RELEASE OF AN ENDOGENOUS PYROGEN IN VITRO FROM RABBIT MONONUCLEAR CELLS*

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Plates 34 and 35

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There is now considerable evidence that most if not all microbial agents produce fever indirectly by liberating a circulating pyrogen of endogenous origin (EP) from tissues of the host (1). To date, the source of this pyrogen can be inferred with some certainty only in fevers induced by the endotoxins of Gram-negative bacteria. When endotoxins are incubated with granulocytes from acute exudates, an EP is released in vitro (2). Furthermore, when given in moderate doses, these bacterial agents fail to cause fever in rabbits that are made severely granulocytopenic with nitrogen mustard (3). Since such animals respond normally to EP given intravenously, these experiments suggest that the granulocyte plays an essential role in mediating endotoxin-induced fever.

Although saline extracts of many tissues in addition to granulocytes, are pyrogenic when given in sufficient dosages (4), the role of such pyrogens in causing various experimentally induced or clinical fevers has not been ascertained. To date, therefore, there has been no firm evidence that cells other than granulocytes are capable of being activated to produce a pyrogen either in vivo or in vitro.[1]

The present study reports the differential ability of mononuclear leukocytes derived from three tissues of the rabbit—lung, spleen, and lymph nodes—to release pyrogen in vitro in two experimental situations: incubation with tuberculin and phagocytosis of heat-killed bacteria. Certain conditions for this reaction have been defined and compared with previously described factors that control release of pyrogen from granulocytes.

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[1] Recent work published from this laboratory, and discussed below, indicates that human blood monocytes will release a pyrogen after phagocytosis of heat-killed staphylococci in vitro (15).

Materials and Methods

General.—All techniques relative to (*a*) preparation of pyrogen-free equipment and solutions, (*b*) selection of rabbits as donors and recipients, and (*c*) assay of pyrogenic activity of preparations by intravenous inoculation were similar to those described previously (5, 6).

Sensitization of Rabbits.—Donor rabbits were sensitized by intravenous injection of 5 to 10 mg BCG (Calmette-Guérin bacillus) by techniques already presented (6). Only animals responding to intravenous inoculation of 50 or 100 mg tuberculin (OT) with fevers of 1.5°C or greater were selected. Animals were generally tested for sensitivity within 10 to 14 days after infection and were not used as donors until several days later to obviate the transient desensitization that occurs in some animals the day following a single large dose of antigen (5).

Old Tuberculin (2000 mg/ml).—This was obtained from the same source as previously (6) and in most experiments was diluted in saline to a concentration of 100 mg per ml just before addition to the tissue suspensions in vitro.

Blood, Spleen, and Lymph Nodes.—Methods used for collection and processing of these tissues were identical with those previously reported (6) except for substitution of heparinized Krebs-Ringer phosphate buffer[2] (with 10–15% normal rabbit serum, heparin 10 units/ml, and glucose 150 mg/100 ml) for physiologic saline as the suspending medium. In several experiments, saline was used instead of buffer and pyrogen release in these two media compared.

Lung Cells.—Mononuclear cells lining the respiratory passages of the lung were collected by a modification of Myrvik's technique (7). After exsanguination, the chest was opened and the heart and pulmonary vessels were dissected out and separated from the surface of the lungs. The trachea was then transected and the lungs removed en bloc, taking care to free up the lungs from the esophagus posteriorly. With sterile gloves, the cut end of the trachea was grasped with forceps and the lungs were suspended over a large beaker packed in ice. 150 ml of cold saline was then infused into the two lungs by a second operator using 50 ml syringes and No. 17 needles placed in the bronchi to distend all lobes to capacity. Following this, the lungs were massaged gently in the upright position and then inverted over the beaker, allowing the contents to drain out the trachea. This process was repeated three times, after which a small additional amount of saline was poured over the surface of the lungs to remove any adherent cells that had spilled out of the trachea during massage. Aseptic technique was used throughout.

The cell suspension was then centrifuged at 2000 rpm in an International Centrifuge, No. 2 (International Equipment, Needhan Heights, Mass.) for 30 min at 4°C and the supernatant discarded. Following this, the sediment was resuspended in the same buffer as used for the other tissues. Cell counts from each pair of lungs generally totaled $1–2 \times 10^9$. In several experiments, lung cells were collected in the same manner from normal rabbits. Counts from these donors averaged somewhat less ($5 \times 10^8–1 \times 10^9$) but differential counts of these cells did not differ significantly from those of BCG-sensitized donors.

Samples of both initial and final cell suspensions from each tissue were taken for culture (before and after incubation with OT) as well as for counting and for differential smears, as reported previously (6). Representative mononuclear cells present in the lung cell preparations are shown in Fig. 1.

In all cases in which tissues were incubated for prolonged periods (18 hr) with tuberculin, penicillin (20,000 units per whole tissue suspension) was added.

Cultures of the lung cell suspensions (both before and after incubation with penicillin) were positive on a number of occasions, the organism most frequently isolated being *Bordetella*-sp. Results with these as well as with other sterile preparations were discarded, if supernatants of control cell preparations (incubated without OT) produced fevers of >0.3°C.

[2] Modified to contain 1/5 the concentration of Ca^{++} and Mg^{++}, to prevent precipitation.

Incubation of Tissues with Tuberculin.—Except where otherwise stated, tissues were incubated with tuberculin in a ratio of 2×10^8 cells per 200 mg OT and the supernatant fluid (usually 5 to 10 ml) which initially contained this number of cells was injected as a single dose.

In several experiments, the initial tissue cell suspension was washed once in saline before resuspension in the buffer but, as pyrogen release appeared poorer after this procedure, it was omitted in the later experiments reported here.

Tissue suspensions (with and without added tuberculin) were shaken for 2 to 3 hr in a Dubanoff incubator at 37°C and then placed in a stationary incubator overnight, except in a few instances in which the total incubation was reduced from 18 to 5 hr. The next morning, the supernatant fluids were separated by centrifugation and injected with 500 units of heparin per dose of cells, either added to the material or given as a separate injection.

Phagocytosis Studies.—A strain of *Staphylococcus albus*, originally obtained from a wound infection, was used in the phagocytosis experiments (8). An 18-hr growth in beef heart infusion broth (previously autoclaved for 2 hr to inactivate any contaminating bacterial pyrogen), was centrifuged, and the bacterial cells washed once with saline. They were then resuspended in saline and the concentration of bacteria determined by agar pour-plates of 100-fold dilutions. In some experiments, numbers of bacteria were estimated by measurement of optical density at 600 mμ, by comparison with a curve constructed from several previous experiments. The suspension of bacteria was then autoclaved for 20 min, and stored at −20°C.

Heparinized rabbit blood (10 units/ml) was obtained by cardiac puncture, and mixed with 2 to 3 parts of 3% Dextran[3] in saline. The dextran had previously been autoclaved for 1½ hr to inactivate contaminating pyrogens. The mixture was allowed to stand for 30 to 40 min, and the leukocyte-rich supernatant then removed and centrifuged at 1500 rpm for 15 min. The cell button was washed once in heparinized Krebs-Ringer-Phosphate buffer (10 units/ml), centrifuged again, and most of the remaining red cells lysed by brief exposure to distilled water (8). The cells were then washed once more in buffer, and suspended in a small volume of buffer to a final concentration of about 5×10^7 per ml.

Incubation.—50 ml Erlenmeyer flasks contained $2\text{–}3 \times 10^8$ white cells in buffer, 10% to 20% serum, glucose 150 mg/100 ml and penicillin 10,000 to 15,000 units. Staphylococci (see above) were added to some flasks at varying multiplicities of bacteria:leukocytes. Total incubation volumes were 15 to 20 ml, except for one experiment with lymph node cells in which 8 ml was used. Flasks were shaken for 2 to 3 hr in a Dubanoff incubator at 37°C, cover slip smears taken, and then the flasks placed in a stationary incubator at 37°C for about 15 hr. Supernatants were removed after centrifugation at 3000 rpm for 30 to 40 min.

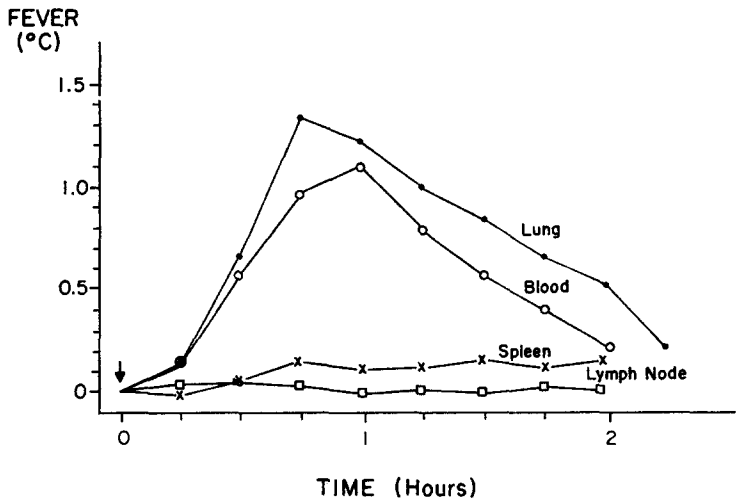
RESULTS

Responses Induced by Supernates of Various Tissues (Derived from a Single BCG-Sensitized Donor) and Incubated with Tuberculin In Vitro.—Text-fig. 1 depicts the mean febrile responses to supernates of various tissues from a single BCG-sensitized rabbit. Tissues were incubated with tuberculin for 18 hr in Krebs-Ringer buffer with 10% normal rabbit serum (see "Materials and Methods"). Each dose was equivalent to 1.7 to 2.25×10^8 leukocytes with 200 mg OT. Prompt monophasic fevers, reaching a maximum at 45 to 60 min, were produced by supernates of lung and blood leukocytes incubated with this dosage of tuberculin; those derived from both spleen and lymph nodes, by comparison, were nonpyrogenic. As the differential count of the lung leukocytes revealed

[3] Dextran, M.W. 100,000–200,000. Nutritional Biochemicals Corp., Cleveland, Ohio.

