O-methyl transferase, with optimum concentration of *S*-adenosyl methionine, the methyl donor, indicate that the daytime activity of this enzyme is several times higher than the daytime activity of pineal *N*-acetyltransferase. On the basis of these measurements, it seems that *N*-acetyltransferase is the rate-limiting enzyme in melatonin synthesis during the day. At night, when *N*-acetyltransferase activity increases 10- to 30-fold, it seems probable that the rate of production of *N*-acetylserotonin exceeds the maximum possible rate of *O*-methylation. Although a small diurnal rhythm in hydroxyindole-*O*-methyl transferase has been observed (*19*), the highest activity reported (*18*) does not exceed the activity of *N*-acetyltransferase at night. This situation might result in the accumulation of *N*-acetylserotonin followed by a gradual conversion to melatonin. Hydroxyindole-*O*-methyl transferase would regulate the rate of production of melatonin under these conditions. The amount of melatonin produced during one 24-hour period, however, would be limited by the amount of *N*-acetylserotonin synthesized by *N*-acetyltransferase.

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Myeloperoxidase: Contribution to the Microbicidal Activity of Intact Leukocytes

Abstract. *Azide and, to a lesser extent, cyanide inhibit the microbicidal activity of myeloperoxidase and of intact normal leukocytes, but they have little or no effect on peroxidase-negative leukocytes. The contribution of the azide-sensitive (peroxidase-dependent?) systems to the total microbicidal activity of normal leukocytes is considerable. The azide-insensitive antimicrobial systems are more highly developed in peroxidase-negative leukocytes than in normal leukocytes, thus suggesting an adaption.*

The importance of intact intraleukocytic antimicrobial systems in the host defense against invading microorganisms is emphasized by the marked increase in susceptibility to infection in patients with severe neutropenia or with functionally abnormal granulocytes (such as in chronic granulomatous disease of childhood). After phagocytosis, rupture of the leukocyte granules occurs with the release of their contents into the phagocytic vacuole (*1*). Among the granular contents are lysozyme (*2, 3*), cationic proteins (*4*), and myeloperoxidase (*3, 5*); all of these have antimicrobial properties. There also is a fall in intravacuolar *p*H (*6*) and the production of H_2O_2 by the cell (*7*). The antimicrobial effect of H_2O_2 is increased considerably by myeloperoxidase and an oxidizable cofactor such as iodide, bromide, chloride, or thiocyanate ions (*8–11*).

The complex nature of the leukocytic antimicrobial systems raises the following questions. Do all the systems described function in the intact cell? Does one antimicrobial system predominate, and, if so, does this system vary with the microorganisms or the functional state of the leukocyte? Do the leukocytes have an overkill capacity and thus a reserve against the decrease or loss of one or other of the antimicrobial systems? Do intrinsic control mechanisms provide a means for increasing the efficiency of one antimicrobial system when a second system is defective? Although definitive answers to these questions cannot be given at the present time, an evaluation of the contribution of myeloperoxidase-mediated antimicrobial systems is possible because of the availability of a patient with a genetic absence of myeloperoxidase (*12*) and of inhibitors of peroxidase-catalyzed reactions such as azide or cyanide.

Heparinized blood was obtained from normal volunteers, from two male patients with chronic granulomatous disease, and from a patient with a genetic absence of myeloperoxidase (*13*). The organisms were grown, washed, and incubated with intact leukocytes or with the isolated myeloperoxidase-mediated system, and the viable cell count and extent of phagocytosis was estimated (*9, 14*).

