mium-treated rats agrees with the results of Nomiyama *et al.* (*6*), who also suggested that the increased excretion of protein and glucose in rabbits continuously injected with cadmium might be associated with renal tubular dysfunction. Treatment with cadmium has also been shown to produce liver damage and a rise in the glycogen-degrading enzyme glycogen phosphorylase, as well as a significant decrease in the activity of hepatic aldolase (*7*, *10*). Our data on the stimulation of the gluconeogenic pathway by cadmium provide an enzymatic basis for the increase in urinary protein and glucose, as suggested in the case of methylmercury and several organochlorine pesticides (*8*, *9*). It is also possible that the cadmium-induced glycosuria might be related to a decreased sugar reabsorption resulting from renal failure (*6*). Our data suggest that the presence of cadmium in the environment could lead to an impairment of hepatic and renal carbohydrate metabolism, and this may account for its environmental toxicity.

RADHEY L. SINGHAL, Z. MERALI
S. KACEW, D. J. B. SUTHERLAND
Department of Pharmacology,
University of Ottawa,
Ottawa, Ontario, Canada K1N 6N5

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Ecto-Enzyme of Granulocytes: 5′-Nucleotidase

Abstract. *The 5′-nucleotidase of guinea pig polymorphonuclear leukocytes is localized exclusively on the plasma membrane of intact cells. The active site of this enzyme faces the external medium, not the cytoplasm.*

Although it is now generally accepted that the 5′-nucleotidase of mammalian cells is localized on their plasma membranes, the evidence is hardly conclusive. Histochemical techniques present many difficulties (*1*), one of which is the possibility that substrate is not penetrating into cells or organelles. Thus the histochemical approach does not rule out the existence of an intracellular 5′-nucleotidase. In biochemical studies, preparations of plasma membrane from rat liver exhibit specific activities of 5′-nucleotidase about 20 times that of the original homogenate (*2*). However, recoveries of this enzyme in the plasma membrane fraction are 10 percent (or substantially less) that of the original homogenate (*3*), so that most of the activity may still be associated with another organelle.

We have proposed an approach to the selection of markers for the plasma membrane (*4*). This approach is based on the circumstance that the plasma membrane is the one cellular structure that can be studied without disrupting the cell. With a suspension of intact cells in vitro the outer surface of the membrane is accessible to biochemical investigation and chemical alteration. We now report our use of intact cells to determine the localization of 5′-nucleotidase in guinea pig polymorphonuclear leukocytes.

Cells were harvested from the peritoneal cavities of guinea pigs as described (*5*). The enzyme (5′-nucleotidase) was assayed in Krebs-Ringer phosphate buffer (KRP), *p*H 7.4, with the use of 1 m*M* adenosine [^{32}P]-monophosphate and 1 m*M* *p*-nitrophenyl phosphate.

The *p*-nitrophenyl phosphate was shown by competition studies to prevent the hydrolysis of adenosine monophosphate (AMP) by nonspecific phosphatases present on the surface of intact cells and in disrupted cell preparations. Further, we showed that, when levamisole, an inhibitor of nonspecific phosphatases (*6*), was included in the reaction mixture, *p*-nitrophenyl phosphatase was inhibited virtually completely, while adenosine monophosphatase activity was identical to that measured as described above. The assay mixture was incubated at 37°C, and the reaction was terminated by the addition of an equal volume of 10 percent (weight to volume) suspension of acid-washed Norit in 10 percent (weight to volume) trichloroacetic acid. The charcoal, which binds AMP but not inorganic phosphate (P_i), was removed by filtration through Whatman No. 1 filter paper, and $^{32}P_i$ in a portion of the filtrate was counted. This assay was found to be linear for at least 30 minutes and over the range of 1×10^6 to 8×10^6 cells per assay vessel. It was used successfully not only with intact or disrupted cells in suspension, but also with cells that had formed a monolayer on a small plastic dish. The diazonium salt of sulfanilic acid was synthesized by the indirect method (*7*). Radioactivity was determined by counting in a toluene-ethanol–based scintillation fluid (*8*). When necessary, cellular fragments were dissolved in NCS solvent (Amersham/Searle) at 37°C.