97% mononuclear and 3% polymorphonuclear cells, each recipient received a total of only 6×10^6 polymorphonuclear leukocytes, a dosage that appears to be inadequate to account for this degree of fever, whether these cells are derived from the blood (see below) or from already activated granulocytes from acute exudates (9).

Febrile Responses to Supernates of Mononuclear Cells Derived from Several Sources and Incubated with Varying Dosages of Tuberculin In Vitro.—Because the striking difference in the capacity of mononuclear cells derived from these three sources to liberate pyrogen could be attributed to differential destruction



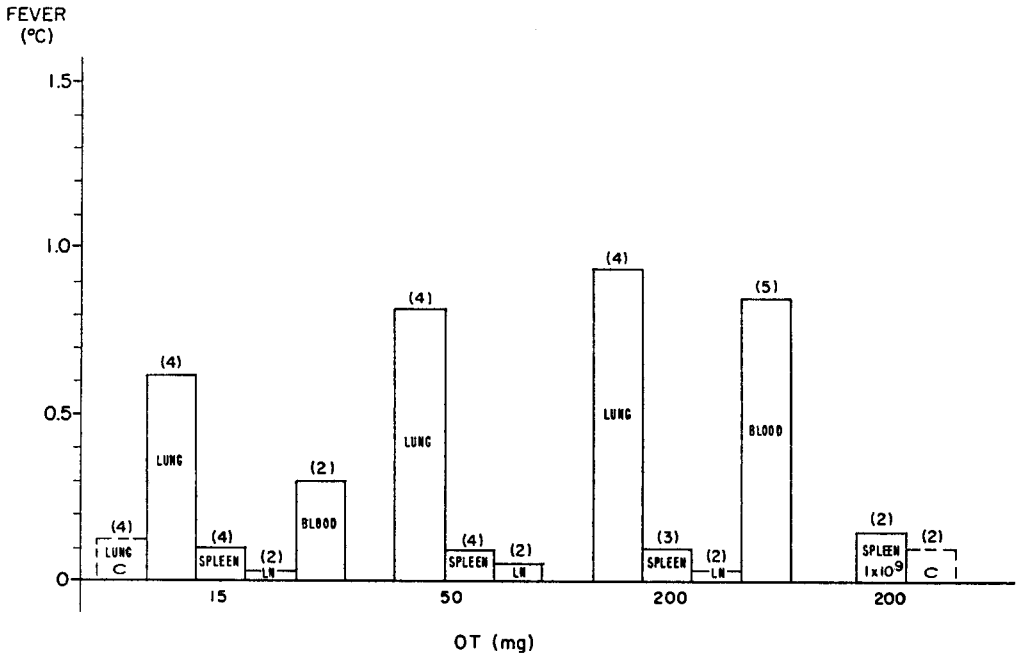
TEXT-FIG. 1. Mean responses of groups of two or three rabbits to supernates of various tissue cells (from a single BCG-sensitized donor rabbit) incubated 18 hr with tuberculin. Each dose was equivalent to about 2×10^8 leukocytes with 200 mg OT. Control injections (supernates of tissue cells incubated without OT) were nonpyrogenic (not shown).

of sensitized cells by the relatively large dose of tuberculin used (200 mg), it next seemed advisable to repeat the experiment with graded doses of tuberculin. Accordingly, samples of lung, spleen, and lymph nodes from similarly sensitized donor rabbits were incubated, in aliquots of about 2×10^8 cells, with one of three dosages of tuberculin—15, 50, or 200 mg—and were compared with blood cells incubated with 15 or 200 mg tuberculin. The results are shown in the bar graphs in Text-fig. 2. Despite a greater than 13-fold variation in the dosage of tuberculin[4], leukocytes derived from both spleen and lymph nodes failed to

[4] In a subsequent experiment, aliquots of 2×10^8 spleen cells were incubated with 500 mg OT for 18 hr in the same media. Supernates of these preparations failed to induce significant elevations of temperature, confirming earlier work with similar dosages of spleen cells and OT incubated overnight in serum or Hanks' solution (6).

release detectable amounts of pyrogen under these conditions. In addition, two recipients remained afebrile when injected with supernates derived from 1×10^9 spleen cells (five times the previous dose) incubated for the same period with 200 mg OT (see Text-fig. 2, extreme right).

Release of pyrogen from lung mononuclear cells and blood was directly related to dosage, although the difference in the febrile responses induced by



TEXT-FIG. 2. Average fevers induced by supernates of various tissue cells (from two BCG-sensitized donors) incubated 18 hr with one of three different dosages of tuberculin. Blood cells were incubated with the smallest and largest dosage only. Each cell dose was equivalent to 2×10^8 leukocytes. C = control cells (incubated without OT). In this and succeeding charts, the numbers in parentheses above or within each bar indicate the number of recipients.

lung cells incubated with 15 or 200 mg tuberculin represents probably less than a twofold increase in amount of pyrogen liberated, as responses of this order fall on the steep part of the dose–response curve for granulocytic pyrogen (9).

Effects of Varying Time of Incubation and Dosage of Mononuclear Cells Incubated with Tuberculin.—In the next experiment, the dosage of mononuclear leukocytes from the same three tissues was varied from 1×10^7 to 2×10^8, on the assumption that factors present in high dosages of spleen or lymph node cells might inactivate any pyrogen liberated during incubation. Each dose of cells was incubated for 18 hr with 200 mg tuberculin in the same medium as used previously.

The results, derived from tissues of a single donor rabbit, are tabulated in Table I. Detectable amounts of pyrogen (producing responses of 0.3°C or greater) were only released by mononuclear cells from the lung. Over this dosage range there was a small but definite correlation between the magnitude of the

TABLE I

*Febrile Responses Induced by Supernates of Leukocytes from Various Sources (from a Single BCG-Sensitized Donor) Incubated 18 hr with Tuberculin In Vitro**

Source of leukocytes	Number of cells (+ OT)			Control cells
	1×10^7	5×10^7	2×10^8	2×10^8
	ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)
Blood	—	—	1.0	0‡
Lung	0.51	0.55	0.83	0
Spleen	0.01	0.20	0.05	0
Lymph nodes	0.02	0.06	0‡	—

* Each dose is equivalent to the stated number of cells with 200 mg tuberculin. Each figure represents an average of 3–5 recipients.

‡ 2 recipients.

TABLE II

*Responses Induced by Supernates of Spleen or Lymph Node Cells (from Four BCG-Sensitized Donor Rabbits) Incubated for 5 or 18 hr in Buffer or Saline with Tuberculin**

Donor	No. of recipients	Spleen‡				Lymph nodes‡			
		5 hr		18 hr		5 hr		18 hr	
		B	S	B	S	B	S	B	S
		ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)	ΔT (°C)
A	3	0.05	0.12	0.15	0.18	—	—	—	—
B	2	0.08	0.10	0.10	0.05	0.20	0.15	0	0.03
C§	3	0.05	—	0.18	—	—	—	—	—
D	3	0.08	—	0.19	—	0.05	—	0.10	—

* Each dose is equivalent to 2×10^8 cells with 200 mg tuberculin. B = KRP buffer with 10% normal rabbit serum + 150 mg% glucose. S = Physiologic saline.

‡ Figures in each column represent average values for indicated number of recipients.

