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Lipid Peroxidation in the Killing of Phagocytized Pneumococci

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To directly examine the role of hydrogen peroxide in the killing of bacteria after ingestion by granulocytes, we have studied some of the events of phagocytosis of a mutant strain of pneumococci which is relatively deficient in peroxide production. The hydrogen peroxide-deficient pneumococci and the otherwise identical wild type were grown with [^{14}C]arachidonic and [^{3}H]palmitic acid labels to label their lipids with unsaturated and saturated fatty acids, respectively. They were then incubated with both normal and chronic granulomatous disease granulocytes. The rates of ingestion and bacterial killing and the stability of fatty acids in the cell-bacteria complex were followed. Radioactive carbon dioxide released from glucose was also independently followed to measure glucose oxidation. Ingestion was similar for all cell-bacteria combinations. Chronic granulomatous disease cells killed the peroxide-positive wild pneumococci much more effectively (20-fold) than the peroxide-deficient mutant. Normal cells killed both peroxide-positive and -negative strains effectively. A considerable loss of [^{14}C]arachidonic acid (~40%) consistent with lipid peroxidation of this unsaturated fatty acid was observed in all normal cells and in chronic granulomatous disease cells with peroxide-positive pneumococci. However, no loss of [^{14}C]arachidonic acid occurred in chronic granulomatous disease cells with the peroxide-deficient pneumococci. No loss of tritiated palmitic acid occurred in any cell-bacteria combination. Glucose oxidation was impaired in the chronic granulomatous disease cells in comparison to normal cells at rest and was especially impaired in chronic granulomatous disease cells ingesting the peroxide-deficient mutant pneumococci. This defect was partially corrected after phagocytosis of the peroxide-positive strain. These data directly support the hypothesis that bacterial killing is partially dependent upon an intact peroxide-generating system in the leukocyte-bacteria complex. Moreover, they indicate that bacterial lipid peroxidation is associated with the generation of peroxide during phagocytosis. Finally, they suggest that such peroxidation may contribute to effective phagocytic bacterial killing.

A single specific mechanism of the intracellular killing of bacteria by phagocytes has not been defined. Indeed, it is likely that several specific mechanisms with different organisms and different ingesting cells exist. The liberation of cationic proteins from granules in some animal systems (37), the transfer of digestive enzymes (14), the production of hydrogen ions (in Metchnikoff, as cited in ref. 19), the generation of amino acid aldehydes (18), and the oxidative generation of H_2O_2 (3) have all been implicated in the killing phase of phagocytosis by polymorphonuclear leukocytes (PMNs). The bacterial fixation of iodine coupled to the action of myeloperoxidase and the generation of H_2O_2 is closely associated with bacterial killing in some cell-bacteria systems (22). Whether, for live bacteria, this halide damages bacterial protein or lipid, or both, is not clear, although Elsbach has shown that specific bacterial lipid changes can occur after the ingestion of killed *Escherichia coli* (10).

Some of the strongest data supporting the importance of peroxidation as an essential process in bacterial killing is derived from experiments with chronic granulomatous disease (CGD) cells (16). These abnormal phagocytes ingest bacteria adequately but are unable to

produce peroxide or adequately kill most bacteria. They can, however, kill those bacteria which themselves yield net hydrogen peroxide and thus supply it for the cell-bacteria complex. CGD cells, however, cannot kill bacteria which have high catalase activities, since such bacteria hydrolyze their own hydrogen peroxide as soon as it is generated. These indirect observations suggest that peroxidation is essential for bacterial killing (23). However, these studies suffer from the fact that they utilize different bacterial species to demonstrate the importance of the presence of catalase, and hence hydrogen peroxide, in the bacteria. Thus, the objection may be raised that some other, unrecognized species characteristic may modify the phagocytes' ability to destroy these bacteria.