Azide has a marked inhibitory effect on the killing of *Lactobacillus acidophilus* by normal leukocytes (Table 1). Comparable results were obtained with *Staphylococcus albus* and *Candida tropicalis*. Cyanide also was inhibitory although less so than azide. An inhibition of the fungicidal activity of leukocytes by cyanide has been reported (*15*). Azide and cyanide did not inhibit phagocytosis, and they were not microbicidal under the conditions employed. Azide and cyanide form complexes with the iron of heme-containing enzymes and, as a result, enzyme activity is lost. Myeloperoxidase is a hemeprotein, and the inhibitory effect of azide and cyanide on the microbicidal activity of myeloperoxidase and iodide ions at *p*H 5.0 is shown in Table 1. The addition of H_2O_2 was not required since *L. acidophilus* is a H_2O_2-producing organism. Azide was a more effective inhibitor than cyanide in the cell-free system, as was observed with intact cells.

The leukocytes of patients with chronic granulomatous disease have an impaired ability to kill certain microbial species (*16*), whereas other organisms

(such as streptococci, lactobacilli, pneumococci) are readily killed by these cells (*14, 17*). Chronic granulomatous disease leukocytes stain normally for peroxidase; however, H_2O_2 formation following phagocytosis is decreased (*18*). This defect may be responsible, in part at least, for the impairment in microbicidal activity since the introduction into the cell of a H_2O_2-generating system results in an increase in functional capacity (*14, 19*). The effect of azide and cyanide on the microbicidal activity of leukocytes from patients with chronic granulomatous disease was comparable to the effect on normal leukocytes when *L. acidophilus*, a H_2O_2-generating organism, was employed; that is, azide and cyanide decreased microbial killing.

Five patients with a genetic absence of myeloperoxidase have been described (*12, 20*). Lehrer *et al.* (*12*) have reported that the leukocytes of their patient had decreased fungicidal and bactericidal activity. Our results are in essential agreement; *S. albus, L. acidophilus*, and *C. tropicalis* were killed less well by peroxidase-negative leukocytes than by normal leukocytes over a 3-hour period (Fig. 1). Phagocytosis by peroxidase-negative leukocytes is normal (*12*). Azide (or cyanide) had little or no effect on the microbicidal activity of peroxidase-negative leukocytes in contrast to the inhibitory effect on normal leukocytes (Fig. 1). The loss of microbicidal activity on the addition of azide to normal leukocytes was greater than the loss resulting from a genetic absence of myeloperoxidase. As a result, the peroxidase-negative leukocytes were more efficient microbicides than normal leukocytes in the presence of azide.

Table 1. Effect of azide and cyanide on the microbicidal activity of intact leukocytes and the isolated myeloperoxidase-iodide system. The organism was *L. acidophilus*. The myeloperoxidase-iodide system consisted of myeloperoxidase, 15 units; iodide, $1 \times 10^{-6}M$; and sodium lactate buffer at *p*H 5.0, 0.6*M*. Azide or cyanide were added in the concentrations indicated. Incubation period, 1 hour.

Antimicrobial system	Inhibitor (*M*)	Viable cell count (10^6 organism/ml)	
		Azide	Cyanide
None	0	3.6	3.6
Intact leukocytes	0	0.004	0.004
	10^{-3}	1.6	0.3
	10^{-4}	1.8	0.008
	10^{-5}	0.6	0.004
	10^{-6}	0.02	0.003
Myeloperoxidase + iodide	0	0.17	0.17
	10^{-3}	3.4	1.7
	10^{-4}	4.0	0.93
	10^{-5}	3.9	0.19
	10^{-6}	0.16	0.11

These data suggest that the antimicrobial systems of normal leukocytes can be divided into two categories: azide-sensitive and azide-insensitive. The following evidence suggests that the azide-sensitive antimicrobial systems are, in large part, dependent upon myeloperoxidase. (i) Azide and cyanide inhibit the myeloperoxidase-mediated antimicrobial systems, and azide is a more effective inhibitor than cyanide in both the intact cell and the cell-free system (Table 1) (*9, 10*). (ii) Azide and cyanide inhibit the iodination of microorganisms by myeloperoxidase, iodide, and H_2O_2 (*9*). Iodination occurs in intact normal leukocytes after the ingestion of bacteria (*9*), and this cell-associated iodination also is azide- and cyanide-sensitive (*11*). Myeloperoxidase-negative neutrophils do not iodinate well (*21*), which suggests a peroxidase requirement for the iodination reaction in intact cells.

