TEMPORAL CHANGES IN pH WITHIN THE PHAGOCYTIC VACUOLE OF THE POLYMORPHONUCLEAR NEUTROPHILIC LEUKOCYTE

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ABSTRACT

Although previous workers have established that the pH of the phagocytic vacuole of the polymorphonuclear (PMN) leukocyte changes from neutral to acid, the time course of conversion has not been investigated. The present experiments were initiated to study pH changes immediately after phagocytosis. Peritoneal exudates were induced in rats; 4 h later, yeast stained with pH indicators was injected intraperitoneally, and the exudate was retrieved at 30-s intervals and examined by light microscopy. Results revealed that (*a*) within 3 min, pH dropped to ~6.5, as indicated by the change in color of neutral red-stained yeast; (*b*) within 7–15 min, pH dropped progressively to ~4.0, as indicated by color change in bromcresol green-stained yeast; (*c*) pH did not fall below 4, since no color change was observed up to 24 h when bromphenol blue-stained yeast was used. The finding that intravacuolar acidity increases rapidly after phagocytosis is undoubtedly important with respect to PMN leukocyte function in killing and digesting microorganisms, for many PMN leukocyte granule enzymes (i.e., peroxidase and lysosomal enzymes) are activated at acid pH (~4.5). It follows that temporal changes in pH and maximal pH depression should be considered in studies of intraleukocytic microbicidal mechanisms, since a defect in these factors could result in impaired PMN leukocyte function.

INTRODUCTION

The pH within phagocytizing cells has long been considered to be acid. In 1893, Metchnikoff observed that particulate litmus ingested by Protozoa changed from blue to red, and postulated that an acid reaction within the cell might be responsible for the death of ingested organisms (1). In 1925, Rous (2, 3), in pioneer studies on mammalian leukocytes, utilized various indicator dyes and concluded that intracellular pH may be as low as 3.0. More recently, Sprick and others (4–7), using leukocytes and indicator-stained organisms, have suggested that intracellular pH may not be as low as originally conceived by Rous, but have definitely supported the premise that intracellular contents are acid. In none of these studies, however, was the time course of conversion from neutral to acid pH emphasized—observations generally being made 1–96 h after phagocytosis.

It is now known that during phagocytosis, polymorphonuclear (PMN) leukocytes internalize particles by invagination of the plasma membrane to form a membrane-bound vacuole. Within 2 min degranulation occurs, and PMN leukocyte granules, which contain agents capable of killing and digestion of microorganisms, empty their contents into the phagocytic vacuole by membrane fusion (8–11). In addition, a burst of metabolic activity occurs within several minutes,

characterized by an increase in O_2 consumption, lactate and H_2O_2 production, and hexose monophosphate shunt activity (12–14). Furthermore, within 6–20 min, many ingested microbes have been killed (15, 16), and within 1–2 h, extensive degradation can occur (16).

Because of the rapidity of the intracellular response to phagocytosis, and because many enzymes considered necessary for killing (14) and digestion (17) are activated at an acid pH, we considered it important to determine not only the maximum fall in pH within the phagocytic vacuole but also the time course of acid development. Hence we observed the phagocytosis of yeast stained with various indicator dyes in order to define the early sequential changes of pH within the newly formed phagocytic vacuole.

MATERIALS AND METHODS

Materials

Sprague-Dawley rats (Sprague-Dawley, Inc., Madison, Wis.) were used in all experiments. Baker's yeast was obtained from Standard Brands, Chemical Industries Inc., New York. Endotoxin (lipopolysaccharide β, *Salmonella typhosa*) was obtained from Difco Laboratories, Inc., Detroit, Mich. All glassware and slides were acid-washed and thoroughly rinsed in distilled water. The pH of buffer solutions was measured with the Corning pH meter (Corning Glass Works, Corning, N. Y.), using a calomel reference electrode on Ag/AgCl internal electrodes. The pH of exudate fluid was measured anaerobically with a Radiometer capillary pH electrode.

Indicator dyes—bromphenol blue (BPB), bromcresol green (BCG), bromcresol purple (BCP), and neutral red (NR)—were obtained from Fisher Scientific Co., Fair Lawn, N. J. Dyes were chosen so that their dissociation constants (pK) would be on successively lower portions of the pH scale, between pH 3 and 8. The sulfonphthalein dyes (BPB, BCG, BCP) are particularly suitable because they are known to be stable and are less affected by the presence of neutral salts and proteins than many other indicators (5, 18).