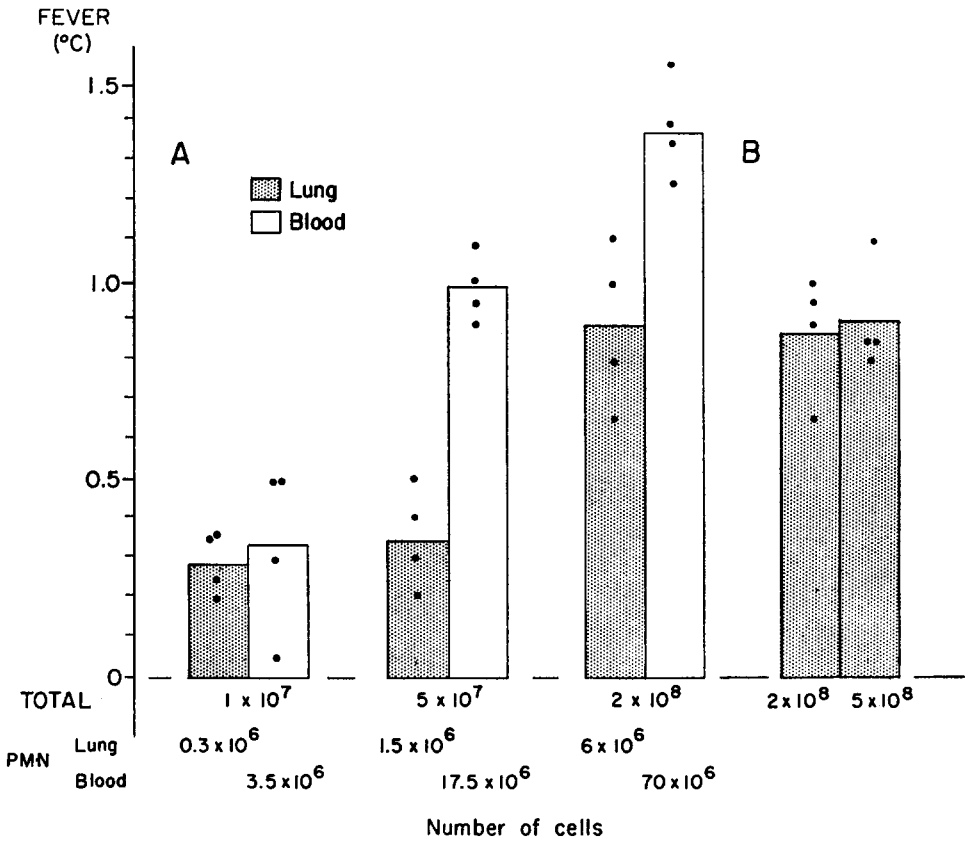
§ Cells from this donor were incubated in aliquots of 2×10^8 with 100 mg OT.

pyrogenic response and the number of cells from which it was derived (compare responses to 1×10^7 and 2×10^8 cells).

To rule out the possible loss of pyrogen with prolonged incubation, spleen and lymph node cells from four donor rabbits were incubated in either buffer or saline for 5 or 18 hr with tuberculin, and supernates given in dosages equivalent to 2×10^8 cells and 100 or 200 mg tuberculin (see Table II). Under these

conditions, there was again no evident release of endogenous pyrogen from mononuclear cells derived from these sources.

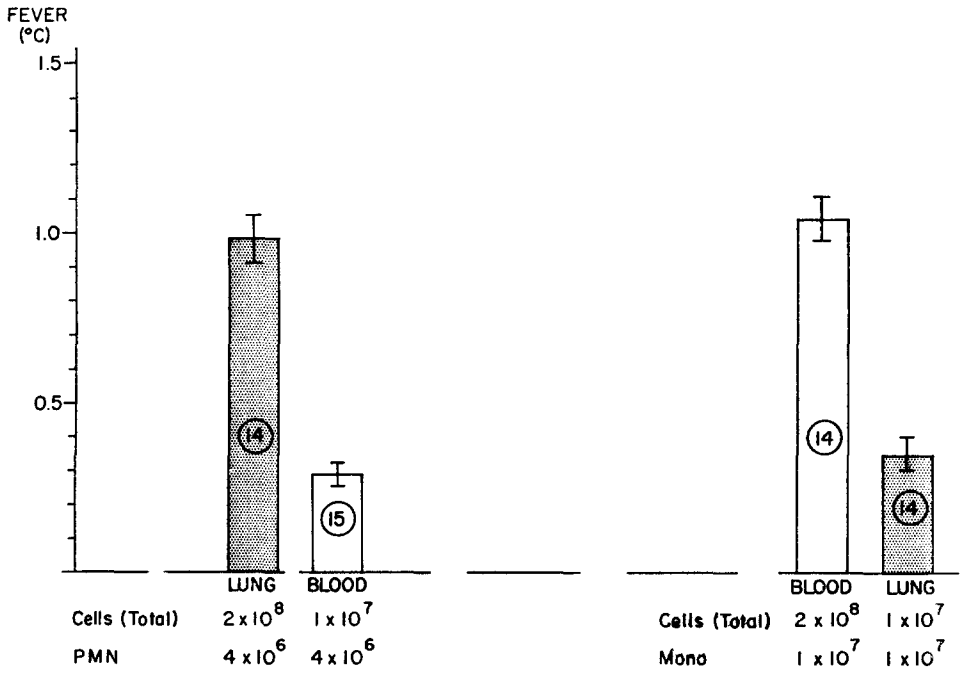
Comparison of Pyrogen Release by Lung and Blood Leukocytes Incubated with Tuberculin In Vitro.—Because leukocytes from the blood and lung were the



TEXT-FIG. 3. (*A*) Mean responses of two groups of four recipients, each given supernates from graded dosages of either lung or blood cells (from a single BCG-sensitized donor) incubated with tuberculin. Each dose is equivalent to the stated number of leukocytes with 200 mg OT. Values for both the total number of cells and number of granulocytes (PMN) contained in each dose are given at the bottom of the chart. (*B*) Mean responses induced by unpooled lung cells (derived from two additional donors) incubated with tuberculin in the two higher cell dosages shown and given to the same four recipients. Individual responses to each dosage of lung or blood cells are indicated by dots.

only tissues of those tested that appeared capable of releasing pyrogen when incubated with tuberculin in vitro, the comparative ability of these cells to release pyrogen when incubated in varying dosages with tuberculin was investigated next. Text-fig. 3 shows the results of incubating three doses of lung and

blood leukocytes from the same donor for 18 hr with 200 mg tuberculin in vitro. With cells from both tissues there was a stepwise increase in the mean responses as the numbers of cells were increased. Comparison of the bar graphs in this figure indicates that approximately 2 × 10^8 lung cells were required to



TEXT-FIG. 4. Left-hand bars (each pair): Mean fevers (± SE) induced by supernates of lung or blood cells (from the same sensitized donors) incubated for 18 hr with tuberculin. Each dose was equivalent to 2 × 10^8 cells with 200 mg OT. Right-hand bars (each pair): Responses induced by supernates of the opposite tissue incubated with tuberculin in a dosage of cells calculated to contain the same approximate number of PMN (blood) or monocytes (lung) as present in the corresponding lung or blood samples, respectively (shown in the left-hand bars of each pair). Figures for total number of cells as well as the number of PMN and monocytes in the tissue samples from which each injected supernate was derived are given at the bottom of the chart.

produce fevers equivalent to those induced by 5 × 10^7 blood leukocytes. The numbers of granulocytes in each preparation are shown under the bar graphs.

Correlation of Mean Fevers Induced by Incubation of Equivalent Numbers of PMN and Mononuclear Cells with Tuberculin.—Because the last experiment raised the possibility that some or most of the pyrogen released by incubation of tuberculin and the largest dose of lung cells (2 × 10^8) might be derived from the small residual numbers of PMN's in such preparations (generally 3–6 ×

10^6), tuberculin was incubated with aliquots of blood cells calculated to contain this number of PMN's. In addition, to determine the possible contribution of monocytes to the pyrogen evolved by the leukocytes in blood preparations (2×10^8) tuberculin was added to an equivalent number of monocytes from the lung (1×10^7). The results of a representative group of such experiments are shown in the bar graphs presented in Text-fig. 4. From the data presented in this figure it seems clear that the pyrogenic responses to supernates of either the lung or blood cells incubated with tuberculin cannot be ascribed simply to the small number of contaminating cells (granulocytes and monocytes, respectively) present in these preparations. These data suggest, therefore, that

TABLE III

*Mean Fevers Induced by Supernates of Lung and Blood Leukocytes Incubated 18 hr with 200 mg Tuberculin In Vitro**

Donor	Response				Cell dose per recipient			
	Lung		Blood		Lung		Blood	
	No. of recipients	ΔT (°C)	No. of recipients	ΔT (°C)	Total $\times 10^8$	PMN $\times 10^6$	Total $\times 10^8$	M $\times 10^6$
A	2	1.35	3	1.07	2.0	6.0	1.7	17.0
B	2	1.13	3	0.84	1.5	1.5	2.0	6.0
C	4	0.89	4	1.38	2.0	6.0	2.0	6.0
D	2	0.80	2	1.20	2.0	2.0	1.6	8.0
E	2	0.58	2	1.10	1.7	18.7	1.5	6.0
F	3	0.38	3	1.15	1.2	9.6	1.5	3.0

* Results from six BCG-sensitized donors are shown together with the equivalent number of cells (granulocytes and monocytes) in the lung and blood preparations, respectively.

PMN, polymorphonuclear leukocyte. M, monocyte.

tuberculin can mobilize pyrogen in vitro from both the granulocytes and mononuclear cells of the host.

Further evidence is presented in Table III that the release of pyrogen by tuberculin from mononuclear cells of the lung is not correlated with the number of PMN's in such preparations. In this table, six representative rabbits are arranged in descending order of the pyrogenicity of their lung cells when incubated with tuberculin. Also shown are the mean responses to equivalent numbers of blood cells from the same donors, incubated with tuberculin under the same conditions. From the differential cell counts, the absolute numbers of granulocytes and monocytes contained in each dose of lung or blood cells, respectively, have been calculated. It is apparent from the data assembled in this table that the pyrogenicity of the lung cells from various donors is not correlated with the number of PMN's present in these samples. The second

highest mean response to lung cells (1.13°C in rabbit B) was produced by supernates of cell suspensions containing only 1.5×10^6 PMN's, whereas the two lowest responses (0.58°C in rabbit E and 0.38°C in rabbit F) were from preparations containing, respectively, 18.7×10^6 and 9.6×10^6 PMN's per dose. The additional lack of correlation between the pyrogenic responses to lung and blood cells from the same donor (cf. donor B with donor F, where the relative pyrogenicity of lung and blood cells is reversed) suggests that the mobilization of pyrogen by tuberculin from these two tissues varies independently, as might be expected if two different cell sources are involved. These observations were repeatedly confirmed with the blood and lung cells from a number of other sensitized donor rabbits not included in this paper.