Intact guinea pig polymorphonuclear leukocytes hydrolyzed AMP added to the medium at the rate of 316 nmole per 10^7 cells in 15 minutes. We showed that this activity was due neither to leakage of enzyme from the cells nor to broken cells; that the adenosine monophosphatase of intact cells was associated with the polymorphonuclear leukocytes and was not attributable to contaminant cell types (monocytes, lymphocytes, eosinophils, and erythrocytes); and that washing the cells ten times with KRP or sucrose did not affect the hydrolysis of AMP by intact cells.

The first experiment to determine whether the intact cell adenosine monophosphatase is an ecto-enzyme—that is, localized exclusively on the plasma membrane, with the active site oriented to the medium—was based on the reasoning that, if a protein reagent that does not penetrate into the intact cells can inhibit the activity of the enzyme, the protein involved must be located on the plasma membrane. Cell monolayers were treated with the diazonium

salt of sulfanilic acid, a nonpenetrating reagent (*7*). Monolayers may be easily and quickly washed in beakers of KRP (*9*), thus allowing rapid removal of the diazonium salt. Lactate dehydrogenase, which is found in the soluble fraction from these cells as with most other cell types, was used as an internal indicator for penetration of the reagent. In sonicated suspensions of cells lactate dehydrogenase and adenosine monophosphatase were equally susceptible to inhibition by the diazonium salt of sulfanilic acid. When intact cells were treated with different concentrations (1×10^{-7} to $1 \times 10^{-3} M$) of the diazonium salt for 30 minutes, the higher concentrations that inhibited the adenosine monophosphatase by 90 percent did not significantly affect the lactate dehydrogenase. Thus, when intact cells were treated with the reagent at a concentration of 3.5 m*M* for 30 minutes at 37°C, the adenosine monophosphatase of the intact cells was inhibited about 90 percent, but the lactate dehydrogenase assayed after the cells were scraped off the monolayer plates and sonicated was inhibited only about 6.8 percent (Fig. 1).

The simplest explanation for these results is that the enzyme involved in hydrolysis of AMP by intact guinea pig polymorphonuclear leukocytes is located on the plasma membrane and has functional groups located outside the permeability barrier presented by this structure. Such results do not of themselves provide evidence that the active site of the adenosine monophosphatase faces the external medium (*10*). The best evidence that the active site faces outward comes from localization of the P_i produced, as described below.

A thick cell suspension in KRP was incubated in the presence of 0.5 m*M* [^{32}P]AMP for 2 minutes, which was long enough to hydrolyze all of the substrate. The cells were then pipetted into a finely calibrated hematocrit tube and centrifuged. [^{14}C]Inulin was present in the incubation mixture to serve as a measure of the extracellular space in the sedimented portion after centrifugation (*11*). The ^{32}P and ^{14}C were determined in the supernatant and the resuspended pellet by differential scintillation counting. Virtually all (99.3 ± 1.4 percent) of the ^{32}P-containing products (shown by paper chromatography to be P_i) was localized outside the cells. Since it is conceivable that the $^{32}P_i$ was formed intracellularly and subsequently transported out of the cells, the following experiment was performed: The cells were allowed to take up $^{33}P_i$ for 30 to 45 minutes at 0°C and then thoroughly washed. The experiment described above was then repeated with these $^{33}P_i$-loaded cells. One change was the use of inulin labeled with ^{3}H instead of ^{14}C so that this compound could be distinguished from both ^{32}P and ^{33}P. If the $^{32}P_i$ produced by the hydrolysis of AMP was at any time inside the cells, it would presumably mix with the pool of $^{33}P_i$. Such mixing would result in similar distributions of ^{32}P and ^{33}P in the extra- and intracellular fluids. The distributions of these isotopes were strikingly different (Table 1).