Methods

STAINED YEAST SUSPENSIONS: 0.5 g yeast were heat-killed in 10 ml modified (19) Krebs-Ringer phosphate (KRP) buffer, pH 7.4, at 100°C for 60 min, after which 0.05 g indicator dye was added. The solutions were centrifuged, washed with KRP, resuspended at a final concentration of 10^4–10^5/ml, and used within 2 days. Under these conditions, all of the yeast particles were stained.

COLOR CHANGES OF STAINED ORGANISMS: To determine more accurately the pH represented by observed color changes, yeast particles stained with indicator were placed in Michaelis buffer solutions ranging from pH 3.0 to 8.0, and the color changes of the yeast were recorded (Table I). These color changes were reversible, since color indicative of an acid pH reverted to its basic hue when replaced in buffer at pH 7.4.

Production of Exudate and In Vivo Phagocytosis

Each rat (rectal temperature 38°C) was injected intraperitoneally with 1 μg endotoxin in 20 cm^3 sterile isotonic saline to produce an exudate. To obtain an adequate sampling of PMN leukocytes, we induced an acute (4 h) peritoneal exudate contain-

TABLE I

Color of Stained Yeast at Various pH

	Color of organisms			
pH of buffer	Neutral red	Bromcresol purple	Bromcresol green	Bromphenol blue
8.0	Yellow			
7.4	Amber	Blue		
7.0	Red-amber	Blue	Blue	
6.5	Red	Blue-grey	Blue	
6.0	Red	Grey-green	Blue-green	
5.5		Yellow-green	Green	
5.0		Yellow	Green	
4.5			Yellow-green	Blue
4.0			Yellow-green	Blue-green
3.5			Yellow	Blue-green
3.0				Yellow

ing ~15,000–20,000 leukocytes per mm^3, 90% of which were PMN leukocytes. The rat was lightly anesthetized with ether (rectal temperature 39.5°–40°C) and the stained yeast suspension (0.75 ml) was injected intraperitoneally. Exudate was retrieved by gravity drainage through a 16-gauge needle onto a glass slide, which was then enclosed with a glass cover slip and sealed with petrolatum. Samples were obtained at frequent intervals up to 120 min after injection of particles. Some were immediately viewed for brief periods with the light microscope and others were observed continuously for 2 h and occasionally 24 h later. Most experiments were conducted in a warm room (38°C), but a few were performed using a Zeiss heating stage (38.5°C) (Carl Zeiss, Inc., New York).

Microscopy

Cells were examined by bright-field microscopy and photographed at original magnifications of × 400 with a Zeiss photomicroscope (Carl Zeiss, Inc.) equipped with a × 100 planapochromat objective. Kodachrome II daylight film with a blue conversion filter (no. 80A, Eastman Kodak Co., Rochester, N. Y.) was used.

RESULTS

Injection of indicator-stained yeast provided an initial ratio of 1–2 leukocytes per yeast particle, and initiated phagocytosis in vivo. Phagocytosis proceeded rapidly; within 2 min, about 50% of the yeast was within leukocytes. By 7–9 min, about 95% was intracellular. During the entire course of the experiment, all extracellular yeast particles were observed to stain the basic color of the indicator, corresponding to the actual pH of the exudate, which was determined to be 7.35–7.40. Trypan blue (0.025%) did not stain the leukocytes, indicating that the cells remained viable during the usual 2 hr span of the experiment.

Details of our observations with four representative indicators are presented below and are illustrated in Figs. 1–12.

Neutral Red

NR-stained yeast is amber at neutral pH and changes to red at ~pH 6.5 (see Table I). During the first 2 min of observation, all yeast particles, both intracellular and extracellular, were amber. By 3–4 min, a few unmistakably red-amber and red intracellular yeast particles were observed (Fig. 1), denoting an intravacuolar pH in the range of 6.5–7.0 or less. The proportion of intracellular acid-staining yeast steadily increased to a maximum of 70–90% by 15–20 min (Fig. 4). However, some of the intracellular yeast did not change to the acid color during the course of the experiment. Indeed, we occasionally found one cell which had ingested two yeast particles, one staining red and the other amber (see Fig. 1), long after initiation of phagocytosis.

