STUDIES ON THE PATHOGENESIS OF FEVER

XIII. The Effect of Phagocytosis on the Release of Endogenous Pyrogen by Polymorphonuclear Leucocytes*

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An endogenous pyrogen, apparently derived from polymorphonuclear leucocytes, has been shown to play a central role in the genesis of fevers caused by acute bacterial infections in rabbits (1, 2). A similar, if not identical, pyrogen has been found in the blood of rabbits during fevers induced by large intravenous injections of both living and heat-killed bacteria (3). Since one of the principal functions of polymorphonuclear leucocytes in bacterial infections is to phagocyte invading bacteria, both in the tissues (4) and in the blood stream (5), the effect of phagocytosis upon the release of leucocytic pyrogen has been examined.

Methods

General.—Details of the methods used to assure pyrogen-free glassware, to prepare suitable suspensions of rabbit granulocytes from acute peritoneal exudates and from blood, and to assay the pyrogen released from the cells have already been described (6).

Suspending Media.—The cells were suspended at a concentration of 3.5×10^7 per ml in: (*a*) modified Hanks' solution (MH),[1] (*b*) heparinized[2] plasma, or (*c*) plasma anticoagulated with EDTA.[3] For the latter two media, blood was obtained by intracardiac puncture from normal rabbits or from rabbits with sterile peritonitis (6). The blood was drawn in syringes previously rinsed with either heparin or EDTA and was collected in chilled, unsiliconized glass

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[1] Sodium, 139.5 meq/liter; potassium, 17 meq/liter; chloride, 139 meq/liter; phosphate, 9 meq/liter, pH 7.5.

[2] Heparin sodium injection, Lederle Laboratories, Pearl River, New York (100 units/ml), final concentration in whole blood, 10 units/ml.

[3] Ethylenediaminetetraacetic acid used as 0.1 M solution adjusted to pH 7.5 with sodium hydroxide and added to whole blood to make a final concentration of approximately 0.005 M in the plasma.

tubes. Following centrifugation at 2000 *g* for 30 minutes at 4°C, the supernatant plasma was aspirated, kept cold, and used within 1 to 2 hours.

Phagocytic Particles.—Three types of particles were used for phagocytosis: living rough pneumococci (unencapsulated), heat-killed rough pneumococci, and polystyrene spherules.

Cultures of rough pneumococcus, strain PNR36NC, were incubated at 37°C in beef infusion broth containing 0.2 per cent glucose and 10 per cent rabbit serum. At the end of 16 hours the cultures were chilled and the organisms were removed by centrifugation and washed 3 times with cold MH before being brought to the desired concentration as determined by direct counts in a Petroff-Hausser chamber.

Analogous suspensions of heat-killed organisms were prepared by heating the final MH-pneumococcus mixture to 100°C for 5 minutes. About 80 per cent of the pneumococcal cells, after heating, were Gram-positive.

The polystyrene particles,[4] which were of the same approximate diameter as the pneumococci, were cleansed of their emulsifying fluid by repeated washings in either isotonic saline or MH. Final suspensions of the desired count were made in MH.

Phagocytic System.—In each experiment the particles to be phagocyted were added in appropriate numbers (see column 4, Table II) to either 3.5 or 1.0 $\times$ 10^8 leucocytes in a 10 ml volume of suspending medium. The 90 ml siliconized centrifuge tube containing the mixture was first warmed in a water bath for 5 minutes to bring its contents to 37°C. Immediately thereafter it was rotated for 30 minutes at 9 RPM in the incubator to promote phagocytosis (7). Finally it was removed from the rotator for additional stationary incubation, after which release of pyrogen from the cells was measured. The total incubation time listed in the tables includes the 30 minutes of incubation on the rotator but not the 5 minute warming period.

Pyrogen Assay.—At the end of the period of incubation the cells were removed from the medium by centrifugation in the cold (4°C) at 200 *g* for 10 minutes, and the bacteria were removed by a second period of cold centrifugation at 2500 *g* for 1 hour. Aliquots of the supernatant fluid were then assayed for leucocytic pyrogen by the usual method based on the fever induced in trained rabbits by intravenous injection (8). The total amount of pyrogen released by the 3.5 (or 1.0) $\times$ 10^8 leucocytes in each experiment was calculated by multiplying the fever index (FI_{120}) obtained in the sensitive range of the dose-response curve ($FI_{120} < 15$) (8) by the reciprocal of that fraction of the total 10 ml volume which was used in the test. Thus the calculated total fever index values not infrequently exceeded the upper limits (*circa* 15) of the sensitive dose-response range (see Tables II and IV).

