STUDIES ON THE PATHOGENESIS OF FEVER

II. CHARACTERIZATION OF FEVER-PRODUCING SUBSTANCES FROM POLYMORPHONUCLEAR LEUKOCYTES AND FROM THE FLUID OF STERILE EXUDATES*

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In the course of a survey of the effect of injection of extracts and suspensions of normal tissues upon body temperature in rabbits (1), it was found that the intravenous inoculation of suspensions of whole polymorphonuclear leukocytes, extracts from these cells, or supernatant fluids obtained from sterile peritoneal exudates containing granulocytes produced febrile responses in normal rabbits. The present report describes experiments designed to characterize the agent or agents responsible for the rise of body temperature.

Materials and Methods

Rabbit leukocytes were collected from peritoneal exudates after infusion of physiologic saline as described in the preceding paper. A standard procedure was adopted for preparing suspensions and extracts of the cells collected. After separation of the cells and supernatant fluid of an exudate, the leukocytes were centrifuged in calibrated 15 ml. tubes at 2000 r.p.m. for 30 minutes and the packed cell volume was recorded. The cells were then ground with physiologic saline in a TenBroeck grinder, the debris was separated by centrifugation, and the supernatant extract was diluted with physiologic saline solution to a volume corresponding to 10 ml. for each 1 ml. of leukocytes. Two ml. of such an extract, corresponding to 0.2 ml. of leukocytes (roughly 400 million cells), injected intravenously into a normal rabbit produced approximately the same temperature response that followed injection of 10 ml. of supernatant fluid from the original exudate. In this manner an extract and a supernatant fluid from each exudate obtained were prepared and stored at 4°C.

Testing and recording of temperatures were carried out in normal rabbits as described in Paper I, and strict precautions against contamination with bacterial pyrogen were observed throughout (1). Thorotrast, nitrogen mustard, heparin, sodium citrate, physiologic saline, and enzyme preparations were injected intravenously into rabbits and demonstrated to be non-pyrogenic before use.

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

Effect of Polymorphonuclear Leukocytes Obtained from Whole Blood.—

It appeared desirable to determine whether fever-promoting activity could be demonstrated in polymorphonuclear leukocytes derived directly from circulating blood.

Nine rabbits under nembutal anesthesia were bled by cardiac puncture into 50 ml. syringes moistened with heparin, yielding a total of 1200 ml. of blood. This was placed in 250 ml. bottles and centrifuged at 2000 R.P.M. for 30 minutes. Buffy coats were removed by gentle suction, pooled, centrifuged again at 2000 R.P.M. for 30 minutes in 40 ml. tubes, and the leukocyte layers were again removed and placed in a 15 ml. calibrated centrifuge tube. After centrifugation at 2000 R.P.M. for 30 minutes, the volume of leukocytes recovered was found to be about 5 ml. Because of firm agglutination of the cells, accurate total leukocyte counts were not possible but stained smears revealed more than 75 per cent polymorphonuclear leukocytes. This material was ground in a TenBroeck grinder and the extract obtained was diluted to 50 ml., the equivalent of 10 ml. for each 1 ml. of buffy coat material. The cellular debris was resuspended in 50 ml. of physiologic saline.

Two ml. doses of the cellular extract were injected intravenously into 6 normal rabbits. Febrile responses, similar to those obtained with leukocytes from peritoneal exudate, were elicited. Heating at 90°C. for 30 minutes inactivated the extract. Injection of the suspension of cellular debris did not affect the body temperature of normal rabbits. It should be men-
tioned that in testing extracts prepared from buffy coat material, sudden death occasionally followed intravenous injections in rabbits; this could be prevented by premedication with heparin or by giving small "desensitizing" doses beforehand (2).

An extract of 6 ml. of buffy coat material was prepared from 1500 ml. of whole blood obtained from 11 normal rabbits using 3.8 per cent sodium citrate solution as an anticoagulant. This was also potent in producing fever and was inactivated by heating at 90°C. for 30 minutes. Fig. 1 shows the results of two typical experiments with extracts of leukocytes from heparinized and from citrated whole blood.