Bromcresol Purple and Green

At physiologic pH, BCP-stained yeast is purple (Fig. 2) and BCG-stained yeast is blue (Fig. 3); they both change to yellow at pH 5 and 3.5, respectively (see Table I). Contrary to the results with NR, there was no change in the color of intracellular yeast during the first 7 min of observation (Fig. 2). By 7–10 min, intravacuolar pH dropped to about 4.5–5.0, manifest by a change to yellow in many intracellular BCP-stained yeast particles (Fig. 5) and a change to green and yellow-green (Figs. 6 and 9) in many of the BCG-stained particles. By 20–25 min, further depression of pH to about 3.5–4.0 had occurred, as demonstrated by a large percentage of yellow (15%), yellow-green (55%), and green (15%) intracellular BCG-stained yeast particles. As mentioned previously, not all of the intracellular yeast changed to the acid color of the indicator; that is, about 15% remained blue (Fig. 6).

Bromphenol Blue

BPB-stained yeast is blue at physiologic pH (Fig. 7) and changes to yellow at pH 3 (see Table I). At no time during the course of the experiment was there a visible change in the color of intracellular yeast, even when ingested yeast was observed at intervals up to 24 h after the onset of phagocytosis (Fig. 8). As time progressed, the intensity of the blue stain decreased slightly (probably reflecting diffusion of the indicator into the medium), but a green or yellow color was never apparent, indicating that intravacuolar pH never dropped to 4 or below.

Additional Studies

RAPIDITY AND DURATION OF PH DEPRESSION: To determine how quickly changes in color occurred within individual phagocytic vacuoles, and the duration of color change, we observed single cells continuously for periods up to 2 h. Several times we visualized the entire se-

quence of phagocytosis, degranulation, and changes in color within a given vacuole. With NR, BCP, and BCG, the change in color from basic to acid hue developed over a period of minutes; that is, there was never an abrupt color change. Once the intracellular particle had turned the acid color, it did not change during the remaining 2 h period of observation.[1]

CHALLENGE WITH PARTICLES DURING TWO SEPARATE COURSES OF PHAGOCYTOSIS: As previously mentioned, not all ingested yeast changed to the acid color, and indeed, it was not unusual to find a PMN leukocyte which had ingested two particles, one acid and the other basic (see Fig. 5). Studies were thus carried out to determine if this reflected the sequence of ingestion, i.e., if the first particle ingested became acid whereas the second particle could not. Initially, unstained yeast particles were injected intraperitoneally and were rapidly internalized. 10 min later, BCG-stained yeast were injected, providing two distinct populations of intracellular yeast. Within 15 min, we observed single cells which contained both unstained yeast and BCG-stained yeast which stained the acid color (yellow-green). This demonstrated ability of PMN leukocytes to acidify the second ingested particle indicates that some explanation other than sequence of ingestion must underlie our previous observation that not all internalized yeast becomes acidic.

