrombi" at 1 hour, and finally, dissolution of vessel walls and interstitial hemorrhage at the 4th and 6th hours.

The remaining four lesions which had been excised at each test period were treated as follows: The epidermis was removed carefully with a razor blade and the rest of the tissue was ground with fine sand or alundum in a mortar and pestle, small amounts of physiologic saline being added to facilitate this process. After addition of more saline solution to bring the total volume to approximately 20 ml., suspensions were centrifuged at 3000 R.P.M. for 30 minutes, after which the slightly blood-tinged supernatant fluids were decanted, and stored at 4°C. Similar extracts were prepared from areas of normal skin as controls.

Each of the extracts from a pool of four lesions was divided into four equal parts. Two of these were then tested by intravenous injection into 2 normal rabbits, each animal, therefore, receiving the equivalent of one lesion. Extracts prepared in this fashion were tested in 64 rabbits, six from normal skin, six before the intravenous provocative injection, and four each from lesions $\frac{1}{2}$, 1, 2 $\frac{1}{2}$, 4, and 6 hours after intravenous injection.

All but two of the twenty-six preparations from Shwartzman lesions produced prompt rises in temperature of 0.8 to 1.7°C. in the test animals. No fever resulted from injection of one of the 2 $\frac{1}{2}$ hour and one of the 6 hour extracts.

Both P-35 polysaccharide and the *S. marcescens* filtrate employed in producing the skin lesions are potent bacterial pyrogens. In order to test the possibility that residual toxin at the prepared skin site was responsible for the observed febrile responses, the remaining two doses of each extract were heated in a water bath at 90°C. for 30 minutes, a temperature which should have no influence upon fever production by the heat-stable bacterial pyrogen. Extracts treated in this manner failed in every instance to produce temperature rises in test animals, thus indicating the presence in them of some fever-producing material other than the bacterial endotoxins. The results of this experiment are depicted in Fig. 1. It is not possible on the basis of these data to discriminate the amount of fever-promoting activity present in the lesions procured at various time intervals.

The Arthus Reaction.—

In view of the finding in the previous experiment that the mild inflammatory reaction caused by the skin-preparatory dose of Shwartzman toxin yielded a

temperature-elevating substance, it was decided to employ the Arthus reaction in a similar study. Here it would be possible to induce an acute local inflammatory reaction at a previously normal site by a single injection of non-pyrogenic antigen.

Six rabbits were given three intramuscular injections of 1 ml. of normal horse serum² at 2 day intervals. Twenty-two days after the last of these sensitizing injections, each animal was tested for hypersensitivity by the intracutaneous injection of 0.2 ml. of horse serum in

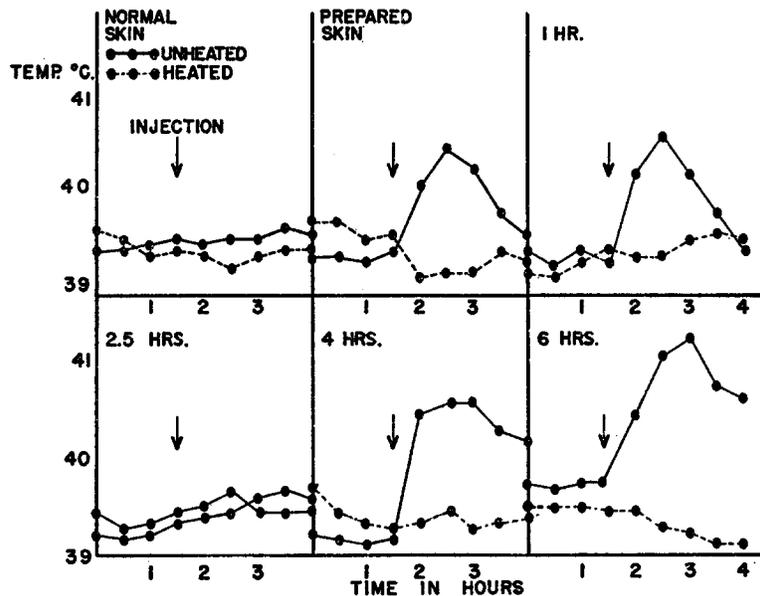


FIG. 1. Effect of injection of saline extracts of normal subcutaneous tissue, Schwartzman-prepared skin, and Schwartzman lesions at various stages of development upon temperature of normal rabbits. All tissues were excised from the same donor animal. In this experiment, extracts of lesions at $2\frac{1}{2}$ hours failed to produce fever (see text). In other experiments, extracts of $2\frac{1}{2}$ hour lesions were pyrogenic.