To examine this question more directly, Pitt and Bernheimer studied the events accompanying phagocytosis and intracellular killing of a mutant *Pneumococcus* which, rather than destroying peroxide by catalase, made very small amounts in the first place (30; J. Pitt, H. P. Bernheimer, and S. Fikrig, Progr. Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 11th, Atlantic City, N.J., Abstr. 134, p. 67, 1971). For comparison, they also studied these events in the wild parent strain, which does produce hydrogen peroxide but which is otherwise identical to the mutant. Similar studies with streptococci were conducted by Holmes and Good, who showed reduced killing of the peroxide-deficient strain by the CGD cells (15). The purpose of the current study was to examine changes in the disposition of the fatty acids in these systems and to correlate any such changes with differences in the bactericidal capacity of these cells. We prelabeled these bacteria with saturated and unsaturated fatty acids to study the possible effects of lipid peroxidation within the bacteria during subsequent phagocytosis. We found preferential loss of bacterial unsaturated fatty acid only after phagocytosis associated with hydrogen peroxide generation and successful bacterial killing. We also found that this lipid loss was associated with the production of acyl diene fragments typical of lipid peroxidation.

The results confirm the previous observations that an intact peroxidation system in the phagocyte-bacteria complex is essential for optimal killing of pneumococci by human PMNs. They further indicate that bacterial lipid peroxidation occurs during such bacterial killing.

Part of this material has been presented previously (S. B. Shohet, J. Pitt, R. L. Bachner, and D. Poplack, J. Clin. Invest. **51**:89a).

MATERIALS AND METHODS

Bacterial selection and characterization. A hydrogen peroxide-deficient mutant of the type 1 *Pneumococcus* strain SV1 was selected after treatment of the wild strain by *N*-methyl-*N*-nitrosoguanidine. Mutant colonies which did not produce peroxide were detected on the surface of blood agar plates containing benzidine as nonpigmented colonies. The details of the selection process and the maintenance of stable mutant lines have been published elsewhere (30). Characteristics of the wild and mutant strain are summarized in Table 1. (For convenience in the tables and figures the peroxide-deficient mutant is referred to as peroxide [−], whereas the wild strain is referred to as peroxide [+].)

Preparation of WBC and bacteria. Blood (50 ml), anti-coagulated with 200 U of heparin, was repeatedly obtained from a normal donor (S.B.S.) and a patient with known CGD (L.B.). Clinical and laboratory data substantiating the diagnosis of CGD in this patient have been previously reported (4). Both the CGD patient and the normal donor were afebrile and clinically free from infection at the times of sampling, which spanned a 4-day period. The leukocytes (WBC) were isolated by gravity sedimentation after the addition of 20 ml of 5% dextran (average molecular weight, 60,000) and then washed two times with isotonic Krebs-Ringer phosphate buffer (KRP; pH 7.4) as previously described (21). The WBC were then resuspended to a concentration of approximately 2×10^7 cells/ml. Typical differential counts showed the normal donor had 76% PMNs and the CGD patient had 82% PMNs. For metabolic studies, the erythrocytes were eliminated by ammonium chloride treatment (3). Erythrocyte contamination in these final suspensions was less than 1 erythrocyte per 10 WBC.

Wild-type pneumococci and the peroxide-deficient mutant derived from the same strain were inoculated into brain heart infusion containing either [^{3}H]palmitic acid or [^{14}C]arachidonic acid bound to albumin as previously described (34). Preliminary experiments showed that more than 95% of the radioactive acids could be found in esterified form in the isolated bacteria; hence active incorporation of the acid into bacterial lipids had occurred. The arachidonic acid was labeled in the C-1 position, whereas the palmitic acid was labeled in the 9 and 10 positions. The initial bacterial inoculum was 4×10^5 organisms/ml. The bacteria were harvested during the logarithmic stage of growth at a concentration of approximately 2×10^8 organisms/ml, washed twice in KRP (10,000 rpm for 10 min in a Sorvall RC2B angle-head centrifuge), and resuspended to 6×10^8 colony-forming units/ml. When equal amounts of either of these bacterial suspensions and the WBC suspension were subsequently combined in the phagocytosis experiments, the ratio of bacteria to WBC was approximately 30:1.