Measurement of Per Cent Phagocytosis.—The degree of phagocytosis which occurred during the 30 minutes of rotation was estimated by making smears of aliquots removed from the tubes at the end of the 30 minute period. The smears in the pneumococcus experiments were stained with methylene blue,[5] whereas no staining in the polystyrene experiments was necessary because of the refractile quality of the particles. The per cent of cells containing one or more particles was counted in each smear.

Because of the fact that the cells which had phagocyted particles in the heparinized plasma tended to stick to the sides of the glass incubation tubes, a modification of the "flying coverslip" technique (9) had to be used to estimate the degree of phatocytosis. In all experiments with heparinized plasma a pyrogen-free coverslip was introduced directly into the cell-particle mixture at the start of incubation and was removed, along with its adherent cells, at the end of the first 30 minutes of incubation. The phagocytic counts were performed on the cells adhering to the coverslip.

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[4] Supplied by the Physical Research Division, Dow Chemical Co., Midland, Michigan. Diameter, 0.814 μ $\pm$ 0.01 μ SD.

[5] Loeffler's alkaline methylene blue.

RESULTS

Inhibition of Pyrogen Release in Plasma and MH.—The amounts of pyrogen released by leucocytes incubated for 4 hours[6] in the 3 suspending media used in the phagocytic experiments are recorded in Table I. It will be recalled that incubation of exudate leucocytes in isotonic sodium chloride resulted in the release of large amounts of pyrogen, whereas similar treatment of blood leucocytes caused little pyrogen to be released (6). In comparison to isotonic sodium chloride (6, 10), all 3 of the suspending media markedly inhibited the release of pyrogen from exudate leucocytes (Table I). The degree of inhibition was of the order of 50-fold. As was to be expected from their behavior in saline, the blood

TABLE I

Comparative Amounts of Pyrogen Released by Exudate and Blood Leucocytes Incubated in Plasma and in Modified Hanks' Solution (MH) for 4 Hours at 37°C

Origin of cells*	Incubation medium	Mean fever index‡	SE
Exudate	Plasma (heparinized)	3.0	±0.43 (21)§
"	" (EDTA)	5.6	±1.6 (4)
"	MH	4.1	±1.2 (5)
Blood	Plasma (heparinized)	1.8	±0.3 (14)
"	MH	2.6	±1.2 (6)

* 3.5×10^8 cells incubated in each experiment.

‡ Computed from 120-minute fever curves obtained in 3 to 4 rabbits.

§ Figures in parenthesis indicate number of experiments.

leucocytes also gave up very little pyrogen when incubated in plasma or MH (Table I).

The inhibitory effect of the Hanks' solution (MH) on the release of pyrogen has previously been shown to be due to the presence of potassium ions (10). The similar action of plasma appears to involve, not only potassium, but also calcium ions, since the latter have been found to potentiate the inhibitory effect of potassium (10). It is not surprising, therefore, that EDTA plasma, in which the calcium ions are chelated, is slightly less inhibitory than heparinized plasma, in which the ionized calcium is free ($P < 0.05$).

Effect of Phagocytosis on Pyrogen Release.—That phagocytosis causes a marked increase in the release of pyrogen from leucocytes incubated in heparinized plasma is clearly shown by the data summarized in Tables II and III. The higher the ratio of particles to cells, the greater was the degree of both phagocytosis and release of pyrogen (Table II).

[6] To assure adequate mixing during incubation each tube was either placed in the rotator or was agitated manually at frequent intervals.