Heating at 90°C . for 30 minutes regularly destroyed the fever-producing effect.

the flank. This was followed by the development within 30 minutes of local reddening and edema which progressed to the point that by the 8th hour, four of the six animals exhibited lesions with hemorrhagic centers at least 1 cm. in diameter. Three days later these four animals were shaved, and 0.2 ml. of horse serum was injected intracutaneously into multiple sites over the trunk. The animals were anesthetized with nembutal and groups of five lesions were excised at $\frac{1}{2}$, 1, $2\frac{1}{2}$, 4 and 8 hours. Extracts of these lesions were prepared in the manner described in the last experiment, sections also being taken for histologic study.

Histologic examination of these lesions showed a progression from mild edema with almost no inflammatory infiltration to a picture indistinguishable

² Supplied by Sharpe and Dohme, Inc.

from the Shwartzman reaction at 8 hours. The principal difference was one of relative slowness of progression of the Arthus reaction as compared with the Shwartzman response.

None of the 30 minute or 1 hour extracts produced a rise in temperature. However, two of four extracts of 2 1/2 hour lesions produced fever and all four extracts of 4 and 8 hour lesions were found to contain a fever-promoting substance. Heating extracts at 90°C. for 30 minutes completely abolished the pyrogenic effect. See Fig. 2.

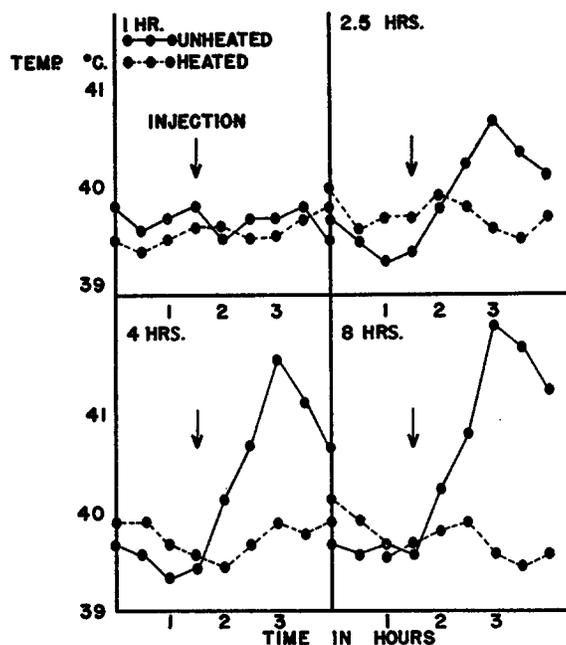


FIG. 2. Effect of injection of extracts of Arthus lesions at various stages of development. All lesions were excised from the same donor animal.

3. Attempts to Demonstrate a Fever-Producing Factor in Cells of Single Types Erythrocytes—Hemolysis or Other Injury *in Vivo*.—

Blood was collected by cardiac puncture into syringes containing heparin or 3.8 per cent sodium citrate solution; the plasma was separated by centrifugation at 2500 R.P.M. for 20 minutes. The cells were washed once with physiologic saline containing 0.8 mg. of streptomycin and 160 units of penicillin per ml., centrifuged at 2000 R.P.M. for 20 minutes in calibrated tubes, and the supernatant fluid discarded. The following methods of injury were then employed:

Method 1.—Four ml. of packed erythrocytes was added to 6 ml. of physiologic saline, shaken mechanically for 2 hours with glass beads, and injected (total volume, 10 ml.) intravenously into a test animal.

Method 2.—To 4 ml. of packed erythrocytes in 6 ml. of physiological saline, 1 or 2 mg. of saponin³ was added and the mixture was incubated for 2 hours at 37°C. This caused lysis of

³ J. T. Baker Chemical Co., lot No. 33144.

most of the cells present. This mixture (total volume, 10 ml.) was injected intravenously into a test animal.

Method 3.—Four ml. of packed erythrocytes was hemolyzed by mixing with 6 ml. of distilled water, incubated for 2 hours at 37°C.; and this material was injected intravenously into a test animal.