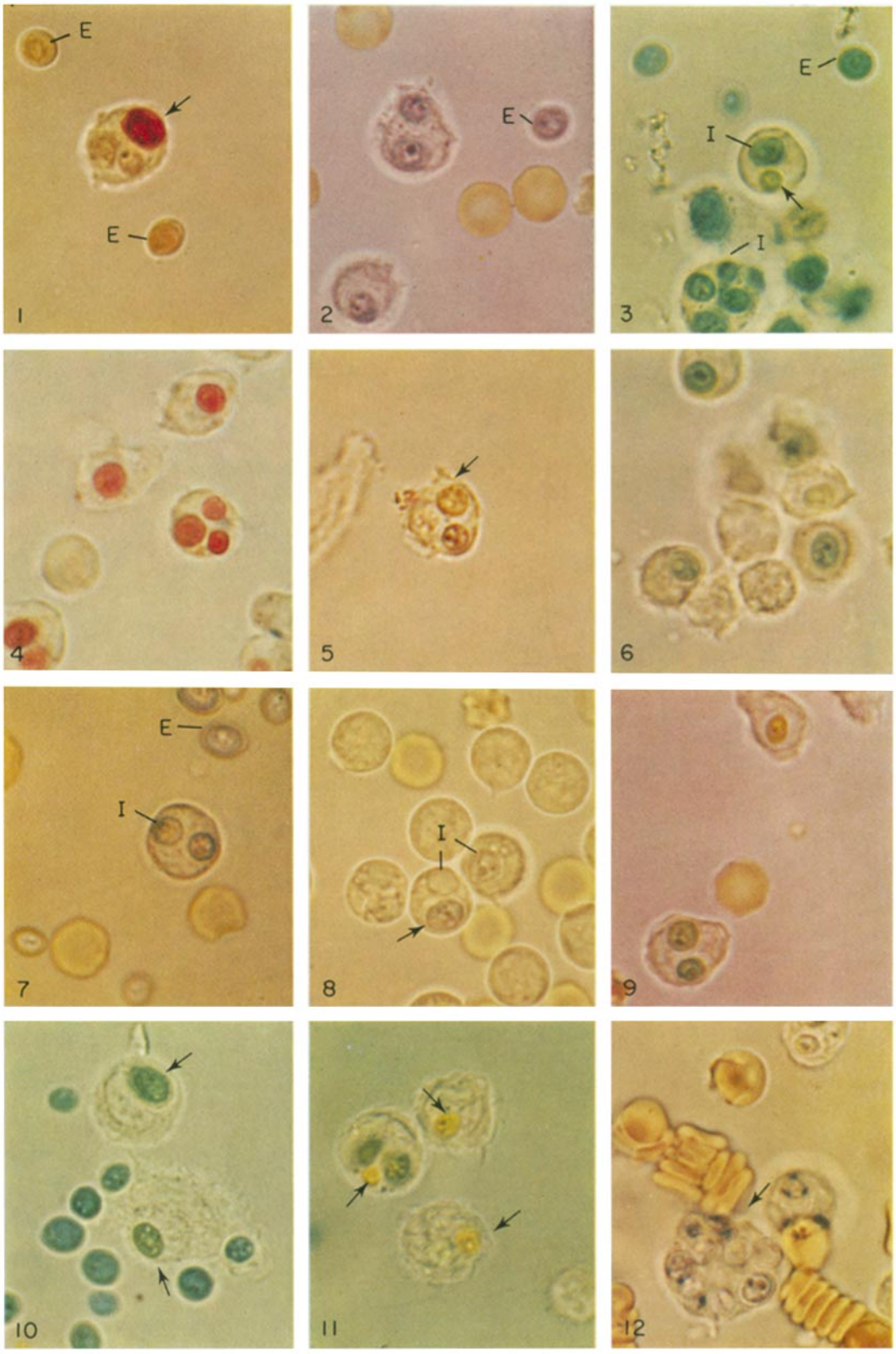
[1] This situation is different from that encountered previously in food vacuoles of certain Protozoa (20), for example, Amoebae and Heliozoa (21), in which there is an initial drop in pH followed by a gradual rise to about pH 7 in 1 h.

FIGURES 1–12 The following abbreviations are used in Figs. 1–12: *E*, extracellular yeast; *I*, intracellular yeast.

All figures are light photomicrographs obtained by bright-field microscopy of rat leukocytes at brief intervals after the initiation of in vivo phagocytosis using yeast stained with various indicator dyes. Figs. 1–9 are PMN leukocytes, and Figs. 10–12 are mononuclear phagocytes. Color changes with time were as follows: (*a*) Figs. 1 and 4. Yeast stained with *neutral red*, in which a color change from amber to red denotes an intravacuolar pH of ~6.5 or lower. No changes were seen before 3 min. Fig. 1 shows a PMN leukocyte after 4 min containing three yeast, two of which are amber and the other bright red (arrow). Two extracellular yeast (*E*) are amber, the color of the indicator at physiologic pH (~7.4). Fig. 4 shows that after 10 min most of the yeast are red, denoting a pH of 6.5 or below. (*b*) Figs. 2 and 5 are yeast particles stained with *bromcresol purple*, and a change from purple to yellow denotes a drop in pH to ~5 or lower. No changes were seen before 7 min. Fig. 2, after 4 min, shows two PMN leukocytes containing purple-staining yeast, one similar extracellular yeast (*E*), and several erythrocytes. Fig. 5, after 15 min, shows two yeast particles in a PMN leukocyte. One is still purple, the basic color of the indicator, and the other has turned yellow (arrow), the acid color (~pH 5). (*c*) Figs. 3, 6, and 9 are yeast particles stained with *bromcresol green*, and a change from blue to yellow-green denotes a drop in pH to 4.0–4.5. No changes were seen before 7–10 min, and by 20–25 min most of the intracellular yeast had turned to yellow-green (55%) and yellow (15%) denoting a pH in the range of 3.5–4.5 or lower. Fig. 3, after 8 min, most of the intracellular yeast particles (*I*) are blue, the basic color, but one particle has changed to yellow-green (arrow) (~pH 4.0–4.5). Figs. 6 and 9, after 15 min, show that many of the intracellular yeast particles have changed to green (~pH 5.0–5.5), yellow-green (4.0–4.5), and yellow (3.5 or lower). One is still blue (pH 6.5 or greater). (*d*) Figs. 7 and 8 are yeast particles stained with *bromphenol blue*, and a change in color from blue to blue-green denotes a drop in pH to 4.0 or lower. No changes were seen at any time, even 24 h after introduction of the stained yeast. Fig. 7, after 4 min, shows intracellular (*I*) and extracellular (*E*) yeast particles, which are blue, the basic color. Fig. 8, 24 h later, shows one intracellular yeast particle is still blue (arrow). Two others (*I*) appear to have faded. No change from blue was observed, indicating that pH did not fall to pH 4.0 or below.