Additional Release of Pyrogen from Lung Cells Reincubated in Buffer Without Added Tuberculin.—The release of pyrogen from blood leukocytes incubated with tuberculin appears to be an active process that is both time- and temperature-dependent (6). When such cells have been already activated by an initial exposure to tuberculin and are then reincubated in a fresh medium without added tuberculin, an additional "harvest" of pyrogen can be obtained. To see if mononuclear leukocytes from the lung behaved in a similar fashion, the following experiment was devised.

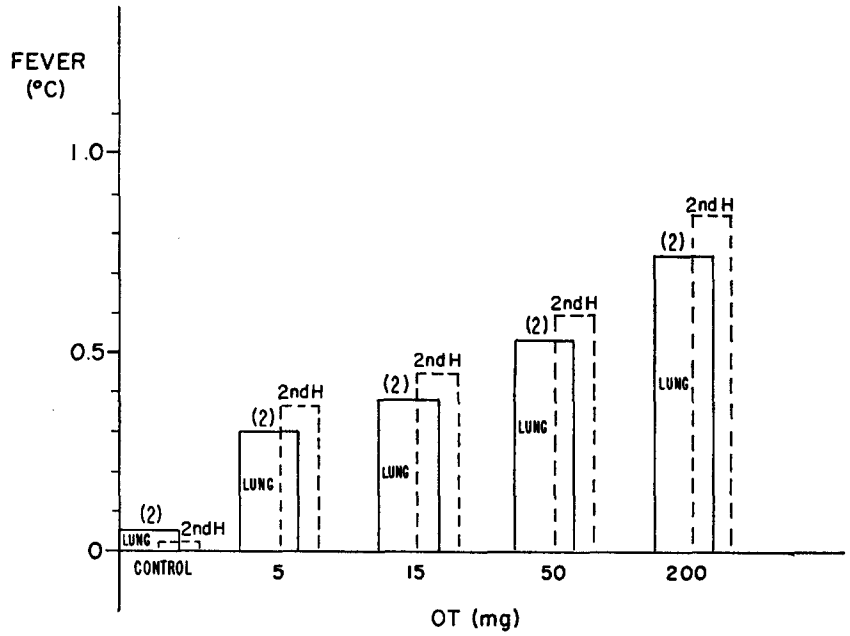
Lung cells were obtained from a single donor rabbit and divided into five aliquots each containing two doses of 2×10^8 cells. Four of the aliquots were incubated with tuberculin to give injected doses equivalent to 5, 15, 50, or 200 mg per 2×10^8 cells. The fifth aliquot was incubated without tuberculin and served as two control doses. After an initial incubation of 18 hr, the cells were centrifuged, the supernatant ("first harvest") removed and the cells resuspended in fresh buffer without tuberculin for an additional 2½ hr. At the end of this period, the cells were recentrifuged and the supernatant collected as a "second harvest."

The first and second harvest from each sample of cells were then injected into a single group of recipients on two successive days. The results are shown in Text-fig. 5. As before, the amount of pyrogen released by the first harvest was directly related to the dose of tuberculin added. In each instance, the second harvests (after brief reincubation—2½ hr) produced responses that were slightly higher (0.05–0.1°C) than were those of the first harvests, indicating continued activity of the cells and suggesting that some of the pyrogen evolved may have been inactivated during the prolonged first incubation period (18 hr).

Influence of the Medium and Duration of Incubation on Release of Pyrogen.—Previous studies have shown that the release of pyrogen by tuberculin from blood cells of BCG-sensitized donors was similar, whether the cells were in whole blood or washed and resuspended in saline. When the duration of incubation was extended from 5 to 18 hr, a significant increase in pyrogen release was evident (6). To determine the effect of the medium and duration of incu-

bation on release of pyrogen from the lung mononuclear cells, the following experiment was devised.

Cells from several donor rabbits were divided into aliquots of 2×10^8 and incubated in either the normal serum buffer medium with added glucose or in



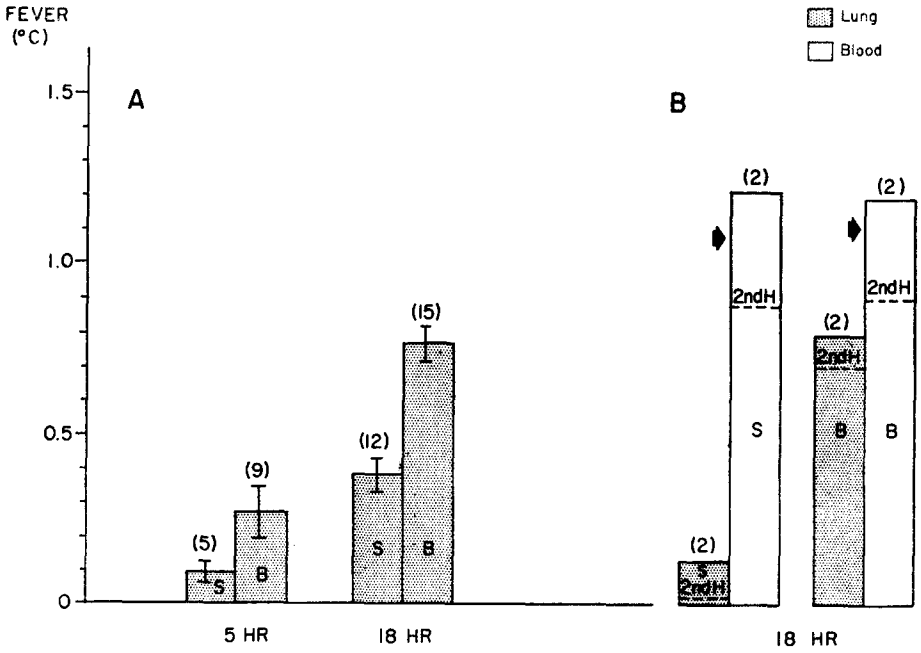
TEXT-FIG. 5. Mean febrile responses to supernates of lung cells (from a single BCG-sensitized donor) incubated 18 hr with one of four graded dosages of tuberculin as shown. After removal of the first supernate, cells were reincubated for 2½ hr in KRP buffer without additional tuberculin to give a "second harvest" (2nd H). Responses to these injections are shown in dotted bars to right of each solid bar. Injections were given to the same four recipients in the following order:

R 1 and 2:	First harvest	15, 200 mg dosages
	Second "	5, 50 mg "
R 3 and 4:	First harvest	5, 50 mg "
	Second "	15, 200 mg "

pyrogen-free saline. In some instances, pyrogen release in the two media was compared at 5 hr, in others at 18 hr, and in still others the release in one or the other medium was compared at 5 and at 18 hr.

The composite results of thus varying both the medium and time of incubation are shown in Text-fig. 6 (bar graphs under *A*). It can be seen that little or no pyrogen was released in either medium after 5 hr; by 18 hr, pyrogen release was still minimal from cells in saline (mean fever of 0.33°C) whereas the

same donor cells incubated in serum-glucose buffer evoked responses of 0.78°C. It is apparent, therefore, that mononuclear cells from the lung, unlike exudate granulocytes (10–13), require a supportive medium to release detectable amounts of pyrogen under the conditions of these experiments. This fact is



TEXT-FIG. 6. (*A*) Mean fevers (± SE) produced by supernates of lung cells (from BCG-sensitized donors) incubated with tuberculin in either saline (S) or KRP buffer with added serum and glucose (B) for the two intervals shown. (*B*) Mean fevers induced by supernates of both lung and blood cells (from a single BCG-sensitized donor) incubated 18 hr with tuberculin in either saline or the KRP buffer medium. Responses induced by reincubation of cells in the same medium for an additional 2½ hr (without tuberculin) are shown by a dashed line within each bar marked 2nd H (= second harvest). Arrows indicate average responses (group of nine recipients each) to supernates of blood cells from three BCG-sensitized donors incubated with tuberculin for 18 hr in either saline or the buffer medium. In all experiments, each dose is equivalent to about 2×10^8 cells and 200 mg OT. Control injections (supernates of cells incubated without OT) were nonpyrogenic (<0.3°C).

further illustrated in Fig. 6 *B*, which shows the results of incubating lung and blood cells from a single donor in both buffer and pyrogen-free saline for 18 hr. As previously shown, the lung cells released pyrogen only when suspended in the serum-glucose buffer medium but the blood cells released equal amounts of pyrogen in the two media. Furthermore, when the blood cells were reincubated without added tuberculin in the same medium used initially (to give "second

harvests") there was only a slight reduction in the amount of pyrogen released. Similarly, the second harvest from the lung cells resuspended in buffer was nearly as pyrogenic as was the first, whereas the second harvest of lung cells in saline, like the first, was nonpyrogenic. These results supply further indirect evidence that a large part, if not most, of the pyrogen evolved from blood leukocytes incubated with tuberculin in vitro comes from a different cell type—presumably the granulocyte.