Table 1. Localization of $^{32}P_i$ formed by hydrolysis of AMP after the intact cells were loaded with $^{33}P_i$. The amount of radioactivity used in these experiments resulted in 2×10^4 to 10×10^4 count/min per sample.

Substrate	Total percentages within the cells		
	Water (by volume)	$^{33}P_i$	$^{32}P_i$
None	50.2	89.9	
AMP	55.1	93.6	1.2

The 5′-nucleotidase of intact guinea pig polymorphonuclear leukocytes is thus an authentic ecto-enzyme. Furthermore, this appears to be the only enzyme in these cells that specifically hydrolyzes AMP and other 5′ nucleotides. This conclusion is based on the following observations.

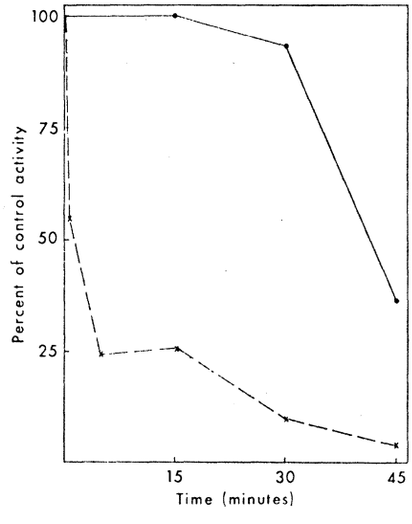

Fig. 1. Monolayers were incubated at 37°C with the diazonium salt of sulfanilic acid (3.5 m*M*) for varying intervals of time. The reaction was terminated by washing the monolayers in five beakers of KRP. Some of the monolayers were then used to assay adenosine monophosphatase activity of intact cells. The cells were scraped off other monolayers, sonicated, and assayed for lactate dehydrogenase. (●) Lactate dehydrogenase; (X) adenosine monophosphatase of intact cells.

1) Sonication of the cells (100-watt ultrasonic disintegrator, Measuring and Scientific Equipment Ltd., London, England; setting 4 to 6 μm; 0°C) for up to 300 seconds had no effect on adenosine monophosphatase activity. The sonication effectively disrupted the plasma membrane and the nuclei (observed microscopically) and the mitochondria [release of glutamate dehydrogenase (*12*)]. The latency of lysosomal hydrolases decreased to less than 40 percent compared to 80 to 90 percent in a carefully prepared homogenate—an indication of substantial disruption of these organelles. Further, cells previously treated with diazotized sulfanilic acid to inhibit ecto–adenosine monophosphatase showed no increase in that enzymatic activity upon sonication, that is, no internal enzyme was revealed.

2) Treatment of intact guinea pig polymorphonuclear leukocytes with 0.5 percent (weight to volume) saponin (the concentration giving maximal effect) caused a 60 percent increase in adenosine monophosphatase activity. When intact cells were first treated with diazotized sulfanilic acid, and then with saponin, the increase in adenosine monophosphatase activity was only 60 percent of that remaining after the treatment with the nonpenetrating reagent—that is, rather than of the original activity of untreated cells. Saponin does stimulate adenosine monophosphatase, such as that of rat liver plasma membranes (*13*), but does not reveal any intracellular enzymes capable of hydrolyzing AMP in these cells.

3) After the cells were homogenized (*12*), limited fractionation studies showed that all the adenosine monophosphatase was sedimentable. However, none was associated with the nuclear fraction (*12*). Upon centrifugation in a density gradient, adenosine monophosphatase distributes quite differently from marker enzymes for mitochondria and lysosomes (*14*).

Further studies demonstrated that the ecto–adenosine monophosphatase of guinea pig polymorphonuclear leukocytes is inhibited by EDTA and sulfhydryl reagents. It has an apparent Michaelis constant (K_m) of $1 \times 10^{-5} M$. This enzyme hydrolyzes all of the ribonucleoside monophosphates in the order adenosine ≧ uridine > cytidine > guanosine > inosine, but does not act

on other phosphates such as ribonucleoside triphosphates or *p*-nitrophenyl phosphate.