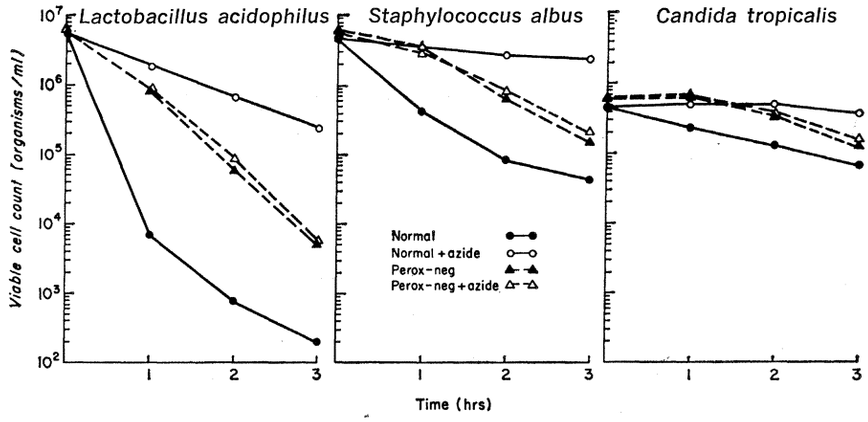

Fig. 1. Comparison of normal and peroxidase-negative leukocytes. Azide concentration, $1 \times 10^{-3}M$. Mean of six experiments.

Cyanide increases the accumulation of H_2O_2 by guinea pig leukocytes (*22*), and cyanide and azide increase oxidation of [1-C^{14}]glucose by rat leukocytes (*23*) after phagocytosis presumably by decreasing H_2O_2 utilization through the inhibition of hemeproteins, including myeloperoxidase. These studies suggest that intracellular myeloproxidase-dependent reactions can be inhibited by azide or cyanide. (iii) Azide and cyanide have little or no effect on the microbicidal activity of peroxidase-negative leukocytes (Fig. 1). The presence of an azide-sensitive antimicrobial system in leukocytes from patients with chronic granulomatous disease is apparent when a H_2O_2-generating organism such as *L. acidophilus* is used. The nature of the azide-insensitive antimicrobial systems in normal leukocytes is unknown. Acid, H_2O_2 (acting without myeloperoxidase), lysozyme, or cationic proteins may be involved. The increase in H_2O_2 concentration on the inhibition of hemeproteins (*22*) is of particular interest in this regard in view of the antimicrobial activity of H_2O_2 at relatively high concentrations in the absence of myeloperoxidase and the possibility of a control mechanism by which a decrease in the microbicidal activity of the peroxidase systems is offset, in part, by an increase in H_2O_2 concentration.

If the inhibition of the microbicidal activity of normal and chronic granulomatous disease leukocytes by azide is due in large part to the inhibition of myeloperoxidase, then the contribution of myeloperoxidase to the total microbicidal activity of these cells is considerable. Yet, of the five patients described with a genetic absence of myeloperoxidase, only one had an increased susceptibility to infection, in this instance to *Candida albicans*; and even this patient reached adulthood without serious problems (*12*). How can this discrepancy be explained? One possibility is that the leukocyte, by virtue of a large overkill capacity, can perform its function adequately under most circumstances despite the loss of one of its antimicrobial systems with the associated decrease in total microbicidal potential. A second possibility is that the patient with a genetic absence of myeloperoxidase has adapted to this condition with an increase in the activity of the nonperoxidase antimicrobial systems of his leukocytes. The finding that the azide-insensitive microbicidal activity of the peroxidase-negative leukocytes is

greater than that of normal leukocytes suggests that this may be the case. It further suggests that a patient suddenly deprived of his peroxidase-mediated antimicrobial systems may be less able to combat infection than a patient with a genetic absence of myeloperoxidase.