Suppression of Pyrogen Release by Puromycin.—The liberation of pyrogen from granulocytes has been shown to be an active process that is temperature-dependent (11–12) and that can be suppressed when the cells are incubated with inhibitors of enzymes with SH-reactive groups (13). Since disrupted granulocytes contain much less pyrogen than is evolved by activated cells (12), and since granulocytic pyrogen is a protein, agents that block protein synthesis might be expected to suppress production of pyrogen from such cells.

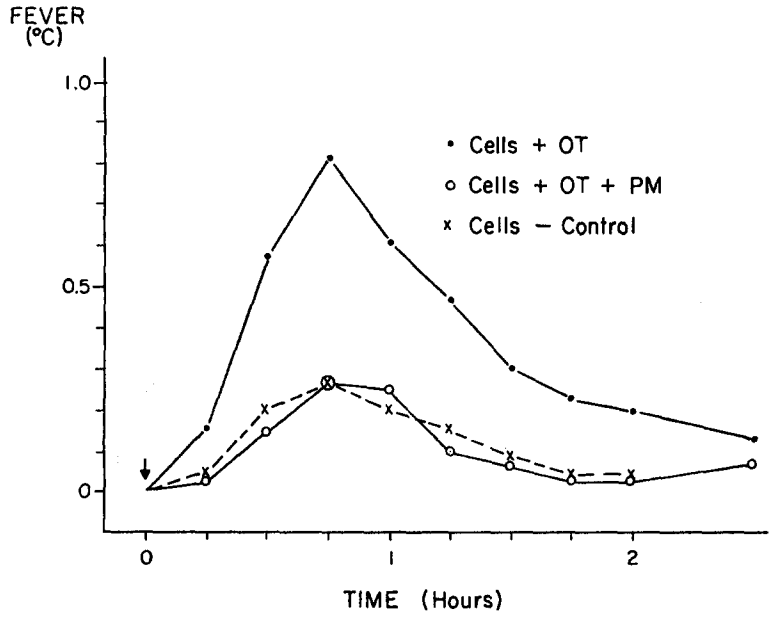
The following experiment was performed to determine if the pyrogen release by mononuclear cells was affected by the suppression of protein synthesis. A suspension of lung mononuclear cells from a single donor was divided into three aliquots, each containing four doses of 2×10^8 cells. Tuberculin (100 mg/ml) was then added in a dosage of 200 mg per 2×10^8 cells to two aliquots; the third aliquot served as a control. Immediately after addition of tuberculin to one of the aliquots of cells, puromycin was added to a final concentration of 10^{-4} M (14).

All three aliquots were then incubated at 37°C for 18 hr, after which the cells were centrifuged at 3000 rpm for 30 min and the supernates injected into normal recipient rabbits.

The results are shown in Text-fig. 7. Very little pyrogen was liberated by the aliquot of cells incubated with puromycin, suggesting that pyrogen release from these mononuclear cells, like that from granulocytes, is enzymatically controlled and requires protein synthesis. To ensure that puromycin had not inactivated any pyrogen that might have been liberated by cells exposed to tuberculin, puromycin was added in a tenfold higher concentration (10^{-3} M) to samples of pyrogen derived from both lung and blood leukocytes previously incubated 18 hr with tuberculin. The puromycin-pyrogen mixtures were then reincubated for an additional 18 hr. There was no evident reduction in activity of these samples as compared with control samples of pyrogen from the same two sources.

Effect of Temperature on Release of Pyrogen In Vitro.—In a further experiment, the effect of temperature on release of pyrogen was investigated. Lung cells from a single donor were divided into two aliquots, each containing four doses of 2×10^8 cells. Tuberculin was added (200 mg per dose of cells) to both samples, after which one was incubated at 37°C for 18 hr while the other was kept for the same period at 4°C. After centrifugation, the supernates of both

samples were injected into the same four recipients and their fevers recorded. From the febrile responses recorded in Table IV, it is apparent that no detectable pyrogen was released from the cells which remained at 4°C. Similar results were obtained with blood leukocytes derived from the same donor and treated the same way (Table IV).



TEXT-FIG. 7. Mean febrile responses induced by supernates of lung cells (from a single BCG-sensitized donor) incubated 18 hr in KRP buffer with tuberculin, with or without puromycin (10^{-4} M). Control injections were derived from the same number of cells incubated alone. All injections were given to the same four recipients.

Role of Sensitized Sera in Activation of Lung Monocytes.—To investigate the role of serum factors in the activation of lung mononuclear cells by tuberculin the following experiment was designed.

Lung cells from each of two sensitized donor rabbits were divided into two aliquots, one of which was incubated with tuberculin in the usual buffer–normal rabbit serum medium; the other was placed in the same buffer but with substitution of serum of BCG-sensitized rabbits for normal rabbit serum. Both aliquots were then incubated overnight as in previous experiments. Supernates of cells in these two media were injected into the same recipients to obviate differences in reactivity of individual rabbits. There did not appear to be a significant difference in the febrile response induced by supernates of cells incubated in sensitized as compared with normal sera. The mean fever induced

in five rabbits was as follows:

cells with sensitized sera: 0.54°C
cells with normal sera: 0.48°C

Under these conditions, therefore, factors in the sera of specifically sensitized donor animals did not appear to play a role in the activation of rabbit lung macrophages by OT.

Role of Complement in Activation of Sensitized Leukocytes by Tuberculin In Vitro.—To assess the possible role of complement in the release of EP from sensitized leukocytes incubated with tuberculin in vitro, the following experiment was devised.

Blood and lung cells were obtained from a single BCG-infected donor rabbit. The blood was then divided into four aliquots, centrifuged at 2000 rpm for

TABLE IV

*Fevers Induced by Supernates of Lung and Blood Cells (from a Single BCG-Sensitized Donor) Exposed to Tuberculin for 18 hr at Either 37° or 4°C**

Source of leukocytes	Number of recipients	37°C	4°C
		ΔT (°C)	ΔT (°C)
Lung + OT	4	0.61	0.08
Controls (cells only)	2	0.05	0
Blood + OT	3	0.72	0.15
Control (cells only)	1	0.10	—

* Each dose was equivalent to 2×10^8 cells and (except controls) 200 mg tuberculin.

30 min at 4°C and the plasma removed from each aliquot. The remaining cell buttons were resuspended in the following media:

(*a*) Normal serum (freshly obtained)
(*b*) " " + OT
(*c*) " " + OT + EDTA[5] 10 mg
(*d*) Heat-inactivated serum (56°C for 60 min) + OT

The initial saline suspension of lung monocytes was similarly divided into four aliquots and processed in the same manner.

All aliquots were shaken for 3 hr in a Dubanoff incubator at 37°C, incubated overnight, and the supernates of these cell suspensions injected. The results, shown in Table V, indicate that pyrogen release from both lung and blood leukocytes was unaffected by techniques known to inactivate complement.

Under these conditions, then, complement does not appear to play a role in the activation of these cells by tuberculin in vitro.

[5] Sodium versenate® (trisodium salt of ethylenediamine tetraacetic acid) 5 ml ampoules containing 1.0 g/5 ml in water for injection. Riker Labs., Inc., Northridge, Calif.

Comparison of Pyrogen Release by Lung Cells from Sensitized and Normal Rabbits Incubated with Tuberculin In Vitro.—To determine whether lung monocytes from normal rabbits, like blood leukocytes (6), were activated when incubated for prolonged periods with tuberculin, the following experiment was devised.

Lung cells from donors either normal or sensitized by BCG were processed in the usual manner. They were incubated for 4 hr, 9 to 10 hr, or 18 hr with tuberculin, and the supernates injected. The fevers induced by these supernates are shown in Table VI.

TABLE V

*Mean Fevers Induced by Supernates of Either Lung or Blood Leukocytes Incubated for 18 hr with Tuberculin In Vitro**

	Cell dose	No. of recipients‖	ΔT (°C)
Blood cells + NS‡	1.2×10^8	4	0.08
" " " + OT	"	4	0.47
" " " " + EDTA	"	4	0.68
" " + HIS§ + OT	"	4	0.68
Lung cells + NS	1.4×10^8	2	0.25
" " " + OT	"	2	1.00
" " " " + EDTA	"	2	1.22
" " + HIS + OT	"	2	1.17

* Each dose is equivalent to stated number of cells + 200 mg OT.

‡ Normal serum.

§ Heat-inactivated serum.

‖ The same recipients were used in all experiments with each tissue.

From these data, it may be concluded that cells from normal donors release pyrogen when incubated in vitro with tuberculin, but to a lesser degree than do cells of sensitized donors incubated for comparable intervals.

As shown above with sensitized lung cells, the amount of pyrogen evolved is directly related to the duration of incubation, and takes place more slowly than from blood leukocytes obtained from the same types of donors (6).