These results indicate that the presence of a fever-producing substance is not peculiar to the granulocytes of an induced peritoneal exudate. Furthermore, they suggest that the fever produced by injection of the supernatant fluids from exudates may be due to presence of material originating in the leukocytes of the exudate.

Effect of Polymorphonuclear Leukocytes Collected from Pleural Exudates.—

Over a period of 3 hours, 50 ml. of physiologic saline solution was infused by slow drip into the right pleural cavities of 2 normal rabbits. Four hours after completion of the infusion, both animals were sacrificed by intravenous injection of nembutal, the chests were opened, and all fluid was removed by gentle suction. The pooled exudates totaled 45 ml. containing 4,800 polymorphonuclear cells per mm.³ Ten ml. portions of the supernatant fluid were injected into 3 normal rabbits and produced typical febrile responses. An extract prepared by grinding the leukocytes of the exudate in 2 ml. of physiologic saline produced fever in a normal rabbit. The supernatant fluid heated at 90°C. for 30 minutes was not pyrogenic for a normal rabbit.

These results indicate that the presence of fever-producing material in exudates is not limited to inflammations of the peritoneum.

Heat Stability.—Boiling for 10 minutes or heating at 90°C. for 30 minutes destroyed the fever-producing capacity of leukocyte extracts or supernatant fluids from peritoneal exudates. A more detailed study of the effects of heating was undertaken.

Duplicate 10 ml. samples of the supernatant fluid and 2 ml. samples of leukocyte extract from an exudate were heated in a water bath for 30 minutes at 56, 65, 70, 80, 85, and 90°C. Two samples of supernatant fluid and of extract were exposed at each temperature and injected intravenously into normal rabbits. The fever-producing property of the extract disappeared from samples heated to 70°C. or higher, whereas samples of supernatant fluid continued to cause febrile reactions even after exposure to 85°C. Heating at 90°C., however, effectively destroyed the fever-promoting factor in these. The experiment was repeated with another exudate, and similar results were obtained.

These findings suggested the possibility that there might be different fever-producing substances in the extracts and supernatant fluids of peritoneal exudates. However, since leukocyte extracts prepared in the manner described above were found to have a pH of 4.5–5.0 while the supernatant exudate fluids showed a pH of 7.2–7.4, it seemed possible that the difference in heat stability might be due only to this factor.
Extracts were adjusted to pH 7.2 and supernatant fluids to pH 4.5 using phosphate-buffered saline and samples were tested after heating. At pH 7.2, the leukocyte extract remained stable at 70°C, the fever-producing capacity disappearing between 85 and 90°C. At pH 4.5, supernatant fluids lost potency after heating for 30 minutes at 70°C.

On the basis of these findings, it was concluded that the fever-producing materials in the extracts and supernatant fluids were similar in heat stability.

Effect of pH.—Extracts and supernatant fluids retained potency over a pH range from 2.0 to 10.5. There was no evidence of a gradual lessening of febrile response with increasing alkalinity or acidity within this range.

Stability at 37 and 4°C.—Incubation at 37°C. for 24 hours or storage for as long as 6 months at 4°C. had no detectable effect upon the ability of extracts or supernatant fluids to cause fever in normal rabbits.

Dialysis.—After dialysis through cellophane against distilled water or physiologic saline for as long as 5 days, with frequent changing of the bath, the fever-producing material was found to remain within the cellophane bag.

Protein and Polysaccharide Content.—

Analysis by a modification of the biuret method of Gornall et al. (3) of leukocyte extracts and supernatant fluids revealed a total protein content of 38 to 51 mg. per 100 ml. for leukocyte extracts and 389 to 511 mg. per 100 ml. for supernatant exudate fluids. Total polysaccharide, determined by a modification of the anthrone method (4), was 1.7 to 3.0 mg. per 100 ml. (calculated against a glucose standard) for extracts and 2.7 to 4.2 mg. per 100 ml. for supernatant fluids.