TABLE II

Increase in Release of Leucocyte Pyrogen in Plasma and in MH Resulting from Phagocytosis of Heat-Killed Pneumococci and Polystyrene Spherules

Origin of cells	Particles phagocyted	Medium	Ratio of particles to cells	Phagocytosis*	Increment in mean fever index‡	
					Incubation time§	
					1 hr.	4 hrs.
				per cent		
Exudate	Heat-killed pneumococci	Plasma (heparin)	1/1	30–40	1.0 (2)‖	5.6 (4)
"	"	"	5/1	80–90	4.6 (4)	8.1 (5)
"	"	"	50/1	95+	6.6 (2)	24.8 (3)
Exudate	Heat-killed pneumococci	Plasma (EDTA)	50/1	95+	—	35.5 (3)
Exudate	Heat-killed pneumococci	MH	5/1	80–90	—	13.2 (2)
Blood	Heat-killed pneumococci	Plasma (heparin)	50/1	95+	—	23.2 (3)
Exudate	Polystyrene spherules	Plasma (heparin)	10/1	95+	—	0.1 (3)
"	"	"	300/1	95+	—	6.3 (3)

* Percentage of cells containing one or more particles after 30 minutes' incubation.

‡ Increment obtained by subtracting mean fever index of control (no particles added) from calculated mean fever index following phagocytosis (see Methods). Each mean fever index was based on 120-minute fever curves of 3 to 4 rabbits.

§ Includes initial 30 minutes during which most of the phagocytosis occurred.

‖ Figure in parenthesis indicate number of experiments.

TABLE III

Increase in Release of Leucocytic Pyrogen in Heparinized Plasma Resulting from Phagocytosis of Living and Heat-Killed Pneumococci

Origin of cells	Particles phagocyted	Ratio of particles to cells	Phagocytosis*	Increment in mean fever index‡	
				Incubation time§	
				1 hr.	2 hrs.
			per cent		
Exudate	Heat-killed pneumococci	5/1	80–90	4.2 (2)‖	11.9 (2)
"	Viable pneumococci	5/1	80–90	3.0 (2)	13.6 (2)

For explanation of footnotes see Table II.

Regarding the possible mechanism of the increased pyrogen release, it was noted that, following phagocytosis at high particle-cell ratios in heparinized plasma, a spectacular agglutination[7] of the leucocytes occurred within 15 to 30 minutes. Examination of the flying coverslip smears stained by the Gram method revealed that the innermost cells in the clumps contained most of the phagocyted bacteria. Many of these cells also stained with eosin Y,[8] suggesting that they were "non-viable" (11). In addition, their cytoplasm was shown with bromthymol blue ($pK_1 = 7.0$) to be relatively acid in comparison to that of the cells at the periphery of the clumps. Since no such clumping occurred in either EDTA plasma or MH, it appeared that leucocytes which have ingested bacteria agglutinate in the presence of divalent cations and once sufficiently clumped together accumulate the acid metabolites generated as a result of phagocytosis (12, 13) and become permeable to eosin Y. That this process, however, was not responsible for the release of pyrogen is clearly indicated by the fact that post-phagocytic pyrogen production was just as efficient in EDTA plasma, and even more efficient in MH (no divalent cations), despite the fact that little, if any, clumping occurred in either medium.

Effect of Particle Characteristics on Pyrogen Release.—Although no significant difference was noted in the amount of pyrogen released by leucocytes which had ingested viable pneumococci, as compared to heat-killed pneumococci (Table III, $P > 0.3$), very different values were obtained in the experiments performed with polystyrene spherules. As shown in Table II, even at relatively high particle-cell ratios, when nearly every cell had ingested a large number of the spherules, surprisingly little pyrogen was released.

Effect of Phagocytosis on Pyrogen Production by Blood Leucocytes.—Inasmuch as blood leucocytes incubated in isotonic sodium chloride produce much less pyrogen than exudate leucocytes (6), it was anticipated that a similar relationship might be observed in the phagocytosis experiments. As shown in Table II, however, following phagocytosis of heat-killed pneumococci, blood leucocytes produced just as much pyrogen as exudate leucocytes. The implications of this finding are discussed below.

Comparative Kinetics of Phagocytosis and Release of Pyrogen.—It will be seen from the data in the upper section of Table II that at each multiplicity (particle-cell ratio) the amount of pyrogen released at the end of 1 hour is significantly less than that released at the end of 4 hours ($P < 0.001$). Since preliminary experiments had revealed that practically all of the phagocytosis took place during the 1st hour, when the suspensions were being rotated, it was concluded that most of the pyrogen was generated by the cells *after*, rather than during, the phagocytic process. That the two phenomena do not occur simultaneously is strongly suggested by the fact that the amount of pyrogen released in the 1st

[7] Easily visible macroscopically.