Phagocytosis studies. (i) Incubations for lipid disposition studies. Prior to phagocytosis, hyperimmune antipneumococcal type-specific rabbit antiserum (1) was added to equal numbers of arachidonic and palmitic acid-labeled bacteria of either the wild

or mutant strain (6×10^8 colony-forming units of bacteria and a 1:100 final dilution of antiserum). This antiserum opsonized the bacteria for efficient phagocytosis without influencing their continued growth (data not shown). Freshly prepared human serum as a complement source was then added (final dilution, 1:10). WBC (2×10^7) and KRP buffer were then added to a final volume of 1.0 ml. The incubation mixture was warmed rapidly and incubated at 37 C with gentle agitation for 45 and 90 min in a water bath. On occasion, 15-min incubations were also used. In addition to 0-time control samples, nonphagocytic controls were performed by preparing and incubating all materials as usual, except that the WBC suspensions were then momentarily combined at the end of incubation, immediately extracted with lipid solvents, and subsequently handled together with the phagocytosis samples.

(ii) Bacterial ingestion studies. For these studies, stock suspensions of WBC and bacteria prepared as above were diluted to give a final ratio of colony-forming units to phagocytes of 2:1. Incubation tubes were rotated on a Fisher Roto-Rack at 15 rpm. The extent of bacterial ingestion was estimated by two independent methods as follows.

(i) After incubation, the WBC-bacteria complexes were separated from the media by gentle centrifugation ($200 \times g$ for 10 min). Colony counts on the residual bacteria in the supernatant media were made. Following the method of Cohn and Morse (6) an estimation of bacterial ingestion and adhesion was made by subtracting the number of bacteria actually found from the number expected after an incubation without granulocytes and extrapolating this figure to 0 time. Since phagocytosis was very nearly complete, the errors inherent in this calculation were small.

(ii) After incubation, the WBC-bacterial complexes were washed gently three times with Krebs-Ringer phosphate, and the radioactivity of [^{3}H]palmitic acid was determined in the original media, the washes, and the WBC-bacteria pellet. More than 94% of all added radioactivity could be accounted for in the washed-cell pellet. Incorporation into the WBC-bacteria complex in comparison to the specific activity of palmitic acid in the original bacterial mixtures was then taken as a measure of bacterial ingestion or ingestion and adhesion. This measure of phagocytic activity did not differ from the first method by more than 10% in any experiment.

(iii) Bacterial killing studies. Johnston's modification (20) of Quie's method (32) was used. In brief, pneumococci growing in broth in log phase were collected by centrifugation, washed, and resuspended in Krebs phosphate buffer with 10% bovine serum albumin to a density which gave an optical density reading of 0.100 at 600 nm in a spectrophotometer. This suspension was then diluted 1:10 in the same buffer, and 0.3 ml of this suspension was added at 0 time to 2.5×10^6 phagocytes in 0.5 ml of the same buffer with albumin, 0.1 ml of antipneumococcus type 1 rabbit immune serum, and 0.1 ml of fresh human serum. These were incubated in 12- by 75-mm sterile, capped propylene tubes on a revolving wheel at 30 rpm. At each desired time, samples were removed and prepared for plate dilutions after disruption of the WBC by the addition of 5 ml of distilled water.

(iv) Metabolic studies. Independent incubations of bacteria and WBC were conducted under similar conditions to the above experiment. However, [1-^{14}C]glucose was present in the incubation media, and the wild and mutant pneumococci were not radioactively labeled. In these studies the ratio of bacteria to PMNs was greater than 200:1 in order to saturate the system. Glucose oxidation was estimated by collection and determination of $^{14}CO_2$ as previously described (2). The results are expressed as counts per minute per 10^7 PMNs during a 30-min incubation at 37 C. Control experiments with bacteria alone showed minimal $^{14}CO_2$ liberation (<300 counts/min) during the same 30-min incubation period).