On the basis of these determinations it can be stated that a quantity of leukocyte extract (2 ml.) sufficient to cause a sharp febrile response in a rabbit contained a total of 0.76 mg. of protein and 0.054 mg. of polysaccharide.

Effect of Proteolytic Enzymes.—The fever-producing effect of leukocyte extracts and supernatant fluids was unimpaired by incubation for 18 hours at 37°C. with crystalline trypsin, chymotrypsin, or ribonuclease. Furthermore, treatment with trypsin did not cause the active material to become dialyzable. The effect of amylases was not determined because of inability to obtain preparations of these enzymes which were not pyrogenic, presumably due to contamination by bacterial products.

Electrophoretic Pattern.—Supernatant fluids were concentrated to one-fifth their original volume by dialysis against 12 per cent dextran. This was done in order to facilitate electrophoretic analysis, and did not cause reduction of potency in producing fever. Paper electrophoresis revealed patterns like the pattern of normal rabbit serum.

Test for Activation or Inhibition by Plasma.—Paired 10 ml. samples of super-

1 Kindly performed by Miss Pauline Hald.
2 Kindly furnished by Dr. Joseph Fruton.
3 Performed by Dr. H. O. Conn.
natant fluid and 2 ml. samples of leukocyte extract were incubated with 10 
ml. of normal rabbit plasma at 37°C. for 30 minutes. Control samples of super-
natant fluid and of extract diluted with physiologic saline were also incubated. 
Injection of these preparations into normal animals resulted in comparable 
febrile responses, there being no evidence of modification of the potency of 
supernatant fluid or leukocyte extracts by normal rabbit plasma. This experi-
ment was repeated with supernatant fluid and leukocyte extract from another 
exudate, the period of incubation in this instance being 18 hours. Again, the 
febrile response was not modified by the procedure. Similar experiments 
employing rabbit plasma which had been heated at 56°C. for 30 minutes before 
mixing with material from peritoneal exudates failed to give any evidence of 
presence of a plasma activator or inhibitor.

Comparison of the Fever Produced by Polymorphonuclear Leukocytes, by 
Bacterial Pyrogens, and by Menkin's Pyrexin.—Menkin has described pyrexin, 
a material associated with the euglobulin fraction of sterile exudates, which 
produces fever when injected intravenously into rabbits (5, 6). As mentioned 
in the accompanying paper (1), one criticism of Menkin's conclusion that 
pyrexin is responsible for the febrile reaction accompanying acute inflammation 
is his omission of adequate precautions against extraneous contamination by 
bacterial pyrogen during the process of separation of his material from exu-
dates. According to Menkin, the potency of pyrexin is not impaired by boiling. 
It has the further property of producing profound leukopenia within a few 
minutes after injection. Both of these findings are strongly suggestive of bac-
terial pyrogen (7). A series of experiments were therefore undertaken in which 
the properties of leukocyte extracts and supernatant fluids from peritoneal 
exudates were compared with pyrexin and with known bacterial pyrogens.

The bacterial pyrogens employed were piromen, a purified material from Pseudo-
monas; and a triple typhoid vaccine containing 1 billion organisms per ml. 
Through the kindness of Dr. Menkin, approximately 80 mg. of purified pyrexin (lot 235-T 
of 9-17-52) was made available to us. This material was found to produce brisk 
fevers in rabbits when injected in a dose of 0.25 mg. suspended in physiologic saline, 
and this dosage was employed throughout the study. The fever-producing effect of 
pyrexin was found to be unimpaired by boiling for 10 minutes, in contrast to the 
heat lability of leukocyte extracts already described.

(a) Effect of Single Injections.—Fig. 2 compares the fever curves of rabbits after 
a single injection of 2 ml. of leukocyte extract, 0.25 mg. of pyrexin, or 0.125 ml. of 
typhoid vaccine. The febrile responses of rabbits given injections of leukocyte ex-
tracts or supernatant fluids were remarkably uniform in pattern. Within 12 to 18 
minutes there was an abrupt rise to a peak at 1 to 2 hours with a prompt fall to the

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* Obtained from Baxter Laboratories.
* Obtained from the Georgia State Department of Health.
Fig. 2. Comparison of fever curves of normal rabbits after injection of leukocyte extract, typhoid vaccine, and Menkin's pyrexin. Note the prompt rise and fall after leukocyte extract as compared with the more prolonged, biphasic reactions to pyrexin and bacterial pyrogen.