[8] At a concentration of 1/2000 in MH.

hour at the 50 to 1 multiplicity, where the per cent phagocytosis was over 95, was essentially the same as that released after 4 hours in the 1 to 1 system, where the per cent phagocytosis was only 30 to 40.

To make certain that the comparatively low yields of pyrogen observed after 1 hour were not due to inactivation of preformed pyrogen during the period of phagocytosis, supernatants obtained from the cell-particle mixtures at the end of 1 hour's incubation were incubated for 3 hours with known amounts of preformed leucocytic pyrogen. No inactivation of the pyrogen was demonstrable.

Dissociation of Phagocytosis and Pyrogen Release.—In view of the foregoing evidence that phagocytosis precedes the release of pyrogen, the following experiments were performed to study further the apparent dissociation of the two processes.

Heat-killed pneumococci and leucocytes (ratio 5 to 1) were suspended in a medium composed of two-thirds MH and one-third isotonic NaCl, supplemented with 200 mg of glucose. After a total of 1 hour's incubation at 37°C, during the first 30 minutes of which the mixture was rotated to promote phagocytosis, the suspension was centrifuged at 200 *g* for 10 minutes. The supernatant which contained 90 to 95 per cent of the unphagocyted bacteria[9] was separated from the cells and stored temporarily at 4°C. The centrifuged cells were immediately resuspended in fresh MH-NaCl and incubated for an additional 3 hours, at the end of which time the fluid medium was again separated from the cells by centrifugation. Both the first and second supernatants were then centrifuged at 2500 *g* for 60 minutes at 4°C, to remove residual bacteria, and were tested for pyrogenicity. As shown in the top two lines of Table IV, relatively little pyrogen was released during the first period of incubation, when most of the phagocytosis took place,[10] whereas large amounts of pyrogen were generated during the second period of incubation.[11]

When 5×10^{-2} M sodium fluoride, which is known to block phagocytosis (13), but not affect the release of pyrogen from leucocytes incubated in saline (14), was introduced into the medium[12] during the first period of incubation, both

[9] Direct bacterial counts made on supernatants from unincubated bacteria-leucocyte mixtures centrifuged in a similar manner at 4°C revealed that 90 to 100 per cent of the bacteria remained in the supernatant.

[10] Since 90 to 95 per cent of the bacteria not phagocyted in the first incubation was removed by centrifugation before the second incubation was begun, the ratio of bacteria to cells during the second incubation could not have been greater than 0.5 to 1. As shown by the data in Table II, such a low multiplicity would result in relatively little phagocytosis and only a small yield of pyrogen.

[11] It will be noted that the amount of pyrogen released after phagocytosis in MH, without transfer to new medium (Table II, line 5), is only about one-fourth of that released when a transfer to new medium is made (Table IV, line 2). One possible explanation for this difference is that during phagocytosis the cells may release cathepsins, which on further incubation may destroy some of the pyrogen (14).

[12] One-third 0.15 M NaF plus two-thirds MH.

phagocytosis and pyrogen release were markedly inhibited (Table IV, lines 5, 6, and 7). However, if the sodium fluoride was added in the second period only, phagocytosis proceeded normally and there was no inhibition of the release of pyrogen (Table IV, line 3). In contrast, when 1×10^{-4} M sodium arsenite, which blocks saline-induced pyrogen release (14) as well as phagocytosis (12, 13), was added during the second period of incubation, the release of pyrogen from the cells was greatly inhibited, despite the fact that vigorous phagocytosis had occurred during the 1st hour (Table IV, line 4). Thus it may be concluded that the

TABLE IV

Effect of Sodium Fluoride and Sodium Arsenite on Pyrogen Release Stimulated by Phagocytosis of Heat-Killed Pneumococci

First incubation period (0–1 hr.)			Second incubation period (1–4 hrs.)	
Medium*	Phagocytosis	FI‡	Medium	FI‡
	per cent			
MH-NaCl	80–90	1.8	—	—
"	—	—	MH-NaCl	17.3
"	—	—	MH-NaF	18.8
"	—	—	MH-Arsenite-NaCl	3.3
MH-NaF	5–10	0.0	—	—
"	—	—	MH-NaCl	0.5
"	—	—	MH-NaF	0.0

* Containing, in addition, 200 mg per cent of glucose.