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Cyclic Cytidine 2′,3′-Phosphate: Molecular Structure

Abstract. *Monoclinic crystals of the sodium salt of cytidine 2′,3′-phosphate contain two anions in the asymmetric unit. Both bases are in the* syn *conformation, and the nucleotides are stacked together into an antiparallel stranded ribbon with the bases 3.3 angstroms apart. One ribose ring is planar, and the other has oxygen-1′ puckered toward carbon-5′. The phosphorus atoms in the five-membered ester rings are puckered toward the sugars. The conformations about the carbon-4′—carbon-5′ bonds are* gauche-trans *and* gauche-gauche.

Pyrimidine ribonucleotides containing a cyclic 2′,3′-phosphate ester linkage are intermediates in the hydrolysis of ribonucleic acid, a reaction catalyzed by ribonuclease (*1, 2*). The cyclic phosphate ester is then further hydrolyzed to give pyrimidine 3′-phosphate and purine oligo nucleotides. The crystal structures of ribonuclease A (*3*) and ribonuclease S (*4*) are being studied at high resolution, and these structures taken in conjunction with the biochemical studies (*1*) should permit the proposal of precise mechanisms for ribonuclease action. No detailed molecular structures have been reported for the cyclic phosphate esters involved in this process; therefore the results of this study of the crystal structure of the sodium salt of cyclic cytidine 2′,3′-phosphate (2′,3′-CMP) will be useful in evaluating hydrolysis mechanisms.

Crystals of the sodium salt of 2′,3′-CMP (Schwarz BioResearch; lot No. 6802) were prepared by diffusing ethanol into an aqueous solution of the salt over a period of several weeks. The crystals were clusters of clear, thin, strongly birefringent plates which tended to stack together. The crystals are monoclinic, space group $P2_1$, with cell dimensions: a, 6.736 ± .005 Å; b, 11.01 ± .01 Å; c, 19.54 ± .01 Å; and β, 95° ± 0.1°. The measured flotation density (1.66 g/cm^3) suggests four molecules of sodium 2′,3′-CMP and eight molecules of water per cell ($d_{calc} = 1.67$ g/cm^3); there are thus two anions per asymmetric unit. The x-ray diffraction data were collected on a General Electric goniostat with a single crystal orienter with copper radiation. The refinement was based on 1964 nonzero intensities with 2θ less than 125°. These data were collected when the indoor relative humidity was 10 percent or greater; at lower humidities reversible intensity changes were evident, presumably reflecting the loss of some loosely bound water molecules.

One phosphate group and the second unique phosphorus were located in the cell by inspection of a sharpened three-dimensional Patterson synthesis. The phase angles calculated with the use of these atomic positions were refined using the tangent formula (*5*), and a Fourier map based on the refined phases and observed amplitudes revealed the main features of the structure. The trial structure was refined by the method of least squares, with each of the 46 atoms assigned an isotropic temperature parameter. Three cycles of refinement reduced the agreement index (R) to 8.5 percent. The structure described here is that reflected by the coordinates from the isotropic refinement. The estimated errors in bond lengths are ±.02 Å and ±1° in bond angles. We are continuing refinement but most of the unusual features of the structure are clear now. A difference Fourier synthesis is being examined for hydrogen atom peaks; no unexplainable features are evident in this map, and there are no indications of disorder.

From a diagram (Fig. 1) of the 2′,3′-CMP anions looking approximately down the b axis, two features of the structure are apparent. First, the bases are aligned parallel to one another, roughly 3.3 Å apart, and the anions form an anti-parallel stranded base-stacked ribbon. This suggests stabilization of the crystal structure through hydrophobic interactions of the bases; such interactions contribute significantly to the stability of nucleic acid helices (*6*) and are often found in crystal structures of purine derivatives. This stabilization is not as common with pyrimidines; for example, the pyrimidine nucleosides 5-chlorouridine (*7*) and 5-bromouridine (*8*) form