

## Stimulation of Leukocyte Hexose Monophosphate Shunt Activity by Ascorbic Acid

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Ascorbic acid *in vitro* markedly stimulates the hexose monophosphate shunt in normal human neutrophils, rabbit alveolar macrophages, and in neutrophils from a patient with chronic granulomatous disease.

During the phagocytic and bactericidal activities of neutrophils and macrophages, there is a significant increase in the utilization of glucose through the hexose monophosphate shunt (6). The hexose monophosphate shunt activity is not necessary for phagocytosis, and the relation of the temporal sequence of this metabolic burst after phagocytosis to the bactericidal activity of the cell is uncertain. The absence of this increment, however, has been associated with a decrease in the bactericidal capacity of the neutrophil in several instances. In chronic granulomatous disease, an entity characterized by recurrent bacterial infections, phagocytosis is not accompanied by the expected increases in the hexose monophosphate shunt, the utilization of oxygen, and the production of hydrogen peroxide (3). A deficiency in reduced nicotinamide dinucleotide oxidase may be responsible for these defects (1). A patient has been described with recurrent bacteremia, whose neutrophils had defective bactericidal activity *in vitro*, which correlated with the absence of an increase in the hexose monophosphate shunt after phagocytosis; the defect was due to a complete absence of leukocyte glucose-6-phosphate dehydrogenase (2). A deficiency of glutathione peroxidase in human neutrophils also has been associated with abnormal hexose monophosphate shunt activity and defective bactericidal activity *in vitro* (4).

Ascorbic acid, a strong reducing agent, causes a significant increase in the hexose monophosphate shunt activity of red blood cells, perhaps through the production of hydrogen peroxide (5). This communication describes the marked stimulation of the hexose monophosphate shunt by ascorbic acid in normal human neutrophils, normal rabbit alveolar macrophages, and in the leukocytes from a patient with chronic granulomatous disease.

Leukocytes were isolated from the blood of

healthy volunteer subjects and from a patient with chronic granulomatous disease by the method previously described (3). Alveolar macrophages were obtained from the lungs of New Zealand white rabbits (2.5 to 5.0 kg) by the method of Myrvik et al. (7).

Glucose utilization via the hexose monophosphate shunt was estimated by a modification of the method of Holmes et al. (3), employing glucose differentially labeled in the C-1 or C-6 position. Each flask contained 1.0 ml of either glucose-1-<sup>14</sup>C or glucose-6-<sup>14</sup>C (0.05  $\mu$ Ci). Phagocytosis was initiated in an appropriate flask by the addition of 0.15 ml of polystyrene particles (0.81  $\mu$ m). Ascorbic acid (neutralized to pH 7.0) was added to experimental flasks in various concentrations, and an equal volume of water was added to control flasks. The reaction was initiated by the addition of 1.0 ml of cell suspension containing  $5 \times 10^6$  neutrophils or monocytes and allowed to proceed for 1 hr at 37 C. The <sup>14</sup>CO<sub>2</sub> liberated in the course of the incubation was collected in 0.5 ml of hyamine hydroxide and counted in a Packard Tri-carb liquid scintillation spectrometer.

The effects of 0.01 M ascorbic acid on metabolism of glucose via the hexose monophosphate shunt of control neutrophils and rabbit alveolar macrophages are summarized in Table 1. The stimulation of hexose monophosphate shunt by ascorbic acid alone in control neutrophils is greater than the metabolic burst obtained after the ingestion of polystyrene particles. Little increase in hexose monophosphate shunt occurs in macrophages during ingestion of polystyrene particles, but a marked increase is observed after the addition of ascorbic acid.

Table 2 summarizes the results obtained by adding 0.01 M ascorbic acid to the neutrophils isolated from a patient with chronic granulomatous disease. Although the neutrophils of

TABLE 1. *Effects of ascorbic acid (0.01 M) on the hexose monophosphate shunt of human neutrophils and rabbit alveolar macrophages*

Conditions	Counts/min in $^{14}\text{CO}_2^a$			
	Human neutrophil		Rabbit alveolar macrophage	
	Glucose-6- $^{14}\text{C}$	Glucose-1- $^{14}\text{C}$	Glucose-6- $^{14}\text{C}$	Glucose-1- $^{14}\text{C}$
Unstimulated.....	238 (202-303)	994 (884-1023)	351 (319-374)	822 (731-884)
With polystyrene particles.....	331 (294-411)	6228 (5931-6478)	372 (318-422)	1744 (1680-1803)
With ascorbic acid.....	344 (308-392)	8164 (7932-8205)	404 (310-482)	5436 (5260-5632)
With methylene blue.....	320 (264-352)	4798 (4103-5009)		

<sup>a</sup> Each value was determined in triplicate. Numbers in parentheses represent the range.

TABLE 2. *Effect of ascorbic acid (0.01 M) on the hexose monophosphate shunt of leukocytes from a patient with chronic granulomatous disease*

Conditions	Count/min $^{14}\text{CO}_2^a$	
	Glucose-6- $^{14}\text{C}$	Glucose-1- $^{14}\text{C}$
Unstimulated.....	486 (432-522)	944 (890-982)
With polystyrene particles.....	618 (543-677)	1104 (1022-1186)
With methylene blue.....	512 (421-576)	4882 (4878-4886)
With ascorbic acid.....	571 (505-621)	4566 (4307-4871)

<sup>a</sup> Each value was determined in triplicate. Numbers in parentheses represent the mean.

the patient are unable to increase their hexose monophosphate shunt activity after ingestion of polystyrene particles, the shunt is increased over 400% by the addition of ascorbic acid. This is similar to the increment in hexose monophosphate shunt activity which occurs after stimulation by methylene blue.

Figure 1 demonstrates that the increase in hexose monophosphate shunt activity is linear with respect to increasing concentrations of ascorbic acid. As little as 0.0001 M final concentration of ascorbic acid stimulates glucose utilization via the hexose monophosphate shunt.

The mechanism of the hexose monophosphate shunt stimulation by ascorbic acid is currently under investigation. Initial experiments indicate that it may involve reaction of ascorbic acid with hydrogen peroxide to form dehydroascorbic acid.

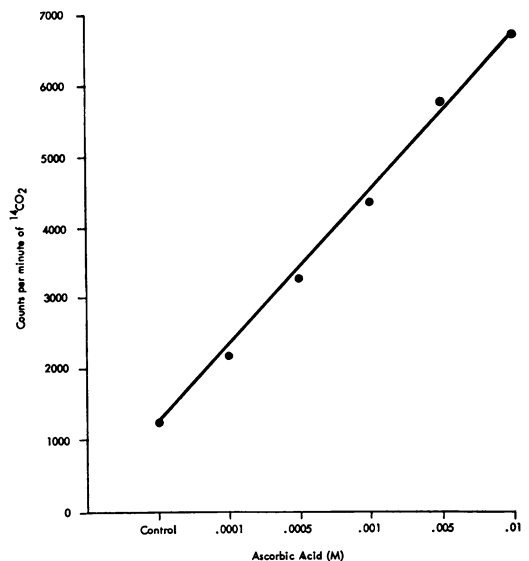


FIG. 1. *Effects of increased concentrations of ascorbic acid on the hexose monophosphate shunt of normal human neutrophils. After phagocytosis of polystyrene particles, 5,626 counts/min were obtained.*

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