Figs. 10–12 are fields of mononuclear phagocytes ingesting indicator-stained yeast particles to illustrate that temporal and maximal changes in pH are similar to those of PMN leukocytes. Fig. 10, *bromcresol green*, 10 min. Note that the extracellular yeast particles are blue, and two intracellular particles (arrows) are green, denoting a pH of about 5.5. Fig. 11, *bromcresol green*, 15 min. Three intracellular yeast particles are yellow (arrows), denoting an intravacuolar pH of ~3.5 or lower. Fig. 12, *bromphenol blue*, 24 h. No changes from blue were observed even after 72 h. Note that one macrophage contains numerous blue particles (arrow). Red cells in rouleaux are also in the field.

In all cases, the time of injection of the yeast is designated as zero. All extracellular yeast (*E*) stain the color of the indicator at physiologic pH (~pH 7.4). All figures, × 1400.



CHALLENGE WITH PARTICLE OTHER THAN YEAST: In order to test the possibility that the color changes observed above were dependent upon the yeast particle itself, PMN leukocytes were challenged with another substance, azolitmin in 2% agar as previously described by Rous (2, 3). The early changes were difficult to follow because, initially, the phagocytic vacuoles were very small with a faint blue color. After 1 h, however, large pink-red vacuoles were present, indicating that the pH of the vacuole was acid. It was concluded that the color changes previously observed were not dependent upon the presence of the yeast.

CONTROL: It has been reported (18) that many indicator dyes may change color when complexed with protein regardless of the actual pH of the solution. Therefore a control experiment was performed to determine if solubilized PMN leukocyte granule contents could change the color of the indicator-stained yeast particles. Isolated granule fractions (11) were resuspended in Michaelis buffer, pH 7.4, frozen and thawed twice, and 1 ml samples containing 4 mg Lowry protein/ml were mixed with indicator-stained yeast (NR, BCP, BCG) for 10 min at 37°C. All yeast particles stained their physiologic pH (7.4) color, indicating that PMN leukocyte granule contents alone (in this concentration) did not change the color of the indicator.

MONONUCLEAR PHAGOCYTES: Because Rous noted color changes with BPB whereas we did not, we considered the possibility that he was observing mononuclear phagocytes rather than PMN leukocytes, since many of his findings were made in 48- and 72-h exudates. We therefore conducted pilot studies on mononuclear phagocytes. To secure a predominant population of this cell type, we waited 5 days after the endotoxin stimulus and then injected yeast particles into the peritoneum and performed experiments similar to that just described for PMN leukocytes. Mononuclear phagocytes obtained in this manner behaved qualitatively very similarly to PMN leukocytes. Phagocytosis proceeded rapidly, and within 10–15 min yellow and yellow-green intracellular BCG-stained yeast particles were seen, indicating an intravacuolar pH of 3.5–4.0 (see Figs. 10 and 11). Again, BPB-stained yeast particles were never observed to change color (see Fig. 12), even after 72 h. We also extended these studies to the mouse, the animal used by Rous (3), and never found color changes with BPB.