‡ Mean fever index in absence of bacteria subtracted from calculated mean fever index in presence of bacteria (see Methods). The pyrogen assayed in each experiment was that released by 1×10^8 cells. (Note that this is a smaller number of cells than used in the preceding experiments, Tables I to III.)

phagocytic event precedes the release of pyrogen and that the latter, once initiated, will continue in the absence of further ingestion of particles by the cell.

Evidence That Phaogcytic Process Alters the Leucocyte Rather Than the Medium.—The release of leucocytic pyrogen induced by phagocytosis may, therefore, be regarded as a sequel to the phagocytic event, which must either alter the cell itself or modify the extracellular environment in such a way as to permit the cell to give up its pyrogen. The latter possibility was investigated by incubating leucocytes in a medium (MH) in which phagocytosis had previously taken place. Since the amount of pyrogen released under these conditions was no greater than when the cells were incubated in the fresh medium ($P < 0.001$), it was concluded that the pyrogen-inducing capacity of the postphagocytic medium had not been significantly altered. Further evidence that the ingestion process altered the cells rather than the medium was provided by the experiments performed with modified Hanks' solution (MH). It will be recalled that

when leucocytes, in the absence of phagocytable particles, were incubated in MH (Table II), relatively little pyrogen was released because of the inhibitory action of the potassium (17 meq/liter) in the medium (10). In contrast, large amounts of pyrogen were generated in this medium by leucocytes which had ingested pneumococci (Table II). Thus it is evident that participation in the phagocytic reaction strikingly altered the responsiveness of the cells to the inhibitory effect of extracellular potassium.

The Pyrogenic Effect of Phagocytosis in Vivo.—When large numbers of bacteria are injected into the blood stream, many of them are promptly ingested, not only by cells of the reticuloendothelial system, but also by circulating poly-

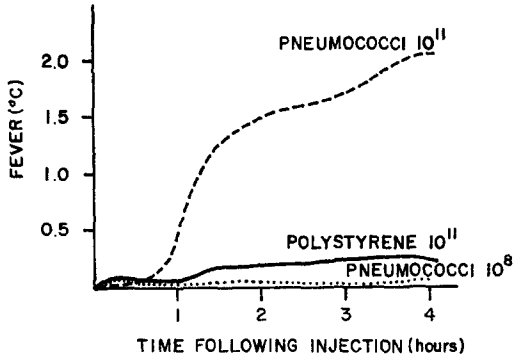

FIG. 1. Mean fever curves of groups of 3 rabbits injected intravenously with: (*a*) 10^{11} washed heat-killed pneumococci, (*b*) 10^{11} washed polystyrene beads, and (*c*) 10^{8} washed heat-killed pneumococci.

morphonuclear leucocytes (5). Because of the observation that phagocytosis *in vitro* causes blood granulocytes to release pyrogen even in plasma (Table II), experiments were performed to investigate the possibility that the *in vivo* phagocytic reaction, which occurs during experimental bacteremia, might also give rise to a febrile reaction in the injected host.

Large numbers (10^{8} and 10^{11}) of washed heat-killed pneumococci suspended in MH were injected intravenously into trained rabbits. The injection of each rabbit was completed in a period of about 1 minute and its temperature was recorded thereafter for a period of 4 hours. Similar experiments were carried out with polystyrene beads.

Several features of the results, which are summarized in Fig. 1, are of interest. First, the injection of 10^{7} pneumococci caused no fever. This finding proves beyond any doubt that the pyrogenicity of the supernatant fluids observed in the foregoing *in vitro* experiments could not have been due to the relatively small numbers of bacteria which they might have contained following intensive centrifugation. Secondly, when 10^{11} pneumococci were injected, a marked febrile

reaction occurred, characterized by: (*a*) an inordinately long latent period of nearly 1 hour, and (*b*) a sustained response which had not begun to subside by the end of 4 hours. Both of these characteristics are in keeping with the kinetics of the pyrogen release which occurs *in vitro* following the phagcytosis of heat-killed pneumococci (Table II). Thirdly, the polystyrene beads (10^{11}) proved to be much less pyrogenic than the pneumococci *in vivo*, just as they had *in vitro* (see also Table II). Taken together, these findings strongly suggest that the febrile reaction induced by the injection of 10^{11} heat-killed pneumococci was due primarily, if not solely, to intravascular phagocytosis of the organisms.