(v) Lipid analyses. The washed bacteria-cell complexes, obtained as in (i) above, were extracted with 20 volumes of 2:1 chloroform-methanol (12). After a 0.2-volume aqueous wash with 0.1 N KCl, the lipid extract was counted for radioactivity and then analyzed by thin-layer chromatography and gas-liquid chromatography as previously described by others (9, 35). The aqueous wash was saved and counted for water-soluble radioactive degradation products. The total recovery of ^{14}C and ^{3}H radioactivity was calculated from the initial lipid extracts. The disposition of [^{14}C]arachidonic and [^{3}H]palmitic acids was determined by radioactive scanning of the thin-layer chromatographic plates, followed by elution of the radioactive spots and scintillation counting of the eluates (33). Confirmation that the recovered radioactivity represented the original fatty acids was secured by gas-liquid chromatography-mass determination of the methyl ester derivatives combined with radioactive counting in a Bendix gas chromatograph equipped with a flow splitting device.

RESULTS

Bacteriological characterization. Several bacteriological characteristics of the wild and mutant strains of pneumococci are summarized in Table 1. As can be seen, with the exception of peroxide production the bacteria were similar in all parameters. In addition, the capsular size of both strains as revealed by Quelling reactions was the same for both strains.

Bacterial ingestion studies. The results of the "ingestion" phase of phagocytosis are summarized in Table 2. In this table, ingestion at 45 min is shown for all cell-bacteria combinations. The data used are those from the [^{3}H]palmitic acid incorporation; nearly identical results were obtained from residual media colony counts. No significant changes were seen in the 90-min samples or in occasional 15-min samples (i.e., ingestion was complete within 15 min).

Bacterial killing. The results of the bacterial

TABLE 1. *Characteristics of wild and mutant Pneumococcus strains*[a]

Pneumococcus	Doubling time in brain heart infusion broth (min)		Blood agar colony size (mm)	Opsonic requirement[b]	Peroxide production[a] per ml of culture[c] (mM)
	By Klett units[d]	By colony counts			
Wild SV1	55	60	2.0	1:16≥ ≤1:32	6.42
Mutant	55	58	1.5	1.16≥ ≤1:32	0.916

[a] Measured by the release of O_2 from purged incubations treated with catalase as previously described (30).
[b] Optimal opsonation by dilution of hyperimmune serum.
[c] Corrected for optical density.
[d] Optical density at 600 nm.

TABLE 2. *Phagocytosis at 45 min*[a]

Pneumococci	Normal cells	CGD cells	0-Time control	45-Min unmixed control
Wild type (H_2O_2+)	86 ± 7	92 ± 3	3 ± 2	5 ± 3
Mutant type (H_2O_2−)	88 ± 5	90 ± 6	4 ± 3	5 ± 2

[a] Data expressed as mean percent of originally added [^{3}H]palmitic radioactivity found in washed cell-bacteria complex at 45 min (± standard error of the mean of four samples). Controls consisted of labeled bacteria which were kept isolated from WBC until immediately before extraction.

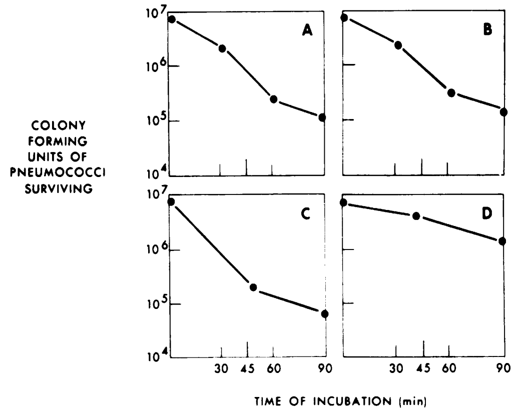

FIG. 1. *Bacterial killing of wild and mutant pneumococci by normal and CGD WBC. Bacterial killing is presented as the number of colqny-forming units per milliliter of media surviving after the addition of sufficient granulocytes to give a phagocyte-bacteria ratio of 2:1. Each point represents the mean of four experiments. (A) Normal WBC; wild pneumococci (H_2O_2+); (B) normal WBC; mutant pneumococci (H_2O_2−); (C) CGD WBC; wild pneumococci (H_2O_2+); CGD WBC; mutant pneumococci (H_2O_2−).*

killing assays of peroxide-positive and -negative pneumococci by normal and CGD cells are plotted in Fig. 1A-D. There was very little difference in the bacterial killing curves of the wild pneumococci in either CGD or normal PMNs (Fig. 1 A and C). These curves were also very similar to the killing curve of the peroxide-deficient mutant in normal PMNs (Fig. 1B). There was, however, a striking difference between bacterial killing in these three systems and in killing of the peroxide-deficient mutant in the CGD cells (Fig. 1D).