Method 4.—Erythrocytes were hemolyzed by quick freezing (in a dry-ice and alcohol bath) and thawing (in an incubator at 37°C.) three times. Four ml. of cells subjected to this treatment was injected intravenously into each test animal.

Method 5.—Erythrocytes were suspended in 50 per cent glucose solution and incubated for 2 hours at 37°C. Microscopic examination at that time revealed many crenated and deformed cells of bizarre shapes. There was usually no hemolysis. Four ml. of cells subjected to this procedure was injected into normal rabbits intravenously.

Table V summarizes the number of samples prepared by these methods and the number of test animals employed. There was no evidence of a fever-producing factor in any of these preparations.

TABLE V
Rabbit Erythrocytes Injured by Various Procedures in Vitro and Injected into Normal Animals Intravenously

Type of preparation	No. of test animals
Mechanical lysis	7
Saponin lysis	5
Distilled water lysis	8
Freezing-thawing lysis	4
Crenation in 50 per cent glucose	3

Erythrocytes—Intravascular Hemolysis.—

Three methods were employed in attempts to produce hemolysis *in vivo*. From 50 to 200 mg. of saponin injected intravenously in 5 ml. of distilled water resulted in hemolysis and a sharp febrile reaction in 4 normal rabbits. However, acid hydrolysis of the saponin sufficient to destroy its hemolytic activity failed to prevent this pyrogenic effect, which leads to the conclusion that the saponin contained extraneous pyrogenic contaminants.

Rabbits were given large amounts of distilled water intravenously (20 to 60 ml.) or intraperitoneally (50 to 200 ml.). This procedure regularly produced intravascular hemolysis and gross hemoglobinuria but no febrile reaction was encountered in 8 animals given distilled water intravenously or in 10 animals which received it by the intraperitoneal route.

Anti-rabbit-erythrocyte serum was prepared by injecting 20 ml. of rabbit red cells intravenously into a normal dog. Serum collected 28 days later produced agglutination of rabbit erythrocytes in a dilution of 1:500 although no hemolysis *in vitro* was demonstrable even in the presence of added complement. The intravenous injection of 10 ml. of this serum into normal rabbits produced definite febrile responses. Heating at 60°C. for 30 minutes reduced the agglutinin titer to zero and eliminated the fever-producing effect. Ten ml. of normal dog serum produced no fever in control animals.

There was no evidence of intravascular hemolysis in test animals given antiserum and all survived. We have observed febrile responses in rabbits as a

result of other antigen-antibody reactions, and instead of interpreting the fever following injection of anti-erythrocyte serum as resulting from specific injury to red cells, we are inclined to attribute it to an immunologic reaction.

Lymphocytes.—

Lymphocytes were obtained from paraaortic and mesenteric lymph nodes excised from normal animals immediately after sacrifice by air embolism. Approximately 1 gm. of lymphoid tissue was minced in 7 ml. of physiological saline and the mixture was shaken gently with glass beads for about 5 minutes. After extraneous connective tissue strands, etc., had been removed by filtration through gauze or a fine wire sieve, total and differential leukocyte counts were determined and the cell suspension was diluted with physiological saline to a concentration of 10,000,000 lymphocytes per ml. Test animals received 10 ml. of this final suspension intravenously, a total of 100 million cells.

In other tests, 1 gm. of lymphoid tissue was ground in a mortar and pestle without added abrasive, suspended in 10 ml. of physiologic saline, and injected intravenously.

TABLE VI
Lymphocyte Preparations Injected Intravenously into Normal Rabbits

Type of preparation	No. of specimens	No. of test animals
Suspension containing 10 million cells per ml.	6	16*
Extract of 1 gm. lymph node	7	7

* Each animal received 10 ml. of suspension.

Table VI summarizes the number of lymphocyte preparations and test animals used. There was no indication of a fever-producing substance in any of these trials.