Fig. 3. Effect of repeated injections of leukocyte extract upon temperature of normal rabbit. Baseline by 3 1/2 to 4 hours. In more than 350 observations, we have never seen a secondary rise after a single injection. This fever curve differed, then, in three respects from that following injection of pyrexin or bacterial pyrogen: the rise began earlier,
the duration of fever was shorter, and secondary elevations were not observed. The similarity of fever curves in rabbits after injection of bacterial pyrogens and pyrexin has been previously pointed out by Grant (8). With repeated injections of leukocyte extract, a series of identical peaks has been obtained as illustrated in Fig. 3 but the duration of fever from a single injection is not prolonged. Increasing the amount of extract injected may increase the height of the febrile response but the duration is little affected.

(b) Test for Development of Tolerance and Its Abolition by Thorotrast.—Rabbits given daily injections of the same dose of bacterial pyrogen rapidly develop tolerance to the fever-producing effect; successive fevers diminish progressively until a “minimal response” is reached after about 1 week (9, 10). Injection of thorotrast or other colloidal materials abruptly abolishes this acquired resistance and animals so treated react to pyrogen with fevers as great or greater than those following the first injection of pyrogen (11). The effects of repeated injections of supernatant fluid from peritoneal exudates and of pyrexin were therefore compared in the following experiment.

Two rabbits were given daily intravenous injections of 0.25 mg. pyrexin in 0.5 ml. of physiologic saline for 9 days; temperatures were recorded for 5 hours after injection on the 1st, 3rd, 5th, 7th, and 9th days. Another pair of animals received daily intravenous injections of 10 ml. of supernatant fluid from pooled exudate, their fever curves also being measured on the same days. On the afternoon of the 9th day, all animals were given 6.0 ml. of thorotrast (Heyden) and injections of pyrexin or supernatant fluid were repeated on the 10th day, 16 hours after thorotrast. In order to facilitate comparison of febrile responses from day to day, they were plotted on 2.5 mm. graph paper, and the area under each temperature curve was measured with a planimeter, the vernier reading being recorded as the “fever index,” an expression of both height and duration of fever (9).

As can be seen in Fig. 4, the febrile response to 0.25 mg. of pyrexin decreased rapidly with successive injections. After injection of thorotrast, however, both animals reacted to pyrexin with higher fevers than did those following the first injection of this material. In striking contrast, the animals given daily injections of supernatant exudate fluid reacted to successive doses with approximately the same fever, and injection of thorotrast failed to modify this reaction. This experiment was repeated with two groups of three animals, with similar results.

(c) Cross-Tolerance to Bacterial Pyrogens.—Rabbits made tolerant by repeated injections of bacterial pyrogen derived from a single species are also resistant to the fever-producing effect of pyrogens of other bacterial species (9, 10, 12, 13). It was of interest, therefore, to test the effect of leukocyte extracts and of pyrexin in animals rendered tolerant to bacterial pyrogens.

Two rabbits were given 0.25 mg. of pyrexin in 0.5 ml. of physiologic saline intravenously and temperatures were recorded for 5 hours after injection; their fever indices were 25 and 36. On the 2nd day, the animals were injected with 0.125 ml. of typhoid vaccine; this resulted in fever indices of 53 and 41. Daily injections of typhoid vaccine were then continued until the 8th day when temperatures were again recorded giving indices of 10.5 and 9, indicating tolerance to the typhoid pyrogen. On the 9th day, pyrexin was again injected;
both animals reacted with mild fevers, the indices being 14 and 12.5, considerably lower than those recorded on day 1. On the afternoon of the 9th day, the rabbits were given 6.0 ml. of thorotrast intravenously and 16 hours later, on day 10, the injection of 0.25 mg. of pyrexin was repeated. Both animals reacted with fevers higher than those recorded on the 1st day, the fever indices being 35 and 54. Fig. 5 shows the results of this experiment. In a similar experiment using 4 rabbits made tolerant by daily injection of 50 μg. of piromen for

Fig. 4. Effect of repeated injections of pyrexin and peritoneal exudate fluid. Note the development of tolerance with pyrexin, abolished by thorotrast injection in the upper graph. Animals given exudate material responded with approximately equal fevers each day and thorotrast did not alter responses on the 10th day.
7 days, animals were found to be resistant to pyrexin and this resistance was abolished by thorotrast injection.