DISCUSSION

The results of the foregoing experiments indicate that phagocytosis causes the release of endogenous pyrogen from polymorphonuclear leucocytes. The amount of pyrogen released by a constant number of cells in a given medium appears to be related both to the degree of phagocytosis and to the nature of the particles ingested. Furthermore, the process occurs in two stages: the first entails the ingestion of the particles; the second, the generation of the pyrogen. The phagocytic phase may be inhibited by both sodium fluoride and sodium arsenite (12, 13), whereas the pyrogen release phase is inhibited only by sodium arsenite (14). When phagocytosis is blocked by sodium fluoride, the release of pyrogen is also blocked, despite the fact that the fluoride does not affect the release mechanism *per se* (14). Thus it may be concluded that, under the conditions of these experiments, the release of pyrogen is dependent upon the phagocytosis but is not synchronous with it. Indeed, once initiated, the release of pyrogen will proceed even in the presence of NaF, when no further phagocytosis can occur.

Concerning the manner in which phagocytosis initiates the release process, it is clear that the ingestion of particles has a direct effect upon the participating cells. That metabolites excreted into the medium by the phagocyting cells (12, 13, 15) are not responsible for the subsequent generation of pyrogen is indicated by the fact that incubation of cells in a medium in which phagocytosis has already taken place causes no greater release of pyrogen than incubation of the cells in a fresh medium. Clumping of the cells which participate in the phagocytic reaction, a phenomenon which is apparently dependent upon the presence of divalent cations (16), likewise does not initiate the generation of pyrogen, since even less pyrogen is released in media in which postphagocytic clumping occurs than in media in which the clumping has been prevented by the elimination of free divalent cations (see Table II).

Why the ingestion of heat-killed pneumococci *in vitro* should act as a much more potent stimulus of pyrogen release than the phagocytosis of a comparable number of polystyrene beads remains unexplained. The reason for this striking difference, which appears to depend upon the nature of the particles ingested,

will probably not be elucidated until more is known about the precise molecular reactions of the release process. The fact that the postphagocytic release mechanism, like that induced by K-free saline (14), is inhibited by arsenite suggests that both release processes entail the action of one or more sulfhydryl-dependent enzymes.

Of particular interest is the behavior of blood leucocytes in the phagocytic system. The pyrogen release mechanism in these cells, in contrast to the process in exudate leucocytes, is not readily activated by incubation of the cells in K-free NaCl (6). Despite this fact, blood cells which have phagocyted pneumococci release large amounts of pyrogen, even in the presence of K^+. Similarly, the postphagocytic release of pyrogen from exudate cells occurs in the presence of "physiological" concentrations of potassium ions. It may be assumed, therefore to operate in the intact host.

In keeping with this assumption is the observation that the intravenous injection of a sufficiently large number of phagocytable particles causes fever (3) The fact that the time required for the *in vivo* response is almost exactly that predictable from the *in vitro* reaction, and the observation that injection of polystyrene beads causes less fever than injection of the same number of pneumococci, both suggest that the resulting fever is caused by phagocytosis of the injected particles by circualting polymorphonuclear leucocytes. This form of intravascular phagocytosis has been repeatedly observed under similar conditions in the rabbit ear chamber (4). The febrile response thus induced *in vivo* is compatible with the finding that the postphagocytic release of pyrogen *in vitro* is uninhibited by physiological concentrations of extracellular potassium. By the same token it may be reasoned that extravascular granulocytes engaged in phagocytosis within infected tissues will contribute significantly to the total production of endogenous pyrogen in acute bacterial infections (1, 2).

SUMMARY

1. Phagocytosis promotes the release of endogenous pyrogen from polymorphonuclear leucocytes.

2. The release of pyrogen, though initiated by the phagocytic event, is not synchronous with it.

3. The postphagocytic release mechanism is not inhibited by sodium fluoride and, therefore, appears not to require continued production of energy by the cell.

4. The release process, on the other hand, is inhibited by arsenite, suggesting the participation of one or more sulfhydryl-dependent enzymes in the over-all reaction.