Release of Pyrogen Following Phagocytosis.—To determine whether a different stimulus, such as phagocytosis, could activate rabbit mononuclear cells to release pyrogen, preparations of lung, spleen, and lymph node cells from BCG-sensitized rabbits were incubated for 18 hr in a serum-buffer medium with heat-killed staphylococci. For comparison, blood cells from one of the same rabbits were also incubated with staphylococci. After incubation, the flask contents were centrifuged at 3000 rpm for 30 min, and supernatant derived from 1×10^8 cells injected into each rabbit.

The results of two such experiments are shown in Text-fig. 8. Pyrogen was released after addition of bacteria to all leukocyte preparations. Control cells, incubated without bacteria, did not release significant amounts of pyrogen (febrile responses to these were 0.0° to 0.3°C). These results are in contrast to those of experiments with tuberculin (see above) in which only lung and blood cells released pyrogen. In subsequent experiments in which bacteria were added to lung and spleen cells, pyrogen release was regularly observed in a total of nine and six experiments, respectively. Lymph node cells, however, were frequently not active; in only three of seven experiments was there clear-

TABLE VI

*Mean Febrile Responses to Supernates of Lung Cells (from BCG-Sensitized and Normal Donors) Incubated for Various Intervals with Tuberculin In Vitro**

	Donor	No. of recipients	4 hr	9–10 hr	18 hr
			ΔT (°C)	ΔT (°C)	ΔT (°C)
Normal cells + OT	A	3	0.08	0.30	0.70
	B	3	0.08	0.28	0.40
	C–G	14	—	—	0.60
	Average		0.08	0.29	0.59
Controls (cells only)	Average	15	—	—	0.16
Sensitized cells + OT	H	4	0.38	0.90	1.00
	I	3	0.33	0.72	1.05
	J–N	10	—	—	0.91
	Average		0.36	0.84	0.96
Controls (cells only)	Average	9	—	—	0.12

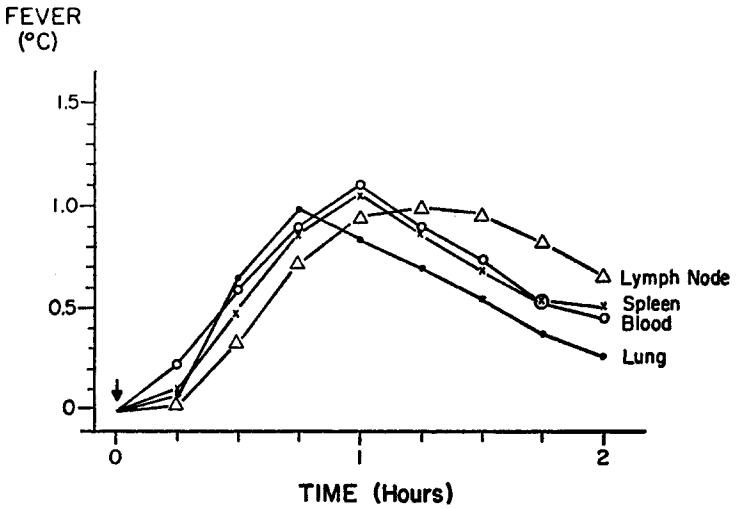
* Each dose is equivalent to 2×10^8 cells + 200 mg tuberculin.

cut evidence of pyrogen release following addition of bacteria. Differences in cell-type, function, or preparation may have been responsible for these differences.

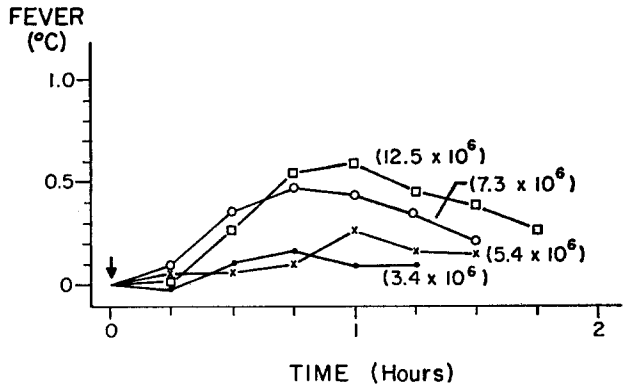
Cover slip smears were made of all leukocyte suspensions incubated with staphylococci. Phagocytosis was clearly seen in most preparations of lung cells, as is illustrated in Fig. 2. In some experiments, >90% of the cells appeared to have ingested bacteria, usually 20 to 30 per cell. However, cover slip preparations of spleen and lymph node cells were frequently unsatisfactory, and on most slides only occasional cells were clearly phagocytic.

It is unlikely that the few granulocytes present in preparations of lung, spleen, or lymph node cells were responsible for the pyrogen production. Differential counts done on the cell suspensions reported in Text-fig. 8 revealed an

average of 2% granulocytes in the lung cell preparations, 0.5% in lymph node, and 4% in spleen. Thus the maximum number of granulocytes contributing to the supernatant of any injection was 4×10^6. In order to test the possibility that these few granulocytes could release large amounts of pyrogen, the following experiments were done.

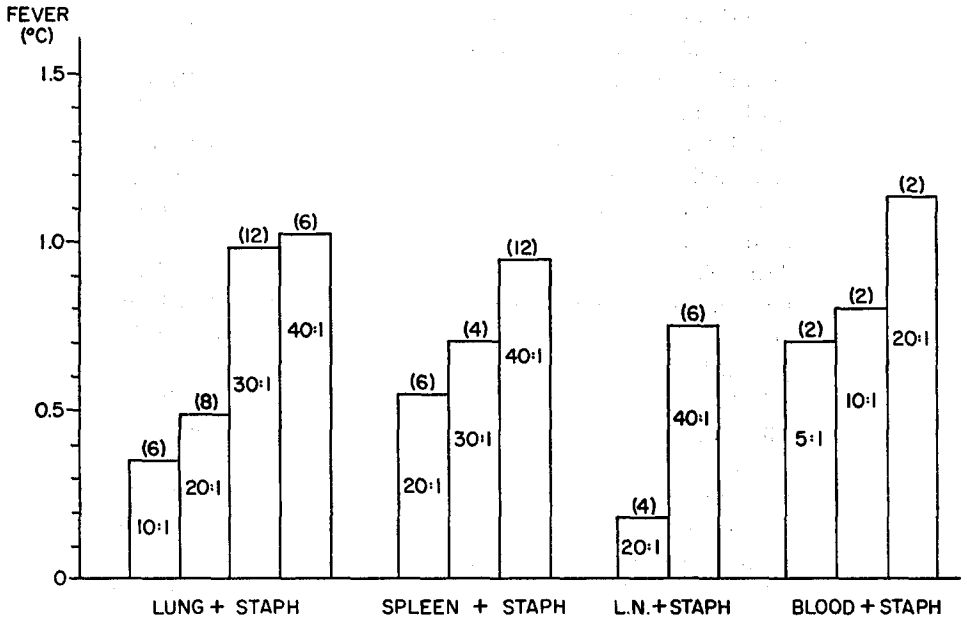


TEXT-FIG. 8. Average febrile responses in two to four rabbits to supernates from 1×10^8 blood, lung, lymph node, or spleen cells (from BCG-sensitized donors) incubated with heat-killed staphylococci for 18 hr. Staphylococci were added in dosages of 4×10^9 to each 1×10^8 lung, lymph node, and spleen cells, and 2×10^9 to the blood cells.



TEXT-FIG. 9. Average febrile responses in two to four rabbits to supernatants from blood leukocytes incubated with 2.5×10^9 heat-killed staphylococci for 18 hr. Numbers of granulocytes contributing to the supernatant for each injection are given in parentheses. These results are the averages of two separate experiments. Four rabbits each (except for the 5.4×10^6 dosage which was given to two rabbits in one experiment).

Normal rabbit blood leukocytes were prepared by dextran sedimentation (see "Materials *and* Methods"). Varying numbers of cells were then incubated under conditions identical to those used for the mononuclear cell preparations. The same large numbers of staphylococci (>4 × 10^9 per flask) were added to ensure an equivalent phagocytic stimulus. After incubation, the supernatant from each flask was divided and injected into two rabbits. In each experiment, the same two rabbits received all supernatants so that a dose–response curve could be constructed. The average responses from two such experiments are



TEXT-FIG. 10. Average maximum fevers of rabbits given supernatants from cells from lung, lymph node, spleen, and blood (obtained from BCG-sensitized donors) incubated with heat-killed staphylococci. Multiplicities of staphylococci to cells are shown for each group. Numbers of recipients are shown in parentheses. Each dosage was equivalent to 1 × 10^8 cells.

shown in Text-fig. 9. When the numbers of granulocytes were 5 × 10^6 or less, very little pyrogen was detected. These experiments indicate, then, that the few granulocytes present in the mononuclear cell preparations (1 to 4 × 10^6) are unlikely to be responsible for most of the pyrogen released by these cells (see Text-fig. 8).

Large multiplicities of bacteria to cells appeared to be required for good release of pyrogen from mononuclear cells. Experiments to determine the amount of pyrogen released with increasing numbers of staphylococci are summarized in Text-fig. 10. The ratios of bacteria to leukocytes are shown within each bar; the number of recipients from which the average fever was obtained is

shown above. Whereas 30 or 40:1 ratios seem to be required by lung, spleen, and lymph node cells, white blood cells (predominantly granulocytes) release comparable amounts of pyrogen at ratios below 20:1. Similar results with human blood cells have been reported previously (15).

