Effects of Caffeine on the Metabolic Stimulation of Polymorphonuclear Leukocytes During Phagocytosis[1] (35656)

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Phagocytosis of bacteria by polymorphonuclear leukocytes (PMN) has been regarded as an important host mechanism in the defense against microbial infection. During phagocytosis certain metabolic changes have been demonstrated in PMN (1–5). The most notable metabolic alterations during phogocytosis appear to be the activation of the hexose monophosphate shunt (HMP) and an increase in oxygen consumption (6, 7). The relationship between particle uptake and metabolic changes has been discussed extensively (8–11).

Many drugs affect the phagocytic activity of PMN (12–16). Phelps and Stanislaw (17) noted that caffeine in concentrations as low as 4.7×10^{-5} *M* inhibits the motility of PMN. It has also been demonstrated that caffeine in doses greater than 0.5 mg/ml decreases the activity of the hexose monophosphate shunt of PMN (18). However, the effect of caffeine on the metabolic activity of PMN during phagocytosis has not been studied.

The purpose of this work was to investigate the effect of caffeine on glucose metabolism and particularly the hexose monophosphate shunt during phagocytosis. Additionally, the effect of caffeine on oxygen consumption was studied.

Materials and Methods. Blood was obtained from normal individuals (hospital volunteers) in silicon-treated tubes and was mixed with heparin (100 U) and a 5% dextran in 0.9% sodium chloride solution (ratio of blood to dextran, 4:1) for acceleration of red blood cell sedimentation. The separation of the PMN was described elsewhere (18, 19). The leukocytes were suspended in a mixture of autologous serum and Krebs–Ringer bicarbonate buffer (3:2). The resultant cell suspension contained from 85–92% PMN.

The incubation was carried out with three flasks. The first was used as a control containing PMN suspended in serum and Krebs–Ringer bicarbonate buffer (pH 7.4) with the addition of glucose-1-^{14}C or glucose-6-^{14}C (New England Nuclear Corporation, Boston, Mass.). The second flask contained the same number of cells, isotope, and latex polystyrene particles (0.81 μ). (Difco Laboratories, Detroit, Mich.). The amount of latex particles was 0.1 ml/10^8 cells. The contents of the third flask were identical to those of the second, with the addition of caffeine sodium benzoate. The cells were incubated for 1 hr using a Dubnoff metabolic shaker. The incubation procedure and chemical determination of the metabolic products is described elsewhere (18, 19).

Radioactivity of the metabolic products was determined with a Beckman liquid scintillation spectrometer (LS 150).

The activity of the hexose monophosphate shunt was calculated from the specific yields of labeled CO_2 from glucose-1-^{14}C and glucose-6-^{14}C using the equation of Wood *et al.* (20). The total glucose utilization was expressed as micromoles of glucose per 10^8 cells per hour.

Oxygen consumption was performed using a Clark type electrode (Oxygen Monitor System, Y.S.I., Model 53). Polymorphonuclear leukocytes were suspended in Krebs–Ringer bicarbonate buffer (pH 7.4) to a concentration of 0.5×10^8 cells/ml. Each cell of the oxygen monitor contained 3 ml of the suspen-

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TABLE I. The Effect of Caffeine on Hexose Monophosphate Shunt and Glucose Utilization During Phagocytosis.

Expt. no.	Hexose monophosphate shunt (%)			Dose of caffeine sodium benzoate (mg/ml)	Glucose utilization (μmoles/10^8 cells/hr)		
		Phagocytosis				Phagocytosis	
	Control glucose	Glucose + latex	Glucose + latex + caffeine		Control glucose	Glucose + latex	Glucose + latex + caffeine
1	1.2	10.6	2.0	2.5	7.2	8.2	5.5
2	1.8	8.9	1.3	2.5	4.6	5.3	3.2
3	1.3	10.0	0.9	2.5	7.2	8.3	5.2
4	2.9	10.2	4.1	1.5	4.9	5.9	5.0
5	3.2	10.0	4.6	1.5	7.8	7.9	5.2
6	1.8	8.9	3.9	1.5	4.6	5.3	3.6
7	1.6	4.0	3.0	1.0	4.7	4.9	4.1
8	3.2	10.0	4.6	1.0	7.8	7.9	6.0
9	1.8	8.9	4.8	1.0	4.6	5.3	3.8
10	1.6	4.0	4.0	0.5	4.7	4.9	4.6
11	1.8	8.9	8.4	0.5	4.6	5.3	5.2
12	2.0	12.5	11.7	0.5	6.5	7.9	7.9
			$(p < 0.01)$[a]				$(p < 0.05)$[a]

[a] For statistical analysis the variance related to condition and dose level of caffeine was used (22).

sion. Latex polystyrene particles, 0.81 μ (0.15 ml) and caffeine sodium benzoate (0.5 or 1.0 mg/ml) were injected through the side groove of the electrode. All experiments were performed at a temperature of 37°.