In striking contrast, tolerance to bacterial pyrogens was found to exert no influence upon the fever-producing potency of the material from polymorphonuclear leukocytes. Fig. 6 portrays the failure of tolerance to typhoid vaccine to modify the febrile response of rabbits to 2.0 ml. of leukocyte extract. Resistance to piromen was likewise without effect upon the fever following injection of leukocyte extract.

**Fig. 5.** The reduction in febrile response to pyrexin on day 9 in animals made tolerant to typhoid vaccine by daily injections on days 2 to 8. Thorotrast resulted in greater fevers after pyrexin on day 10.

(d) Effect upon Circulating Leukocytes.—In this study no observations were made regarding the effect of pyrexin upon circulating leukocytes. Menkin, however, has described leukopenia followed by leukocytosis after injection of some preparations (5, 6). This is, of course, a characteristic response to injection of bacterial pyrogens and has even been suggested as a test for their presence (7). Weisberger et al. (14) have reported that the injection of suspensions of polymorphonuclear leukocytes is followed by leukopenia in rabbits. Our findings with whole cell suspensions were in accord with this. However, the injection of leukocyte extract or the supernatant fluid of peritoneal exudates produced no consistent change in the number of circulating leukocytes, although with some preparations a mild tendency to leukopenia during the first 2 hours after injection was noted. Weisberger et al. noted leukopenia after
injection of leukocyte extracts. The difference in our findings may be a matter of dosage but it should be pointed out that Weisberger et al. did not take precautions against contamination with bacterial pyrogens. Table I shows the effects of leukocyte extract and supernatant fluids from the same peritoneal exudate upon the total leukocyte count of normal rabbits.

**Fig. 6.** The failure of tolerance to typhoid vaccine to modify the febrile response to leukocyte extract on day 9 and the failure of thorotrast to result in increased fevers on day 10.

The **Effect of Amidopyrine.**—Four rabbits were given 10 ml. of supernatant fluid and 4 received 2 ml. of leukocyte extract from the same exudate. Two animals in each group were given 0.5 gm. of a suspension of amidopyrine subcutaneously 1 hour before and 1 hour after injection of the leukocyte materials. Untreated animals showed typical febrile responses while those given amidopyrine remained afebrile. This experiment was repeated on two occasions with the same result.

The **Effect of Cortisone and Amidopyrine upon the Production of the Fever-Promoting Material in Peritoneal Exudates.**—Eleven animals were used in preparation of sterile saline peritoneal exudates, as described previously. Four had received 25 mg. of cortisone acetate intramuscularly daily for 3 days; three

<sup>1</sup> Kindly supplied by Merck and Company.
were given 0.5 gm. of amidopyrine subcutaneously 1 hour before beginning the infusion and 3 1/2 hours later when the infusion had been completed. The remaining 4 rabbits received no therapy. Peritoneal exudates were harvested and extracts and supernatant fluids were prepared and tested. Table II tabu-

TABLE I

Total Leukocyte Counts of Rabbits after Intravenous Injection of Leukocyte Extract or Supernatant Fluid from the Same Peritoneal Exudate