5. Particle for particle, the ingestion of heat-killed rough pneumococci causes the release of approximately 100 times as much pyrogen as the ingestion of polystyrene beads of the same size.

6. The pyrogen release mechanism of polymorphonuclear leucocytes separated directly from blood, unlike that of granulocytes in acute inflammatory exudates, is not readily activated by incubation of the cells in K-free saline. Despite this difference, both blood and exudate leucocytes following phagocytosis release large amounts of pyrogen, even in the presence of K^+. The fact that the postphagocytic reaction is uninhibited by the concentrations of K^+ which are present in plasma and extracellular fluids, suggests that this mechanism of pyrogen release may well operate *in vivo*.

7. As might be expected from the foregoing observations, the intravenous injection of a sufficiently large number of heat-killed pneumococci causes fever in the intact host. Intravenously injected polystyrene beads, on the other hand, are significantly less pyrogenic. Evidence is presented to support the conclusion that the fever in both instances is caused by pyrogen released from the circulating leucocytes which have phagocyted the injected particles.

8. The possible relationships of these findings to the pathogenesis of fevers caused by acute bacterial infections are discussed.

BIBLIOGRAPHY

1. King, M. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. V. The relation of circulating endogenous pyrogen to the fever of acute bacterial infections, *J. Exp. Med.*, 1958, **107,** 305.
2. Bennett, I. L., Jr., Studies on the pathogenesis of fever. V. The fever accompanying pneumococcal infection in the rabbit, *Bull. Johns Hopkins Hosp.*, 1956, **98,** 216.
3. Atkins, E., and Freedman, L. R., Studies in staphylococcal fever. I. Response to bacterial cells, *Yale J. Biol. and Med.*, 1963, **35,** 451.
4. Wood, W. B., Jr., Studies on the cellular immunology of acute bacterial infections, *Harvey Lectures*, (1951–52, **47,** 72).
5. Wood, W. B., Jr., Smith, M. R., Perry, W. D., and Berry, J. W., Studies on the cellular immunology of acute bacteriemia. I. Intravascular phagocytic reactions and surface phagocytosis, *J. Exp. Med.*, 1951, **94,** 521.
6. Kaiser, H. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. IX. The production of endogenous pyrogen by polymorphonuclear leucocytes, *J. Exp. Med.*, 1962, **115,** 27.
7. Robertson, O. H., and Sia, C. H. P., Studies on pneumococcus growth inhibition. II. A method for demonstrating the growth-inhibitory and bactericidal action of normal serum-leucocyte mixtures, *J. Exp. Med.*, 1924, **39,** 219.
8. Bornstein, D. L., Bredenberg, C., and Wood, W. B., Jr., Studies on the pathogenesis of fever. XI. Quantitative features of the febrile response to leucocytic pyrogen, *J. Exp. Med.*, 1963, **117,** 349.
9. Rowley, D., and Whitby, J. L., The bactericidal activity of mouse macrophages *in vitro*, *Brit. J. Exp. Path.*, 1959, **40,** 507.
10. Berlin, R. D., and Wood, W. B., Jr., Studies on the pathogenesis of fever. XII. Electrolytic factors influencing the release of endogenous pyrogen from polymorphonuclear leucocytes, *J. Exp. Med.*, 1964, **119,** 697.

11. Schrek, R., A method for counting viable cells in normal and malignant cell suspensions, *Am. J. Cancer*, 1936, **28,** 389.
12. Sbarra, A. J., and Karnovsky, M. L., The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes, *J. Biol. Chem.*, 1959, **234,** 1355.
13. Cohn, Z. A., and Morse, S. I., Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and consequences of particle ingestion, *J. Exp. Med.*, 1960, **111,** 667.
14. Kaiser, H. K., and Wood, W. B., Jr., Studies on the pathogenesis of fever. X. The effect of certain enzyme inhibitors on the production and activity of leucocytic pyrogen, *J. Exp. Med.*, 1962, **115,** 37.
15. Karnovsky, M. L., Metabolic basis of phagocytic activity, *Physiol. Rev.*, 1962, **42,** 143.
16. Garvin, J. E., Factors affecting the adhesiveness of human leucocytes and platelets *in vitro*, *J. Exp. Med.*, 1961, **114,** 51.