To determine the amount of latex particles engulfed by the leukocytes, the phagocytic index was calculated. It was expressed as percentage of PMN cells containing six or more particles. Two hundred cells from each sample were counted.

Results. The depressant effect of caffeine on the HMP shunt and glucose utilization is shown in Table I. Caffeine doses of 1.0 mg/ml, and greater, depressed the HMP shunt during phagocytosis. At a level of 0.5 mg/ml, the HMP shunt showed no significant change. Caffeine concentrations of 0.5 mg/ml did not affect glucose utilization of polymorphonuclear leukocytes during phagocytosis. Doses of 1.0 mg/ml, and greater, significantly depressed both the HMP shunt and glucose utilization.

The effect of increasing doses of caffeine on the HMP shunt and glucose utilization is presented graphically in Fig. 1. A dose–response relationship is presented as percentage decrease in HMP shunt and dose of caffeine HMP shunt activity declines progressively with the increasing doses of caffeine. A tendency for a decrease in glucose utilization is noted at the higher doses of caffeine (0.8 mg/ml and greater).

A caffeine dose of 0.5 mg/ml inhibited the oxygen consumption which had been stimulated by the addition of latex polystyrene particles (Table II). The same dose did not alter the rate of oxygen consumption as measured in resting condition nor did stimulation occur after particles were added. The oxygen consumption was completely inhibited when a dose of 1.0 mg/ml was used. No stimulation was produced by the addition of latex particles.

The phagocytic index of leukocytes incubated in medium free of caffeine was 75% or more, while those incubated with caffeine 0.5 mg/ml showed an average of 63%. When the dose of caffeine reached 1.0 mg/ml the average phagocytic index was 22%.

Discussion. The metabolic changes during phagocytosis by polymorphonuclear leukocytes have been studied very intensively. Particle uptake is accompanied by increased oxygen consumption and increased HMP shunt activity. The metabolic stimulation of

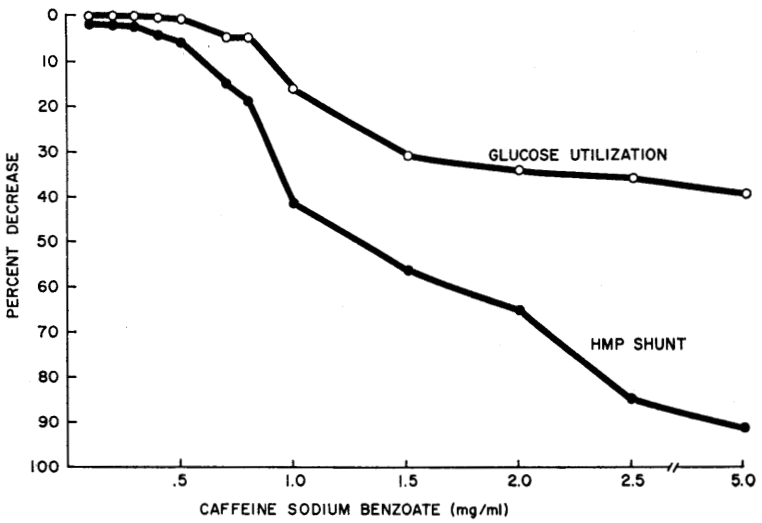

FIG. 1. Relationship between the dose of caffeine and decrease of glucose utilization and hexose monophosphate shunt during phagocytosis.

the HMP shunt occurs as early as 30 sec after phagocytosis (10). Besides the metabolic stimulation Zatti *et al.* (10) have shown some morphological changes in PMN during phagocytosis. It has been demonstrated that glycolytic inhibitors such as sodium fluoride and iodoacetate inhibit phagocytosis, while respiratory inhibitors as potassium cyanide and antimycin A do not affect phagocytosis (2, 11). This indicates that the energy requirement during phagocytosis is derived from glycolysis. Thus the metabolic activities of the phagocyte may be separated into those involved with engulfment (glycolytic) and those stimulated by the particle after it has entered the cell (oxidative). The latter activities are associated with the destruction of the phagocytized material. This phenomenon has been demonstrated with leukocytes from patients with chronic granulomatous disease (21). The leukocytes of these patients can engulf bacteria, but are not capable of destroying them. Such phenomenon is associated with the inhibition of the HMP shunt.