DISCUSSION

We believe these experiments and others involving human leukocytes recently reported from this laboratory (15), supply the first clear evidence that a pyrogen can be mobilized in vitro from mononuclear cells.[6] Ever since the report of Bennett and Beeson in 1953 (16) that a fever-inducing agent could be extracted from sterile suspensions of granulocytes, most work on the in vitro release and characterization of endogenous pyrogen (EP) has been carried out with granulocytes from acute exudates (2, 10–13, 17–20). Previous studies with both human (8, 21) and rabbit (11, 19, 22) blood cells incubated with various stimuli, including tuberculin (6), have shown that pyrogen may also be liberated by blood leukocytes in vitro, but the specific cell type involved in these reactions was not ascertained.

Mononuclear cells obtained from the lungs of rabbits were activated in vitro by tuberculin to release pyrogen. Prolonged incubation (9 to 18 hr) in a fully supportive medium [Krebs-Ringer Phosphate (KRP) buffer with added serum and glucose] appeared to be necessary to produce clear-cut results (see Table VI and Text-fig. 6). Fevers of 0.5°C or higher were regularly obtained only with cell dosages of 2×10^8 and 200 mg tuberculin, unlike the findings with blood cells in which comparable fevers were produced under the same conditions by only one-fourth this dosage of leukocytes (5×10^7 total cells containing approximately 20–25 $\times$ 10^6 granulocytes). Lung cells from four of six donor rabbits shown in Table III were strongly pyrogenic and it does not seem likely that the small number of granulocytes in these preparations was responsible for the pyrogen, since samples of blood cells, adjusted to contain approximately the same number of granulocytes and similarly incubated with tuberculin, produced only equivocal fevers (mean 0.29°C) (see Text-fig. 4). Similar, marginally pyrogenic responses (mean 0.37°C) have been obtained with saline supernates derived from comparable numbers of fully activated exudate granulocytes (9).

Additional indirect evidence to support the hypothesis that the pyrogen released by lung and blood leukocytes incubated with tuberculin is derived largely or wholly from different cell sources may be summarized as follows:

(*a*) Lung cells incubated with tuberculin in saline rather than in the buffer-serum-glucose medium even for prolonged periods, failed to release clearly detectable amounts of pyrogen, whereas blood cells from the same donors re-

[6] In the following paper, W. B. Wood, Jr. and his colleagues have presented similar findings with rabbit peritoneal macrophages (37).

leased as much pyrogen when suspended in saline as in buffer (see Text-fig. 6). Previous studies have shown, moreover, that blood cells of BCG-sensitized donors will release significant amounts of pyrogen after only 5 hr incubation with tuberculin in saline (6).

(*b*) There was no correlation between the amount of pyrogen released by equal numbers of lung and blood leukocytes from the same donor. In a few instances, supernates of lung cells evoked higher fevers than did those of blood cells although, in general, the relative pyrogenicity of the two cell preparations was reversed (see Table III).

(*c*) Perhaps most conclusively, there was no correlation between the number of PMN leukocytes in individual lung cell preparations and their pyrogenic activity (cf. rabbits B and E in Table III, where mean fevers of 1.13°C and 0.58°C were produced by mononuclear cell preparations containing 1.5 and 18.7 $\times$ 10^6 PMN's respectively). This finding provides a strong argument against the possibility that the small numbers of granulocytes present in these preparations are in an abnormally active state and, hence, may produce the observed pyrogenic responses.

Although we believe that this evidence strongly implicates the mononuclear cell rather than the granulocyte as a source of pyrogen from these lung cells, certain requirements for pyrogen release appear to be similar in both types of cells.

(*a*) There was no detectable release of pyrogen from lung mononuclear cells kept at 4°C after addition of tuberculin. Similar results have been previously obtained and were again confirmed here with blood leukocytes under the same conditions (6).

(*b*) Pyrogen release from cells incubated with tuberculin was directly proportional to the duration of incubation. On the basis of dose–response curves established for granulocyte pyrogen (9), two to four times as much pyrogen was present in preparations incubated for 18 hr as compared with 5 hr (see Text-fig. 6).

(*c*) Puromycin almost completely inhibited release of pyrogen from tuberculin-activated mononuclear cells, suggesting that this reaction, like the one resulting in the release of granulocyte pyrogen, is an active, enzymatically controlled process.

In all these respects, the release of mononuclear cell pyrogen differs from that described by Johanovský (23–27) and later, in part by Allen (28). Johanovský has reported in a series of publications that the addition of tuberculin or diphtheria toxoid to mononuclear cells from either the spleen or lymph nodes of specifically sensitized animals (rabbits and guinea pigs) resulted in the appearance of a pyrogen in vitro which he has called "hypersensitivity pyrogen." Although Johanovský has more recently been unable to repeat these findings [ref. (29) and personal communication], Allen has reported

similar but more equivocal results using the spleen and lymph node cells of guinea pigs (28). The findings described here differ from those of both Johanovský and Allen in the following respects: First, unlike these investigators, we have been unable previously (6) and again here to mobilize pyrogen in vitro from either spleen or lymph node cells of BCG-sensitized rabbits incubated with varying amounts of tuberculin for 5 or 18 hr in saline or Hanks' solution (as used by Johanovský) or, as in these experiments, in serum-KRP buffer with added glucose. In view of the positive results obtained when phagocytosis of heat-killed staphylococci was used as the activator of these cells, it seems unlikely that the conditions of incubation are not suitable for pyrogen production, or that any significant amount of pyrogen liberated by tuberculin during this period could have been inactivated by other factors. Second, pyrogen release from lung mononuclear cells in our experiments is clearly an active process, dependent on temperatures above 4°C and presumably involving protein synthesis, whereas in Johanovský's studies "hypersensitivity pyrogen" was produced in vitro by either live or disrupted cells to which antigen had been added at either 4° or 37°C (25, 26). Finally, in our studies, lung cells of both normal and sensitized rabbits released pyrogen in vitro when incubated with tuberculin although, as previously reported with blood cells (6), lung cells from BCG-sensitized donors released significantly more pyrogen at each of the three intervals tested. Johanovský and Allen, on the other hand, have reported that tuberculin reacted only with cells of specifically sensitized animals. In confirming Johanovský's work, Allen has not explored these aspects of cell activation, but the borderline fevers (mean of 0.4°C) produced in guinea pigs by supernates of spleen and lymph node cells incubated with tuberculin achieve significance in Allen's work only by virtue of completely negative control reactions (<0.03°C). By contrast, our control lung cell preparations (incubated 18 hr without tuberculin) frequently induced fevers of 0.3°C and on a few occasions appeared to be spontaneously "activated" producing responses of 0.5°C or higher,[7] as has also occurred with blood leukocytes and spleen cells incubated alone for similar periods (6).

Since tuberculin released significantly more pyrogen from the lung mononuclear cells of BCG-sensitized rabbits as compared with similar cells from unsensitized controls, these studies suggest that this cell may have a role in producing the fever that follows intravenous inoculation of tuberculin in

[7] These experiments have not been included in the Results. (see "Materials and Methods"). In general, markedly positive control reactions (0.5 to 1.0°C) were obtained with cells from donor rabbits whose lungs were heavily infected with Gram-negative bacilli (*Bordetella*-sp.). Since both typhoid vaccine and a purified endotoxin derived from *Proteus vulgaris* (4) mobilize pyrogen from lung macrophages in vitro (unpublished results) it seems likely that control cells in these instances have been activated either by phagocytosis or by endotoxin released by these organisms during incubation in vitro.

specifically sensitized rabbits. Previous studies have demonstrated that blood leukocytes (presumably the granulocytes) of sensitized donors mobilize a significant amount of pyrogen when incubated in vitro with tuberculin for short periods (4 to 5 hr) whereas the cells of normal donors are inactive under the same conditions. With prolonged incubation, the blood cells of normal donors are also activated to produce pyrogen, but to a lesser degree than are those of sensitized animals. Since mononuclear cell pyrogen appears to be produced more slowly, a significant difference in the amount of pyrogen liberated by cells of sensitized and normal donors exposed to tuberculin in vitro was only detectable after 9 to 10 hr incubation. Tuberculin, like other antigens, damages hypersensitive monocytes both in vivo and in vitro (reviewed in ref. 30) and, when injected intravenously, produces a prolonged mononuclear leukopenia as well as a transient granulocytopenia in specifically sensitized recipients (5). It seems possible, therefore, that blood monocytes [from which two-thirds of the lung mononuclear cells appear to be derived (31)] contribute some of the circulating endogenous pyrogen that mediates tuberculin-induced fever (5, 32). Allen has provided indirect evidence to support this hypothesis with the observation that BCG-sensitized rabbits made severely granulocytopenic by nitrogen mustard (33, 34) continue to react to intravenous inoculations of tuberculin with high fevers, although similarly leukopenic animals are unresponsive to Gram-negative bacterial endotoxin (35), an agent known to mobilize endogenous pyrogen from granulocytes (2).

