

## Ascorbic Acid Levels in Phagocytic Cells<sup>1</sup> (37974)

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The human peripheral polymorphonuclear leukocyte (PMNL) has long been known to contain a relatively high concentration of ascorbic acid (1). The function of this vitamin has not been established in this cell, although it has been suggested that ascorbate might play a significant role in the intracellular killing of bacteria (2, 3). The present investigation compares the ascorbate content of leukocytes (both PMNL and alveolar macrophages) from several species to assess whether this postulated killing mechanism might have general applicability.

*Materials and Methods.* Human polymorphonuclear leukocytes were isolated from the venous blood of volunteer subjects by sedimentation with plasma gel (HTI Corp., Buffalo, NY) as previously described (4). Cells were obtained from both normal controls and from patients with a variety of hematologic disorders. The cells were washed once with 0.9% saline and subjected to hypotonic lysis to remove the contaminating red cells. Isotonicity was restored after 20 sec by the addition of hypertonic saline and the cell concentration determined in a counting chamber under phase microscopy. Smears of the isolated cells showed greater than 85% to be polymorphonuclear leukocytes. The cells were centrifuged at 500g and the resulting pellet was suspended in 5% trichloroacetic acid (TCA) to give a nominal concentration of  $1 \times 10^8$  cells/ml. The suspension of cells in TCA was subjected to ultrasonic disruption

to ensure complete cell lysis. The sonicated extract was centrifuged for 30 min at 27,000g to remove precipitated protein, and the clear supernate was assayed for ascorbic acid.

Guinea pig and rabbit peritoneal PMNL were harvested 14-16 hr following the intraperitoneal injection of sterile 12% casein (5). The cells were treated as described for the human PMNL. Following the enumeration of cells in the counting chamber, an aliquot was removed for protein analysis in some cases. The cells were collected by centrifugation at 500g and the packed cell volume was recorded. The cells were then suspended in 5% TCA, sonicated, and centrifuged as described above. Smears of the isolated cell preparations demonstrated that greater than 90% of the cells were PMNL.

Alveolar macrophages were harvested from the lungs of New Zealand white rabbits by the lavage technique of Myrvik *et al.* (6). Both normal controls and animals which had been vaccinated by the injection of heat-killed *Baccillus-Calmette-Guerin* (BCG) were used as the source of cells (7). The macrophages were subsequently treated in the same manner as that described for peritoneal PMNL. Greater than 95% of the cells isolated from normal animals were observed to be macrophages; 75-85% of the cells obtained from BCG-treated animals were macrophages, the great bulk of the contaminating cells being lymphocytes.

The levels of ascorbate in the TCA extracts were determined by the method of Denson and Bowers (8). Each extract was assayed in duplicate at two concentrations

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TABLE I. Levels of Ascorbic Acid in Human Peripheral PMNL from a Variety of Cell Donors.

Diagnosis	Number <sup>a</sup>	Leukocyte ascorbic acid $\mu\text{g}/10^8$ cells
Normal control	15	11.3 (7.3-20.1) <sup>b</sup>
Polycythemia vera	4	11.1 (8.7-16.9)
Chronic granulocytic leukemia	5	10.1 (7.5-14.3)
Chronic granulomatous disease	1	15.9

<sup>a</sup> This represents the number of different cell samples assayed. Each assay represents the mean of 4 determinations.

<sup>b</sup> Numbers in parentheses represent the range.

(0.50 and 1.00 ml); standards containing 0.00, 2.50, and 5.00  $\mu\text{g}$  ascorbate were run with each assay. A standard curve relating absorbance to concentration demonstrated linearity up to 10  $\mu\text{g}$  of ascorbate.

The protein concentration of the disrupted cell preparations was determined by the biuret procedure of Gornall *et al.* (9).

**Results.** The data in Table I illustrate the ascorbic acid content of PMNL obtained from a number of individuals with hematologic diseases. None of the patients was infected at the time of the study and all had essentially normal differential counts. The leukocytes of four patients with polycythemia vera and of five patients with chronic granulocytic leukemia contained essentially normal levels of ascorbic acid as did the leukocytes of one patient with chronic granulomatous disease of childhood when compared with leukocytes from 15 normal controls.

The levels of ascorbic acid in various types of phagocytic cells are compared in Table II. All cell types examined have a relatively high concentration of this compound. The macrophage appears to have an especially high concentration based on cell count, but this is deceptive because of the much greater size of this cell. If the concentration is based on packed cell volume or mg of cell protein rather than simply cell number, the concentration of ascorbate in the alveolar macrophage is much closer to that observed in the PMNL.

There is an apparent decrease in the concentration of ascorbate in the BCG-induced alveolar macrophage as compared with normal, but this again probably reflects changes in size and protein content of the cell. The induced macrophage is larger than normal and contains more protein (10); this results in an apparent decrease in ascorbate concentration when either of these param-

TABLE II. Levels of Ascorbic Acid in Various Types of Phagocytic Cells.

Cell type	Number <sup>a</sup>	Ascorbic acid concentration		
		$\mu\text{g}/10^8$ cells	$\mu\text{g}/\text{ml}$ PCV <sup>b</sup>	$\mu\text{g}/\text{mg}$ protein
Casein-induced guinea pig peritoneal PMNL	21	3.8 (1.9-8.2) <sup>c</sup>	N.D. <sup>d</sup>	N.D.
Normal rabbit	14	54.7 (38.0-82.1)	231 (164-330)	2.2 (1.4-3.3)
BCG-induced rabbit	12	47.1 (25.0-75.3)	112 (71-158)	1.1 (0.6-1.7)
Casein-induced rabbit peritoneal PMNL	6	9.6 (8.0-14.1)	101 (43-154)	1.2 (0.6-1.6)

<sup>a</sup> This represents the number of samples assayed. Each sample was assayed in quadruplicate.