Our results demonstrate that caffeine at concentrations of 0.5 mg/ml (1.2 μmoles/ml), and greater, decreases respiration during phagocytosis (Fig. 1). It appears that respiratory stimulation is blocked severely at this point, after which a continual decrease follows the gradual increase of the

TABLE II. The Effect of Caffeine on Oxygen Consumption During Phagocytosis (μl O_2/min/10^8 cells).

Expt. no.	Resting	Addition of particles	Addition of caffeine (mg/ml) 0.5	Addition of caffeine (mg/ml) 1.0	Addition of particles
1	0.206	0.624	0.005		
	0.220		0.206		0.220
	0.206			0.008	0.008
2	0.145	0.508	0.005		
	0.145		0.145		0.154
	0.154			0.003	0.005
3	0.260	0.630	0.007		
	0.270		0.260		0.260
	0.270			0.005	0.005

dose. The maximum depressant effect of caffeine on respiration during phagocytosis was found at doses of 2.5 and 5.0 mg/ml. At this concentration of caffeine the presence of noticeable glucose utilization, when the HMP shunt is significantly depressed, indicates that the depressant effect of caffeine does not affect the entire vitality of the cells. It should be emphasized that the caffeine doses used are in excess of the amount of one high therapeutic dose. Sodium benzoate used even in very high doses did not affect glucose metabolism. This demonstrates that the noted metabolic changes should be considered only in regard to the caffeine effect. The oxygen consumption test was applied to demonstrate the above-mentioned effect of caffeine. When latex particles are added, oxygen consumption is greatly increased. No further oxygen consumption is noted after the addition of caffeine (0.5 mg/ml). If the same dose of caffeine is followed by the addition of latex particles, no stimulation of oxygen consumption occurs, but the uptake continues at a rate of the resting cells. At this stage, the phagocytic index shows an average of 63%. The addition of a caffeine dose of 1.0 mg/ml inhibits oxygen consumption completely and latex particles do not change this condition (Table II). The phagocytic index is extremely low.

These results indicate that caffeine doses of 0.5 mg/ml do not significantly affect the engulfment of the latex particles. Thus the glucose utilization and oxygen consumption remain unchanged and the phagocytic index shows 63%. The same dose inhibits any metabolic stimulation that may occur during particle ingestion. The activity of the HMP shunt is decreased and oxygen consumption is inhibited. These metabolic deviations during phagocytosis indicate the preferential effect of caffeine on the oxidative activities of the leukocyte (destruction of the particle). The dose of 1.0 mg/ml, or greater, significantly affects both the glycolytic and oxidative activities of the cell.

The above mentioned metabolic changes are also accompanied by a decrease in the phagocytic index. Phelps and Stanislaw (17) described inhibition of PMN motility in the presence of caffeine. These results indicate that the biochemical alterations might also be associated with functional changes. As previously discussed (18) the most probable mechanism for such alteration in the metabolic activity is the direct influence of caffeine on certain enzymes from the HMP shunt.

Our experiments demonstrate that the preferential effect of caffeine during phagocytosis by polymorphonuclear leukocytes is the inhibition of the direct oxidation of glucose through the HMP shunt and a decrease in oxygen consumption. Further investigations are required to elucidate the precise inhibitory effect of this drug on the HMP shunt.

Summary. The effects of caffeine sodium benzoate on glucose utilization, hexose monophosphate shunt (HMP), and oxygen consumption of polymorphonuclear leukocytes (PMN) during phagocytosis were studed. The stimulation of HMP during phagocytosis was depressed when the dose of caffeine was in the range of 0.5–1.0 mg/ml. Caffeine doses of 1.0 mg/ml, or greater, depressed the HMP shunt significantly during phagocytosis; whereas the glucose utilization was affected less. The stimulation of oxygen consumption by latex particles was terminated with a caffeine dose of 0.5 mg/ml. No stimulation occurred if the same dosage was used before the addition of latex particles. Control experiments with glucose and sodium benzoate did not change the HMP shunt and glucose utilization during phagocytosis. The data presented indicate that the preferential effect of caffeine during phagocytosis by polymorphonuclear leukocytes is the inhibition of the direct oxidation of glucose via the HMP shunt. Caffeine inhibits the stimulation of oxygen consumption during phagocytosis.

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