Metabolic studies. The result of the liberation of $^{14}CO_2$ from [1-^{14}C]glucose is shown in Table 3. It can be seen that glucose oxidation was depressed as expected in the CGD PMN at rest and that it was further depressed during phagocytosis of the H_2O_2-deficient mutant. In contrast, there was a twofold increase in the liberation of CO_2 after phagocytosis of the peroxide-positive mutant by the CGD PMNs, indicating probable stimulation of glucose oxidation by the release of some H_2O_2 by the bacteria. Finally, it should be noted that in all four phagocytic combinations virtually identical bacterial ingestion rates occurred (see Table 2).

Lipid analyses. The results of incubation of [^{14}C]arachidonic acid- and [^{3}H]palmitic acid-labeled peroxide-positive pneumococci with normal granulocytes are shown in Fig. 2. For the purposes of this experiment, the entire contents of the incubation mixture were extracted (i.e., cell-bacteria complexes and incubating media); however, as shown in the ingestion studies (see above), ingestion of more than 90% of the labeled organisms was complete within 15 min. Less than 5% of the ^{3}H (palmitic acid) radioactivity could not be accounted for at the end of the 90-min incubation. Conversely, nearly 40% of the ^{14}C (arachidonic acid) radioactivity could not be accounted for in the lipid extract of the cell-bacteria complex after 90 min of incubation. Further analyses of the washed lipid extracts of the incubation mixtures by thin-layer

and gas-liquid chromatography showed that more than 93% of the ^3H and ^{14}C labels which were recovered in the lipid extracts were still in fatty acids. On the other hand, 65% of the apparently lost ^{14}C (originally arachidonic acid) was found in the aqueous washings of the lipid extracts which subsequently chromatographed as diffuse background streaking and multiple small polar compounds. The remaining 35% of the missing ^{14}C radioactivity (about 12% of the overall ^{14}C radioactivity) could not be accounted for.

Due to lack of resolution in the chromatographic system, it was not possible to specifically identify these water-soluble products. However, a sixfold increase in the ratio of absorbance at 235 nm to absorbance at 215 nm of the unwashed lipid extract of normal granulocytes similarly incubated with normal pneumococci (ratio before incubation, 0.047; after incubation, 0.290) implied that these were in part conjugated dienes (24). Restoration of the baseline ratio after aqueous extraction and elimination of the change by adding 1% of the anti-oxidant butylated hydroxytoluene (a gift from the Shell Chemical Co.) to the original incubation strongly supported this implication.

Results of similar incubations of [^3H]palmitic acid and [^{14}C]arachidonic acid with peroxide-positive and -deficient pneumococci by both normal and CGD cells are shown in Table 4. In these studies, the lipids were only analyzed at 45 min. At this time, ingestion was complete in all samples (Table 2), and killing was well advanced in all but the CGD cell-mutant bacteria combination (Fig. 1). The data are presented as percent of original bacterial radioactivity recovered in washed lipid extracts of the cell-bacteria complex.

TABLE 3. *Liberation of $^{14}CO_2$ for [1-^{14}C]glucose at rest and during phagocytosis of unlabeled peroxide+ and peroxide− pneumococci in normal and CGD PMN*

Determination	$^{14}CO_2$ released[a]	
	Normal PMN	CGD PMN
Resting granulocytes	3,258	1,648
After phagocytosis of wild-type pneumococci (H_2O_2+)	50,206	2,932
After phagocytosis of mutant-type pneumococci (H_2O_2−)	32,905	792

[a] $^{14}CO_2$ released is expressed as counts/min of 10^7 cells after 30 min; data represent averages of duplicate determinations.