Macrophages.—

Macrophages were obtained from peritoneal exudates in rabbits by a method similar to that used by Chase in guinea pigs (20). Fifty ml. of mineral oil was injected intraperitoneally and 5 days later, animals were sacrificed by air embolism. The abdomen was massaged for a few minutes and then opened by a midline incision. Approximately 100 ml. of physiologic saline was poured into the abdominal cavity, covering the viscera, and all fluid was then removed by gentle pipetting. Material collected in this manner was kept at 4°C. for 1 hour to allow mineral oil to rise to the surface. Exudates were centrifuged at 2000 R.P.M. for 30 minutes, supernatant fluid was discarded, the packed cells were resuspended in physiologic saline, and total and differential leukocyte counts were performed. Cell suspensions contained from 64 to 96 per cent macrophages, the remainder being composed almost entirely of polymorphonuclear cells (heterophiles). Test animals received suspensions of approximately 100 million macrophages intravenously or were given saline extracts of 500 million macrophages prepared by shaking cells for 2 hours with glass beads or alternate freezing and thawing.

In general, macrophage suspensions and extracts produced no febrile response in test animals. Table VII summarizes the number of preparations tested

and the results obtained. Two of fourteen macrophage exudates consistently produced definite rises in temperature of test animals. However, these contained respectively 28 and 36 per cent polymorphonuclear cells and, on the basis of negative results obtained with purer macrophage preparations and the results described in the next section with polymorphonuclear cells, it was concluded that macrophages contain no fever-producing substance.

TABLE VII
Macrophage Preparations Injected Intravenously into Normal Rabbits

Type of preparation	No. of specimens	No. of test animals
Suspension containing 100 million cells	12	16*
Extract of 500 million cells, mechanical shaking	7	12*
Extract of 500 million cells, freezing and thawing	4	6

* All animals remained afebrile with the exception of two in each group given material from exudates Nos. 6 and 8 which contained respectively 28 and 36 per cent polymorphonuclear (heterophile) leukocytes.

Polymorphonuclear Leukocytes (Heterophiles).—

In view of the technical problem involved in obtaining large quantities of leukocytes from the blood, a method similar to that of Mudd *et al.* (21) and Ponder and MacLeod (22) for collection of granulocytes from sterile peritoneal exudates was employed. Several modifications were tried; the procedure described here gave consistently good yields and was employed in most of the studies to be described.

Rabbits were shaved on the abdomen, and 350 ml. of physiologic saline containing 60 units of penicillin and 0.8 mg. of streptomycin per ml. was infused through 18 gauge needles into the peritoneal cavity, employing standard Fenwal apparatus. The drip was regulated so that the infusion was completed in 3 to 3 ½ hours. The animals were maintained on their backs for 4 hours after termination of saline injection. Then, under nembutal anesthesia, the abdomen was opened through a midline incision and all fluid in the peritoneal cavity was aspirated into a 250 ml. centrifuge bottle placed as a trap in a suction line. In early experiments heparin was added to the infusion fluid but this procedure was discontinued, because clotting of exudates presented no problem. Alteration of the tonicity of the infusion fluid appeared to exert little effect upon the yield of polymorphonuclear cells. The exudates were cloudy, occasionally slightly blood-tinged. Total and differential leukocyte counts were made immediately. After centrifugation for 20 minutes at 2000 R.P.M., the slightly turbid supernatant fluid was decanted into 125 ml. Erlenmeyer flasks, equipped with rubber stoppers. Cells and supernatant fluids were stored at 4°C. until tested. By this method, 100 to 200 ml. of exudate containing up to 10,000 leukocytes per mm.³, over 90 per cent granulocytes, were often obtained from a single animal. The record of an experiment in which this procedure was carried out on twelve animals is given in Table VIII.

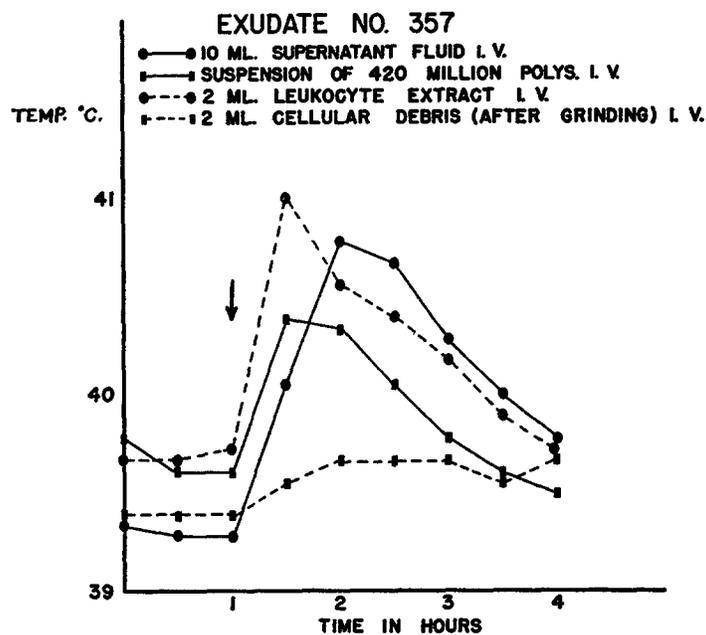


FIG. 3. Temperature response of normal rabbits given intravenous injections of whole leukocytes, leukocyte extract, cellular debris after extraction, and supernatant fluid from the same peritoneal exudate.