<table>
<thead>
<tr>
<th>Animal</th>
<th>Injected with</th>
<th>Preliminary</th>
<th>5 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>1 hr.</th>
<th>2 hrs.</th>
<th>3 hrs.</th>
<th>4 hrs.</th>
<th>5 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 ml. extract</td>
<td>10,400</td>
<td>8,400</td>
<td>7,300</td>
<td>9,100</td>
<td>8,700</td>
<td>10,700</td>
<td>10,500</td>
<td>11,200</td>
<td>9,500</td>
</tr>
<tr>
<td>2</td>
<td>2 ml. extract</td>
<td>11,500</td>
<td>9,450</td>
<td>9,850</td>
<td>8,000</td>
<td>11,200</td>
<td>13,100</td>
<td>11,200</td>
<td>10,100</td>
<td>10,500</td>
</tr>
<tr>
<td>3</td>
<td>2 ml. extract</td>
<td>12,000</td>
<td>9,400</td>
<td>10,150</td>
<td>13,900</td>
<td>11,100</td>
<td>9,200</td>
<td>14,500</td>
<td>13,200</td>
<td>13,800</td>
</tr>
<tr>
<td>4</td>
<td>10 ml. supernatant</td>
<td>12,950</td>
<td>6,450</td>
<td>5,850</td>
<td>6,500</td>
<td>7,900</td>
<td>12,100</td>
<td>11,600</td>
<td>11,700</td>
<td>10,100</td>
</tr>
<tr>
<td>5</td>
<td>10 ml. supernatant</td>
<td>14,300</td>
<td>8,350</td>
<td>8,650</td>
<td>7,700</td>
<td>8,350</td>
<td>12,000</td>
<td>11,400</td>
<td>5,400</td>
<td>13,700</td>
</tr>
<tr>
<td>6</td>
<td>10 ml. supernatant</td>
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<td>7,700</td>
<td>9,100</td>
<td>8,300</td>
<td>5,700</td>
<td>6,800</td>
<td>6,300</td>
<td>9,400</td>
<td>6,900</td>
</tr>
</tbody>
</table>

TABLE II

Effect of Amidopyrine and Cortisone upon Peritoneal Exudates after Infusion of Saline in Rabbits

<table>
<thead>
<tr>
<th>Animal</th>
<th>Volume of exudate</th>
<th>White blood cells per mm.3</th>
<th>Polymorphonuclear leukocytes per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1*</td>
<td>112</td>
<td>6,200</td>
<td>84</td>
</tr>
<tr>
<td>A-2*</td>
<td>90</td>
<td>5,650</td>
<td>92</td>
</tr>
<tr>
<td>A-3*</td>
<td>75</td>
<td>7,700</td>
<td>99</td>
</tr>
<tr>
<td>C-1†</td>
<td>160</td>
<td>4,950</td>
<td>96</td>
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<tr>
<td>C-2‡</td>
<td>90</td>
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<tr>
<td>C-3‡</td>
<td>45</td>
<td>2,800</td>
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<tr>
<td>C-4‡</td>
<td>80</td>
<td>9,150</td>
<td>88</td>
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<tr>
<td>N-1§</td>
<td>110</td>
<td>2,400</td>
<td>91</td>
</tr>
<tr>
<td>N-2§</td>
<td>180</td>
<td>7,200</td>
<td>100</td>
</tr>
<tr>
<td>N-3§</td>
<td>65</td>
<td>9,450</td>
<td>99</td>
</tr>
<tr>
<td>N-4§</td>
<td>50</td>
<td>6,050</td>
<td>96</td>
</tr>
</tbody>
</table>

* Amidopyrine.
‡ Cortisone.
§ Normal controls.

lates the leukocyte counts and volumes of exudate from each animal. All exhibited fever-promoting activity. There was no evidence of suppressive effect by either cortisone or amidopyrine.

Effect of Nitrogen Mustard (HN2).—It has been shown that rabbits treated with HN2 in doses sufficient to suppress the formation of polymorphonuclear leukocytes and to produce severe neutropenia are still capable of reacting to
injection of bacterial pyrogens with high fever (15). We have also observed that rabbits made leukopenic with HN₂ respond with typical fevers to antigen-antibody reactions, to injection of influenza virus, and to injection of various particulate materials. In view of these findings, the effect of HN₂-induced granulopenia upon the production of fever-promoting substances in peritoneal exudates was investigated.