DISCUSSION

Significant Findings

To study the relationship between acidification, degranulation, and the metabolic changes accompanying phagocytosis in PMN leukocytes, we concentrated on the early changes in pH within the phagocytic vacuole immediately after the phagocytic event. In this respect, these experiments differ from the previous experiments of Rous, Sprick, and others (2–7), who concentrated on maximal depression of pH at later times. Our findings, obtained by using indicator-stained yeast, demonstrate that the pH of the phagocytic vacuole is measurably decreased to ~6.5 within 3–4 min after the onset of phagocytosis and approaches the 3.5–4.5 range after 7–15 min. In the majority of phagocytic vacuoles (70–90%), pH was observed to be in this range within 15–20 min, and was found to be constant for at least 2 h.[2] An enigma we cannot explain is the presence of intracellular yeast particles which did not stain the acid color even after 2 h; similar findings have been reported by earlier investigators (5, 7). In addition, we have demonstrated that changes in pH of the phagocytic vacuoles of mononuclear phagocytes are similar to those seen in PMN leukocytes.

Correlation of Acidification with Time of Activation of PMN Leukocyte Granule Enzymes

The finding that intravacuolar acidity is increased within a few minutes after the phagocytic event is of particular importance in respect to the degranulation phenomenon and the function of PMN leukocyte granule enzymes. Rabbit PMN leukocytes are known to contain two types of chemically distinct granules (23–30),[3] azurophils (25%) and specifics (75%) (see Table II). The predominant granule type, the specific granule,

[2] It should be noted, however, that yeast is not rapidly digested by PMN leukocytes (22), and it is possible that the pattern of acidity might vary if other, more rapidly digested microorganisms were studied.

[3] It should be clarified that all of the following work on PMN leukocyte granules was performed on the rabbit. Although comparable data on the rat do not exist, our preliminary analysis of developing rat PMN leukocytes indicates similar granule populations.

contains *alkaline phosphatase* (23–28), the function of which is unknown; *lysozyme* (26, 27), an aminopolysaccharidase which degrades the bacterial cell wall and causes lysis of a few bacterial species; and *lactoferrin* (29), a chelator of iron and copper. Lactoferrin has been suggested to be synergistically involved with the function of lysozyme, since the lytic activity of lysozyme has been reportedly increased and is effective in killing bacteria which are insensitive to lysozyme in the absence of chelators (30). Interestingly, the known contents of specific granules function best at near-neutral or alkaline pH (26, 29, 30, 31). In contrast, many of the contents of the azurophil granule, such as peroxidase and lysosomal enzymes (24–29), have an optimal pH in the acid range. A considerable number of the lysosomal hydrolases are completely inactive above pH 6.0, and several (e.g., hyaluronidase) are largely inactive above pH 4.5 (17, 32, 33). Peroxidase, demonstrated by the recent work of Klebanoff to be a potent bactericidal (34) and virucidal (35) enzyme, has a pH optimum of about 4.5.[4]

Recent evidence from this laboratory (41, 42) indicates that in rabbit PMN leukocytes, specific granules empty into the phagocytic vacuole before azurophils. These data, obtained by electron microscope cytochemistry, indicate that alkaline phosphatase, used as a specific granule marker, is detectable within the phagocytic vacuole within 30 s after ingestion. On the other hand, peroxidase reaction product, used as a marker for the azurophil granule, has not been observed in phagocytic vacuoles at 30 s, is only occasionally seen at 1 min, but is often present after 3 min. Thus our new findings demonstrate that before 3 min, when the pH of the phagocytic vacuole is near neutral, the contents of the specific granule are present and are probably activated (see Table II). Later, between 3–15 min, when the pH is rapidly changing to ~4, azurophil granule contents are also present, and may also be activated. Hence pH change roughly parallels the sequence of granule discharge, providing conditions which would allow for coordinated granule interaction. Other possible consequences of this interaction are that after activation, lysosomal enzymes may digest or alter the other granule contents, or that pH may affect the solubility of various granule contents (43). Finally, it should be mentioned that many species of microorganisms (such as pneumococci) die rapidly when exposed to an acid reaction alone, as discussed by Dubos (44).

TABLE II

Time of Changes in Content and pH of Phagocytic Vacuoles

Early (30 sec–3 min), pH 7.4–7.0 →	Late (after 3 min), pH rapidly changes to 4–5 range
Specific granule content*	Plus azurophil granule content*
1. Alkaline phosphatase (pH 9.4)	4. Lysosomal enzymes (pH 4–5)
2. Lactoferrin (stable at pH 7.4–3.5)	5. Peroxidase (pH 4.5)
3. Lysozyme (pH 7.5)	6. Lysozyme (pH 7.5)
	7. Acid mucopolysaccharide

