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PYRIDINE NUCLEOTIDE OXIDATION BY INTACT HUMAN POLYMORPHONUCLEAR NEUTROPHILS

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SUMMARY

The capacity of human polymorphonuclear neutrophils to oxidize NADH and NADPH before and after phagocytosis was quantitated. Spectrophotometric measurement of pyridine nucleotide oxidation in dialysates of intact cells demonstrated that more NADH was oxidized by phagocytizing cells than by resting cells. In contrast NADPH oxidation did not increase after phagocytosis. This suggests that NADH oxidation may be responsible for the post phagocytic increase in oxygen consumption and hydrogen peroxide production that occurs in human polymorphonuclear neutrophils.

Following phagocytosis, the O_2 consumption and H_2O_2 production of polymorphonuclear neutrophils increased markedly[1,2]. Polymorphonuclear neutrophils utilize endogenously produced H_2O_2 to kill ingested bacteria[3]. An oxidase of reduced pyridine nucleotide appears to be the principal mediator of the reaction, whereby O_2 is consumed and H_2O_2 is formed, but there has been controversy over whether NADH oxidase or NADPH oxidase is the major determinant of the post-phagocytic respiratory burst.

Several investigators have found more activity for the oxidase of NADPH than for the oxidase of NADH in the granular fraction of guinea-pig leukocytes[4–9]. However, KARNOVSKY and co-workers[10–12] found that when 1 mM KCN is introduced into the system, NADH oxidase activity is far greater than NADPH oxidase activity. They state that the enzyme responsible for the respiratory burst should be cyanide insensitive since it is known that phagocytizing polymorphonuclear neutrophils will consume O_2 and produce H_2O_2 normally in the presence of 1 mM KCN[1,13].

BAEHNER AND KARNOVSKY[14] have shown that patients with chronic granulomatous disease of childhood are deficient in NADH oxidase, an enzyme found in the supernatant of alkaline KCl leukocyte homogenates. These authors correlated this deficiency with the abnormal O_2 consumption, decreased H_2O_2 production and inability of these cells to kill bacteria normally previously observed[15].

All of the above studies on the oxidation of reduced pyridine nucleotide were carried out on leukocyte extracts. We employed a method utilizing intact human polymorphonuclear neutrophils obtained from peripheral blood of normal donors. The capacity of these cells to oxidize NADH and NADPH before and after phagocytosis

was measured by spectrophotometrically quantitating the oxidation of the pyridine nucleotides. Studies were performed with and without KCN.

Polymorphonuclear neutrophils were obtained from heparinized peripheral blood that was sedimented with 3 % dextran[16]. 1-μm polystyrene latex balls (Dow Chemical Company) were utilized as particles to be phagocytized at a multiplicity of 100 particles per polymorphonuclear neutrophil. 1 ml of suspension of polymorphonuclear neutrophils in Hank's balanced salt solution, pyridine nucleotide and 10 % autologous serum (with or without particles) were placed in 75-mm plastic tubes and tumbled at 37° for 30 min. This suspension was then dialysed through a cellulose membrane against 1 ml of Hank's balanced salt solution for 1 h in a 37° shaker bath. At the end of this time, absorbance at 340 mμ was measured on the dialysate. By referring to a standard curve, the amount of reduced pyridine nucleotide in the dialysate could be ascertained. Acid lability was used as a check for the purity of the pyridine nucleotide in the dialysate.

TABLE I

OXIDATION OF PYRIDINE NUCLEOTIDES BY INTACT RESTING AND PHAGOCYTIZING POLYMORPHONUCLEAR NEUTROPHILS

$2.5 \cdot 10^7$ polymorphonuclear neutrophils, $2.5 \cdot 10^9$ 1-μm polystyrene balls, and 1.1 mM reduced pyridine nucleotide were utilized to make up suspensions. Baseline values of pyridine nucleotide oxidation were established with boiled polymorphonuclear neutrophil suspension. Phagocytizing polymorphonyclear neutrophils with or without KCN oxidized more NADH than resting polymorphonuclear neutrophils ($P < 0.01$). In contrast, resting and phagocytizing cells oxidized similar amounts of NADPH ($P > 0.2$). Standard errors of the mean are shown.

	μmoles NADH oxidized	*μmoles NADPH oxidized*
Resting neutrophils	30 ± 3.5 ($n = 11$)	20 ± 4.4 ($n = 8$)
Phagocytizing neutrophils	45 ± 5.5 ($n = 11$)	14 ± 5.3 ($n = 8$)
Resting neutrophils + 1 mM KCN	28 ± 6.0 ($n = 8$)	15 ± 3.3 ($n = 10$)
Phagocytizing neutrophils + 1 mM KCN	40 ± 4.5 ($n = 8$)	12 ± 3.7 ($n = 5$)

Intact human polymorphonuclear neutrophils oxidized NADH to a greater extent after phagocytosis of latex particles than when resting (Table I). The addition of cyanide did not change the ability of the intact polymorphonuclear neutrophils to oxidize NADH. Resting polymorphonuclear neutrophils were also able to oxidize NADPH (to a lesser extent) but there was no increment in NADPH oxidase activity after phagocytosis. The finding that hydrocortisone, an inhibitor of NADH oxidase, markedly impairs post phagocytic O_2 consumption and H_2O_2 production[17] is consistent with these data. Thus, our findings are in agreement with the original observations of EVANS AND KARNOVSKY[10] and we conclude that the post-phagocytic increase in O_2 consumption and H_2O_2 production by human polymorphonuclear neutrophils is due to the activity of NADH oxidase.

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