![Graph showing febrile responses of rabbits after injection of supernatant fluid from peritoneal exudates of HN₂-treated rabbits.]

**Figure 7.** Febrile responses of rabbits after injection of supernatant fluid from peritoneal exudates of HN₂-treated rabbits.

### TABLE III

Effect of HN₂ upon Peritoneal Exudates after Infusion of Saline in Rabbits

<table>
<thead>
<tr>
<th>Animal</th>
<th>Volume of exudate</th>
<th>White blood cells per mm.³</th>
<th>Polymorphonuclear leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ML.</td>
<td></td>
<td>per cent</td>
</tr>
<tr>
<td>1</td>
<td>130</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
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<td>0</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>350</td>
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</tr>
<tr>
<td>4</td>
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<td>140</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
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</tr>
<tr>
<td>6</td>
<td>110</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
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</tr>
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<td>95</td>
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</tr>
<tr>
<td>12</td>
<td>40</td>
<td>60</td>
<td>0</td>
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Twelve rabbits were given 5 mg. of HN₂₈ intravenously and 4 days later, saline peritoneal exudates were prepared in the usual way. The cellular response, detailed in Table III, was scant in all animals and in many instances; no polymorphonuclear leukocytes were present in the abdominal fluids. However, the injection of 10 ml. of supernatant fluid from these exudates into normal rabbits was followed in every instance by typical febrile responses, examples of which are shown in Fig. 7. The ability of these fluids to cause fever was destroyed by heating at 90°C. for 30 minutes and was uninfluenced by dialysis or digestion with proteolytic enzymes. This experiment was repeated, using 9 animals, and again it was found that peritoneal fluids contained a fever-producing substance, even in the absence of granulocytes in the exudates or in the peripheral circulation.

**DISCUSSION**

The evidence furnished by these further studies on the production of fever by the components of peritoneal exudates in rabbits indicates that the fever-producing substances contained in polymorphonuclear leukocyte extracts and supernatant fluids are closely related. Conclusive evidence of identity must await chemical definition but the properties thus far studied have been common to both. Neither is dialyzable, even after tryptic digestion. There is no impairment of potency by trypsin, chymotrypsin, or ribonuclease. At pH 7.2, the pyrogenic activity of extracts and supernatant fluids is lost by heating at 90°C. for 30 minutes; at pH 4.5, potency disappears at 70°C. Both preparations retain their activity over a pH range from 2 to 10.5. The fever curves of normal rabbits after injection of whole leukocytes, leukocyte extract, or supernatant peritoneal fluid are the same in time of onset, height, shape, and duration.

It is hoped that the chemical nature of the pyrogenic factor can be defined more precisely, although the problems of obtaining large amounts of material and the necessity that all manipulations be carried out with techniques which avoid contamination with bacterial pyrogen offer severe handicaps. Also the process of identification is tedious because the only method now available for detection of activity is test of effect upon the body temperature of experimental animals.

That the presence of fever-promoting material in peritoneal exudates is not determined by some influence peculiar to the abdominal cavity is established by the demonstration of similar activity in pleural fluids. Furthermore, extracts of acute localized skin inflammations contain a heat-labile pyrogenic material (1).

Although the finding of febrile responses after injection of peritoneal fluids from HN₂₈-treated animals, containing no leukocytes, suggests strongly

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*: Mechlorethamine HCl, Merck.
that the polymorphonuclear leukocyte is not the only source of fever-producing material, the demonstration of such a substance in extracts of polymorphonuclear leukocytes collected from the peripheral blood justifies the assignment of an important role to this cell.