TABLE VIII

Quantity of Leukocytes in Peritoneal Exudates Produced in Rabbits by Slow Infusion of Physiologic Saline Solution

Animal	Volume of exudate	White blood cells per mm. ³	Polymorphonuclear leukocytes
	<i>ml.</i>		<i>per cent</i>
1	110	6,050	100
2	70	9,550	98
3	60	7,100	100
4	95	2,750	95
5	70	4,200	100
6	40	6,400	89
7	55	9,400	95
8	85	10,850	98
9	105	4,450	94
10	80	7,350	100
11	75	2,800	91
12	60	4,050	97

The intravenous injection of polymorphonuclear leukocytes in quantities of 200 to 600 million suspended in physiologic saline regularly produced febrile responses in test animals. Experiments were then performed to determine whether the active material could be extracted from the cells.

Extracts were prepared by shaking 500 million granulocytes in 10 ml. of saline solution with glass beads for 2 hours. After centrifugation at 2000 R.P.M. for 20 minutes, the clear supernatant fluid was decanted and injected into a test animal. The cellular debris, resuspended in 10 ml. of saline solution, was also injected intravenously into a normal rabbit.

In repeated experiments, the extracts were found to contain the fever-producing factor; only in rare instances was there any temperature rise in animals given the cellular debris.

It was also found that the supernatant fluids obtained from the original peritoneal exudates contained relatively large amounts of a fever-producing material. Injections of 10 ml. amounts of these fluids regularly produced fever in normal animals. In later experiments, the supernatant fluid from exudates proved to be a useful source of the fever-producing agent.

Fig. 3 shows the temperature records of animals given whole leukocytes, leukocyte extract, cellular debris, and supernatant fluid from the same exudate.

Heating at 90°C. for 30 minutes destroyed the fever-producing property of whole leukocytes, extracts, and supernatant fluids.

DISCUSSION

To test the hypothesis that products of injured tissue are capable of causing fever, a systematic search for evidence of fever-producing substances in the tissues of the rabbit has been carried out.

Several of the experimental procedures described were designed to simulate types of non-infectious pathological change in man which are usually attended by fever, such as infarction of the heart, lung, or kidney, and acute hemolytic episodes. The fact that it was not possible to induce fever in the rabbit by these procedures cannot be explained at this time.

It is of interest that the polymorphonuclear leukocyte was the only cell type tested which yielded a fever-promoting factor under the conditions described. These cells are likely to accumulate and to be damaged in many of the types of acute injury which are associated with fever. It is possible that the production of fever by extracts of Shwartzman and Arthus lesions as well as the occasional febrile response to bone marrow extracts noted in the experiments was due to the presence of these cells.

A possibility which cannot be excluded is that cells and tissues which appeared inactive in the experiments outlined here could be the source of temperature-elevating factors under other conditions, or in greater amount.

Furthermore, all cell types were not included in this work, a notable omission being capillary endothelium in quantity. As will be shown in the paper which follows, we have obtained evidence that there are sources of fever-promoting factor other than the polymorphonuclear leukocyte.

SUMMARY

Injection of extracts or suspensions of various rabbit tissues was found to be without effect upon the body temperature of normal rabbits.

Occasionally, extracts of bone marrow produced transient fever, and saline extracts of acute inflammatory lesions of the Shwartzman and Arthus types were found to produce fever when injected intravenously.

Suspensions or extracts of polymorphonuclear leukocytes collected from sterile peritoneal exudates contain a heat-labile substance which produced fever whereas those of erythrocytes, macrophages, and lymphocytes failed to do so.

The cell-free supernatant fluid of sterile peritoneal exudates obtained at the stage when polymorphonuclear leukocytes predominate also proved capable of producing fever.

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