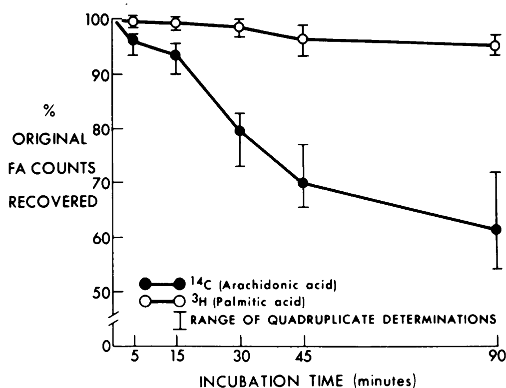

FIG. 2. *Recovery of radioactive fatty acids from doubly prelabeled bacteria after phagocytosis of peroxide (+) positive bacteria by normal WBC. Washed lipid extracts of the entire cell-bacteria complex were counted for ^{14}C (arachidonic acid) and ^3H (palmitic acid) radioactivity. Quench corrections for each isotope were made by the addition of individual internal standards. As in previous experiments more than 90% of the organisms were ingested by 15 min.*

TABLE 4. *Recovery of ^{14}C (arachidonic acid) and ^3H (palmitic acid) radioactivity from washed lipid extracts of 45-min incubations of H_2O_2+ and H_2O_2− pneumococci with normal and CGD cells*

Pneumococci	Normal cells[a]		CGD cells	
	^{14}C	^3H	^{14}C	^3H
Wild type (H_2O_2+)	59 ± 16[b]	96 ± 3	68 ± 6	97 ± 1
Mutant type (H_2O_2−)	64 ± 14	92 ± 4	94 ± 5	94 ± 4

[a] Each cell type was exposed to bacteria of either the wild or mutant type with both fatty acids at the same time.

[b] Data are expressed as percent of total 0-time radioactivity and are presented as means ± the standard error of the mean of four replicate determinations. As noted in the text, the bulk of the missing radioactivity could be found in the aqueous washes of the lipid extracts as small polar molecules.

DISCUSSION

Although circumstantially strong, much of the previous data implicating the role of H_2O_2 generation in the killing phase of bacterial phagocytosis has necessarily been indirect (10, 14, 25). The current experiments were designed to exploit the availability of both a peroxide-deficient pneumococcal mutant and a peroxide-deficient phagocyte (the CGD cell) to directly assess the role of H_2O_2 in phagocytic bacterial killing.

The observations shown in Table 1 indicate

that, except for the ability to generate peroxide, the wild-strain *Pneumococcus* and the mutant were bacteriologically similar. This is not surprising since the mutant was derived from the parent strain by nitrosoguanidine mutagenesis followed by selection of benzidine-negative colonies on blood agar plates; thus, selection for a highly specific characteristic was designed into the mutagenic process. It is also not surprising that the ingestion phase of phagocytosis was very similar for the two strains (Table 2). Also, expectedly, the presence of H_2O_2 in the cell-bacteria complex was not necessary for ingestion.

On the other hand, as two of us have previously shown (30), the data in Fig. 1 show that a normal level of H_2O_2 is necessary for a large proportion of bacterial killing of pneumococci by human granulocytes. In the one system where both cellular and bacterial peroxide were reduced, killing was markedly inhibited. The fact that some killing still occurred indicates that either other bactericidal systems can operate without peroxide or that the slight peroxide formation in the mutant bacteria can be partially effective.

Moreover, these data confirm the previous data of Klebanoff (22, 23), which implied that peroxide derived from either the phagocytic cells or the phagocytized bacteria was sufficient to satisfy the requirements of an active bactericidal system. Previous studies of Johnston and Baehner showing partial reconstitution of CGD cell-bacterial killing capacity with the introduction of a glucose oxidase H_2O_2-generating system also suggested this conclusion (20).