The experiments in which the effects of leukocyte extracts and Menkin’s pyrexin were compared demonstrated important differences in these two substances. The heat-lability of leukocyte extracts, the short, monophasic febrile response following a single injection, the failure of animals to develop tolerance to repeated injections, the lack of potentiation of the response in thorotrast-treated rabbits, and the absence of evidence of cross-tolerance in animals resistant to bacterial pyrogens are all in striking contrast to the findings with pyrexin. Pyrexin resists boiling, the fever following its injection is prolonged and is often biphasic, animals acquire tolerance to its fever-producing effect when given repeated injections, a resistance which is abolished by thorotrast, and animals resistant to bacterial pyrogens show greatly decreased fevers after injection of pyrexin. While it is entirely possible that pyrexin is a product of injured cells having properties similar to those of pyrogenic bacterial endotoxins, the possibility that contamination with bacterial products is responsible for these findings must be taken seriously. Certainly, the present results justify the conclusion that leukocyte extracts contain some substance other than pyrexin which causes fever. In this connection, it may be pointed out that turpentine-induced pleural exudates in dogs, of the type used by Menkin as a source of pyrexin, were found by Bennett (16) to produce fever in rabbits and dogs when injected intravenously. Bacterial pyrogen contamination was avoided in his experiments and it was demonstrated that animals did not become tolerant to exudates with repeated injections and that resistance to bacterial pyrogens conferred no protection against the production of fever by pleural exudates. Furthermore, the febrile response to a single injection of pleural fluid in rabbits was of short duration, resembling closely the fevers after injection of leukocyte extracts and peritoneal fluids described in the present report. It seems possible, indeed probable to us, therefore, that the activity observed by Menkin in the exudates from which he extracts pyrexin is due to the same substance or substances that we are attempting to characterize.

Menkin’s suggestion that pyrexin is the cause of fever in inflammatory states is not easily reconciled with the fact that the animal body rapidly develops a tolerance to its fever-producing effect. The occurrence of long continued fever with inflammation might be explained as due to liberation of increasing quantities of the material, but there is seldom any reason to believe this to be the case; furthermore there is experimental evidence indicating that recovery from most infections takes place without the development of increased resistance to bacterial pyrogens (10, 17). Relapses and
secondary infections are likely to be accompanied by fever; indeed recurrence of fever is usually the signal of such complications.

It is tempting to speculate upon the possible role of a fever-producing substance from polymorphonuclear leukocytes in the fever accompanying various disease states. Leukocytic infiltration at inflammatory sites and the occurrence of peripheral leukocytosis in many febrile disorders might signify a possible relationship. In diseases accompanied by normal peripheral leukocyte counts or leukopenia, the actual turnover of leukocytes is unknown. Certainly, fever in patients with agranulocytosis or aplastic anemia cannot be attributed to a product of leukocytes, and experiments in rabbits given HN₂ suggest that sources of fever-promoting substances other than granulocytes exist in the animal body.

**SUMMARY**

Further studies on fever production by injection of leukocyte extracts or cell-free supernatant fluids from peritoneal exudates in rabbits are reported.

Granulocytes collected from peripheral blood or from pleural exudates contain a heat-labile pyrogenic substance.

The material in extracts of leukocytes and in peritoneal fluids, which causes fever, is destroyed by heating for 30 minutes at 90°C. at pH 7.2 and at 70°C. at pH 4.5. It is active in producing fever over a pH range of 2.0 to 10.5 and maintains potency for as long as 6 months at 4°C.

The fever-producing substance in leukocyte extracts is not dialyzable. Its activity is not destroyed by trypsin, chymotrypsin, or ribonuclease. No evidence of plasma activator or inhibitor was detected.

Significant temperature elevation in the rabbit was effected by a quantity of leukocyte extract containing 0.76 mg. protein and 0.054 mg. polysaccharide.

The febrile response produced by the material under study was compared with that of Menkin's pyrexin as well as with that of bacterial pyrogens. Several significant differences were noted. The properties of pyrexin are similar to those of bacterial pyrogens.

Amidopyrine suppressed the febrile response to injection of leukocyte extracts, whereas neither amidopyrine nor cortisone influenced the appearance of pyrogenic material in induced peritoneal exudates.

Peritoneal fluids collected from rabbits made leukopenic by HN₂ were found to contain a fever-promoting substance. Its character has yet to be determined.

It is concluded that there is present in polymorphonuclear leukocytes of rabbits a heat-labile factor capable of producing fever in rabbits and that the leukocyte is probably not the only source of such a factor.
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