An interesting finding in these experiments was the mobilization of endogenous pyrogen from all three sources of mononuclear cells—lung, spleen, and lymph nodes—when phagocytosis of heat-killed staphylococci was used as the stimulus. Again, the results cannot be correlated with the small numbers of granulocytes in these tissues and it seems safe to conclude that phagocytosis, unlike tuberculin in these studies, is an effective stimulus of mononuclear cells from these two additional tissues as well as from lung macrophages. It is noteworthy that phagocytic ratios of 20:1 or greater (bacteria to leukocytes) were required to release significant amounts of EP with leukocytes derived from the lung, spleen, and lymph nodes, whereas with blood leukocytes, similar results were obtained with ratios as low as 5:1. Quantification of phagocytosis suggests that ingestion of more bacteria per cell is required to stimulate pyrogen release by these large mononuclear cells as compared with leukocytes (presumably granulocytes) present in blood cell preparations.

The differential stimulation by tuberculosis and by phagocytosis of these mononuclear cell populations from the lung, spleen, and mesenteric lymph nodes raises the intriguing possibility that some "activators" react specifically with certain tissues to release pyrogen. Alternatively, the difference in the response of spleen and lymph node cells to tuberculin and to phagocytosis may be due to a relatively greater effectiveness of phagocytosis in releasing pyrogen

from a single mononuclear cell type that is present in much smaller numbers in these two tissues than in the respiratory passages of the lung. Further work will be necessary to establish if there are other activators with specific ability to release pyrogen from certain tissues. Preliminary (unpublished) results with Newcastle disease virus suggest that the intact virus activates all these tissues in vitro (perhaps by a mechanism similar to phagocytosis) whereas a polysaccharide fraction derived from the virus appears to liberate pyrogen only from blood cells in vitro. On the other hand, we have been unable to obtain in vitro release of pyrogen by incubating a purified antigen, human serum albumin, with blood cells of specifically sensitized rabbits (unpublished results). Since these same animals develop marked leukopenias followed by high fevers when injected with the antigen intravenously, the host's cells may be activated only under conditions present in vivo in this form of experimental fever, as appears to be the case in certain clinical fevers associated with immune hemolysis (36).

Since pyrogen may be extracted from the cells of many tissues in addition to leukocytes (4), a search should now be made to determine whether there are specific activators (microbial or endogenous) of various tissue cells—a possibility that may account for fevers present in diseases where blood leukocytes do not appear to be implicated.

SUMMARY

The capacity of rabbit mononuclear cells to release an endogenous pyrogen (EP) in vitro has been studied.

After incubation with tuberculin, preparations of predominantly monocytic cells, derived from the respiratory passages of the lungs of rabbits sensitized with BCG, were activated to release EP. Pyrogen production occurred more slowly with lung monocytes than with blood leukocytes of similarly sensitized rabbits and 9 to 10 hr incubation in a fully supportive medium was required to produce clear-cut results. As previously reported with blood leukocytes, mononuclear cells from the lungs of normal animals were also activated by tuberculin but to a lesser degree than were those from specifically sensitized rabbits.

Under a variety of conditions, mononuclear cells from either spleen or lymph nodes of the same sensitized rabbits failed to release detectable amounts of pyrogen when incubated with tuberculin in vitro but were activated in a majority of instances when phagocytosis of heat-killed staphylococci was used as the stimulus.

Release of pyrogen from lung monocytes appears to be an active process that is both temperature-dependent and requires protein synthesis. Neither serum antibody nor complement appears to play a role in this process.

Evidence is presented that the granulocyte is the main source of pyrogen

evolved by blood leukocytes incubated in vitro with OT or heat-killed staphylococci, whereas the lung macrophage and/or monocyte is responsible for most of the pyrogen released from the lung cell preparations.

From these studies, it is concluded that mononuclear cells can be activated in vitro by several microbial stimuli and must be considered an additional cellular source of EP. The clinical implications of these findings for the pathogenesis of fever in granulomatous diseases where the monocyte is the predominant cell are discussed.

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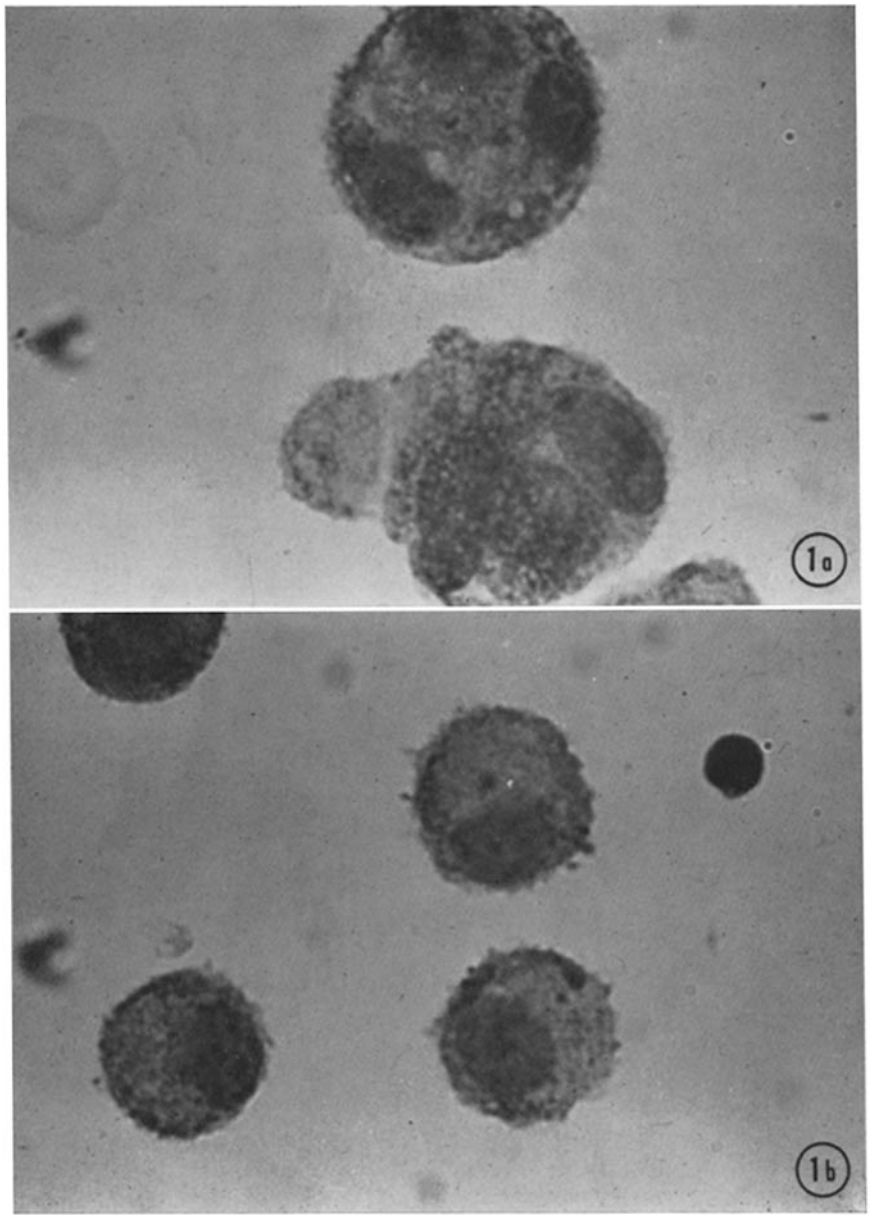
EXPLANATION OF PLATES

PLATE 34

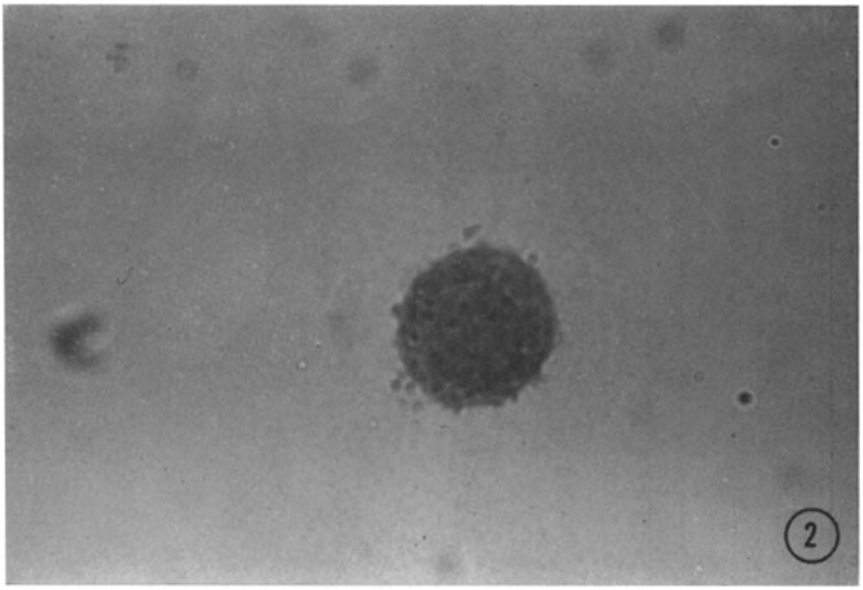
FIG. 1. Lung mononuclear cells: (*a*) macrophage; (*b*) smaller monocytes and lymphocyte (both × 3300).



(Atkins et al.: Endogenous pyrogen from mononuclear cells)

PLATE 35

FIG. 2. Phagocytic cell from lung, incubated with staphylococci. × 3300.



(Atkins et al: Endogenous pyrogen from mononuclear cells)