* With pH optimum of each enzyme

Mechanism(s) of pH Change

The mechanism of intravacuolar pH depression is unknown, but a number of possibilities have been proposed (see reference 32) as follows: (*a*) increased lactic acid formation as a consequence of the metabolic burst that accompanies phagocytosis; (*b*) a change in the Donnan equilibrium; (*c*) the presence of H^+ pumping ATPase; or (*d*) a redox pump. In regard to these various possibilities, Kakinuma (13) demonstrated measurable lactic acid production in phagocytizing PMN leukocytes of the guinea pig within 5 min. While our results correlate well with his findings, it must be recognized that there is no direct evidence that lactic acid is actually present within the phagocytic vacuole. Furthermore, only small increments of lactic acid production have been detected after phagocytosis by rat PMN leukocytes (45).

Coffey and de Duve (46) consider it likely that a certain degree of acidity is normally maintained

[4] Other enzymes known to be present in PMN leukocyte granules but which have not yet been localized to a given granule type are RNase, DNase, cathepsin (11), and lipases (37), all of which have pH optima in the range of 4.5–4.8; and collagenase (38) and elastase (39), with pH optima above 7. Recently, pilot studies of Davies et al. (40) suggest that a neutral protease, with a pH optimum of 7.0–7.5, is located in azurophils. Also, Zeya and Spitznagel have reported the presence of cationic antibacterial proteins in azurophil granules (36), although Baggiolini et al. (26) found phagocytin (citric acid extracts with bactericidal action active at pH 5.6) in both azurophil and specific granule fractions.

TABLE III

Previous Observations on Maximal pH Depression in Phagocytic Vacuoles

Investigator	Maximal fall in pH	Change with BPB	Animal	System
Rous (2, 3)	3.0	+	Mouse	in vivo
Sprick (5)	4.7–5.2	−	Guinea pig	in vivo
Mandell (7)	6.0–6.6	−	Human	in vitro
Jensen and Bainton	3.5–4.5	−	Rat, mouse, rabbit	in vivo

within secondary lysosomes, at least inside those that are sites of digestive phenomena. They have further hypothesized that hydrogen ion segregation may be simply a consequence of a Donnan equilibrium; that is, a differential distribution of charged particles due to the presence of a nondiffusable substance, such as acid mucopolysaccharide, on one side of a semipermeable membrane. As we have already stated, azurophil granules, which contain an acid mucosubstance (47), are present within the phagocytic vacuole by 3 min, and therefore must be considered as a candidate responsible for this change in acidity. Certainly, further work is necessary to clarify the mechanism(s) involved in acidification of phagocytic vacuoles.

Maximal Depression of pH of the Phagocytic Vacuole

Previous studies involving measurement of the maximal pH depression of the phagocytic vacuole using indicator dyes have resulted in interpretations of intravacuolar pH widely varying from 6.0–6.5 (7) to as low as 3.0 (2, 3). Basically, our results are in agreement with those of Sprick (5) and others (4, 6) (see Table III). These workers concluded that the phagocytic vacuole pH in PMN leukocytes is in the general range of 4–5, and were unable to confirm the observations of Rous, who alone noted a change to yellow with BPB, indicating that the pH may be as low as 3. It should be remarked that there were some variations among our own results using the various dyes. Specifically, BPB-stained yeast particles were never observed to change from their basic blue color, indicating that the pH did not go as low as 4.0; whereas some BCG-stained yeast turned yellow, suggesting a pH of 3.5–4.0 or below (see Table I). This emphasizes the generally recognized fact that indicators do not permit a precise determination of pH (18); they are accurate, however, to about ±0.5 pH units (as indicated in Table I).

Other reasons for the variation in results obtained by previous investigators may be due to differences in the species used, the nature of the particle ingested, and whether the studies were performed in vitro (7) or in vivo (2–6) (see Table III). Our own limited studies using stained yeast particles in vivo yielded results in the rabbit and mouse similar to those reported here in the rat in vivo. In the future, we plan to compare in vivo and in vitro systems using the same particle and the same species.

Although it has long been appreciated that the pH of the phagocytic vacuole becomes acid, our work emphasizes the importance of changes in pH in relationship to the function of PMN leukocyte granule enzymes. Since it is possible that antibacterial and lysosomal substances could be present in normal quality and quantity but may not be functional if the intravacuolar pH fails to become acid, temporal changes in pH and maximal pH depression should be investigated in studies of intraleukocytic microbicidal mechanisms, in addition to the other morphologic and biochemical parameters (see reference 14) now employed.

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