The data in Table 3 show that increased glucose oxidation is also partially dependent upon a peroxide-generating system within the phagocytic complex. Indeed, the percentage of the increment in CO_2 released by both normal and CGD cells upon the addition of the bacterial peroxide contribution is very similar. The difference in absolute values in the two cell systems suggests that other factors which regulate the basal metabolic activity of these cells may be operative as well. Whereas these data do not help us to decide if the increase in glucose oxidation is causally related to the peroxide generation (or vice versa), they do show that the respiratory machinery of the cell can be influenced by the type of bacteria which it ingests, and they emphasize the previously well-described requirement for glucose oxidation in bacterial killing (17, 20). They also represent the first metabolic study of granulocytes ingesting live bacteria rather than killed bacteria or latex particles, emphasizing the importance of the metabolic contribution of the ingested particles to the overall process.

The data in Fig. 2 and Table 4 show that bacterial lipid loss occurs during phagocytosis. Moreover, this loss begins very early after phagocytosis at a time when it could influence eventual bacteria killing. Since the loss was found in a polyunsaturated fatty acid (arachidonic) and not in a saturated fatty acid (palmitic) and since it was only found together with the evolution of hydrogen peroxide in the phagocyte-bacteria complex (Table 4), it is reasonable to suppose that this loss represents peroxidation of unsaturated fatty acids. This is also consistent with the previous observations of Mason et al. on the peroxidation of ingested lipid droplets in a system using macrophages rather than PMNs (27). The recovery of approximately two-thirds of this 40% loss of radioactivity as background streaking or small polar molecules in the aqueous washes of the lipid extracts further supports this supposition. Finally, the absence of significant loss of [^{3}H]palmitic acid during the same incubations additionally shows that this represents a particular lipid change which is not solely due to nonspecific membrane lysis after phagocytosis.

The precise mechanism of this peroxidative lipid loss is not shown by the current experiments. The absence of ^{3}H loss from the saturated fatty acid suggests that neither conventional β-oxidation nor peroxidase-dependent α-oxidation (26) starting at the carboxyl end of the molecule is involved. On the other hand, the predominant effect on polyunsaturated fatty acid and the finding of elevated dienes imply that peroxidative cleavage of the acyl chain, starting with attack upon the double bonds as described by Dohle, may be a likely mechanism (7). The recent studies of Fong et al. which demonstrate the peroxidation of liver lysosome membrane lipids after the oxidation of reduced nicotinamide adenine dinucleotide phosphate and xanthine suggest that hydroxy (OH·) free radicals generated by oxidases known to be active during phagocytosis may mediate this process (13).

It should be noted that Elsbach has observed the activation of bacterial phospholipases during phagocytosis (11). The current studies, which only measure total lipid radioactivity in the phagocyte-bacteria complex, do not rule out such activation. Indeed, previous observations in similar systems (31), which indicate some rearrangement of the bacterial saturated fatty acid label amongst phosphatide classes in the phagocytic complex after phagocytosis, sug-

gest that some activation of bacterial phospholipases may occur here, in addition to peroxidation of unsaturated lipids.

As noted above, unsaturated fatty acid lipid loss was not found in the one combination of cells and bacteria where neither normal peroxide generation nor effective bacterial killing occurred (the CGD cell-peroxide-deficient *Pneumococcus* complex; see Table 4). This suggests that peroxidative lipid attack may be a part of the final bacterial killing mechanism. It should be noted, however, that the lipid changes may be a consequence of the death of the bacteria and not a cause, and the current data do not discriminate between these possibilities.

Other peroxide-mediated changes have been shown to occur during phagocytosis. In particular, there is evidence that peroxide-linked iodination of erythrocyte membranes may damage membrane proteins or amino acids (29), and Welton and Aust have noted lipid peroxidation associated with peroxidase-catalyzed iodination (36). The possibility that similar reactions lethally damage bacterial membrane lipids is raised by the current studies. In several model systems, membrane permeability and function is known to be affected by lipid peroxidation (5, 8, 28). Moreover, the presence of short-chain polar peroxidation products (A. L. Tappel, *In* B. F. Trump and A. Arstila, ed., *Pathological Aspects of Cell Membranes*, vol. 1, in press), which may behave like detergents within membranes, would be expected to have highly deleterious effects upon the bacteria.

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