<sup>b</sup>  $\mu\text{g}$  ascorbic acid/ml packed cell volume.

<sup>c</sup> Numbers in parentheses represent the range.

<sup>d</sup> N.D.: not determined.

eters is used to assess the concentration. The ascorbate content of both normal and BCG-induced rabbit alveolar macrophages is similar when calculated on a basis of  $\mu\text{g}/\text{cell}$ .

*Discussion.* Our average value for the ascorbate concentration in the human PMNL ( $11.3 \mu\text{g}/10^8$  cells) is somewhat lower than that generally reported in the literature. Hume and Weyer (11) reported an average value of  $18.9 \mu\text{g}/10^8$  cells, while Loh (12) cited a value of  $25.2 \mu\text{g}/10^8$  cells for a group of 39 university students. Denson and Bowers (8) reported variations in ascorbate content depending on age and ranging from a mean of  $13.4 \mu\text{g}/10^8$  cells in older populations to one of  $35 \mu\text{g}/10^8$  cells in younger individuals. Others have reported values as low as  $6.0 \mu\text{g}/10^8$  cells (13). All reports show a wide range of individual variation and such factors as age, sex, diet, and season of the year may affect the leukocytic levels of this vitamin. It is possible that our values are somewhat low because of leakage during the washing and lysis procedures which were employed to ensure a relatively homogeneous preparation, and the true concentrations might be somewhat higher. No significant differences in ascorbate content were observed between the control cells and those obtained from patients with polycythemia vera, chronic granulocytic leukemia, or from one patient with chronic granulomatous disease of childhood.

It is apparent from the data in Table II that all types of phagocytes examined contain a relatively high concentration of ascorbate. For example, the rabbit PMNL contains 100–200  $\mu\text{g}$  ascorbate/ml of cell water, while the rabbit alveolar macrophage contains 200–400  $\mu\text{g}/\text{ml}$  of cell water. The serum concentration of ascorbate in the same animal is reported to be 9–14  $\mu\text{g}/\text{ml}$  (14) and so the cells actively concentrate the vitamin by a factor of 10–40-fold. Guinea pig PMNL apparently contain only about one-third as much ascorbate as the rabbit PMNL. This is consistent with the observation that guinea pig serum contains only 1–3  $\mu\text{g}/\text{ml}$  ascorbate and thus the guinea pig cell is about as efficient as the

rabbit PMNL in concentrating ascorbic acid.

The primary function of phagocytic cells involves the ingestion and subsequent killing of invading bacteria. In the PMNL, phagocytosis is accompanied by increases in  $\text{O}_2$  consumption, hexose monophosphate shunt activity, and hydrogen peroxide production (15). Although the exact microbicidal reactions are unknown, they are thought to involve, at least in part, the generation of hydrogen peroxide which can react with the enzyme myeloperoxidase to either iodinate the bacterium (16) or to generate the bactericidal aldehydes from amino acids (17). This situation is even less clear in the case of the alveolar macrophage. Although this cell has been shown to respond to phagocytosis by producing hydrogen peroxide (18, 19), its ability to iodinate bacteria or to generate aldehydes is questionable because of the relatively low concentration of peroxidase in the cell (18, 20). An alternate pathway of bactericidal activity involving ascorbic acid has been proposed for the PMNL. This compound has been observed to increase the oxidative metabolism of resting cells in a manner analogous to that seen upon phagocytosis (3). Further, Miller has demonstrated that the combination of ascorbate and  $\text{H}_2\text{O}_2$  is a potent bactericidal mixture at a concentration of ascorbate which is readily achieved within the PMNL (2). The present demonstration that similar levels of ascorbate exist in the phagocytes of cells from several species and in alveolar macrophages as well as PMNL makes the ascorbate-mediated reactions generally applicable.

Intraleukocytic levels of ascorbate do not appear to play a significant role in several hematologic diseases, however. Leukocytes from patients with chronic granulocytic leukemia may have a slight functional defect (21), while cells from patients with chronic granulomatous disease show a profound defect in their ability to destroy certain types of bacteria (22). No significant deviation from normal leukocyte ascorbate levels was observed in five patients with chronic granulocytic leukemia or in our one patient with chronic granulomatous disease (23), and

thus the functional impairment cannot simply be ascribed to a leukocyte ascorbate deficiency. Similarly, although the leukocytes of patients with polycythemia vera have an increased oxidative metabolism (24), this cannot be explained on the basis of ascorbate levels within the cell. Thus, although it is possible that ascorbate is involved in the bactericidal process, it appears unlikely that intraleukocytic levels of this compound play an important role in the human hematologic diseases examined.

*Summary.* The concentration of ascorbic acid was determined in guinea pig peritoneal PMNL, normal rabbit alveolar macrophages, BCG-induced rabbit alveolar macrophages, rabbit peritoneal PMNL, and human circulating PMNL. The high levels of ascorbate seen in all cell types examined are consistent with a role for this compound in the bactericidal activity of the phagocytes. No significant differences from normal were observed in leukocytes from patients with chronic granulocytic leukemia, polycythemia vera, or in one patient with chronic granulomatous disease.

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