We have also found an ecto–adenosine triphosphatase and an ecto–*p*-nitrophenyl phosphatase on these cells (*15*), and others have reported the hydrolysis of added substrates by intact cells of other types (*16*). The existence and characteristics of such ecto-enzymes are of interest in the study of the interaction between cells and their external environment, although the function of such enzymes is still obscure. The ecto–adenosine monophosphatase described above provides a rigorous marker for the plasma membrane of guinea pig polymorphonuclear leukocytes. It remains to be established whether or not the 5′-nucleotidases of other mammalian cells are also ecto-enzymes.

JOSEPH W. DEPIERRE
MANFRED L. KARNOVSKY
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

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The Size of Suspended Particle Matter in Air

We agree with Lee (*1*) that the size distribution of particles suspended in the air is important. However, we disagree with several of his conclusions. His data, obtained with an Anderson cascade impactor operated at five times the design flow rate, are plotted exclusively on logarithmic-probability coordinates. A log normal distribution was assumed, the points were fitted to a straight line, and mass median diameters were calculated.

Examination of the graphs presented shows that from 50 to 85 percent of the collected aerosol mass was found on the first stage and the after filter, and was therefore not sized. However, even though only 15 to 50 percent of the collected mass was sized, Lee assumed that the size distributions could be approximated in all cases with a log normal distribution. Examination of Lee's figure 3 indicates that the data for Cincinnati, Ohio, and Washington, D.C., would be fitted better by a curve than by a straight line.

Whitby *et al.* have presented evidence that the mass distribution of atmospheric aerosol is usually bimodal with one mode occurring below 1 μm and another in the 5- to 15-μm range (*2*). If a reasonable curve is drawn through Lee's points, and the resulting data are transformed into a graph of $\Delta m/\Delta \log D_p$ on a linear scale against log D_p, it is seen from Fig. 1 that the Lee data may also generate a bimodal distribution. Thus, we believe that in general the mass distribution of atmospheric aerosols is not log normal. In particular, we believe that Lee's data should not be used as evidence for a single mode log normal distribution.

Obtaining mass median diameters by extrapolation of the data on a log normal plot to sizes below the last data point leads to some unusually low mass median diameters, such as 0.02 μm for

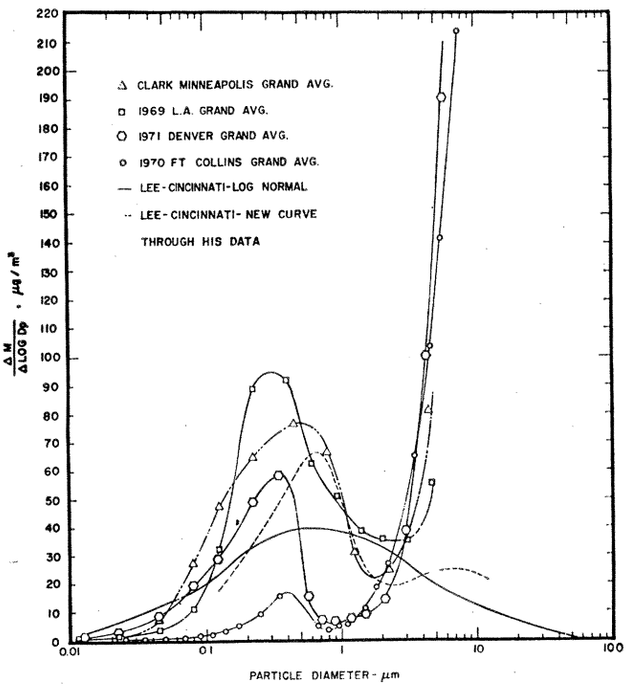

Fig. 1. Mass distribution graph of the grand averages from four cities as measured with the Minnesota aerosol analyzing system compared with Lee's six-city log normal size distribution average. The dashed line shows the Cincinnati average distribution redrawn so that the curve goes through the actual points measured rather than those obtained on the assumption that it is log normal. The bimodal nature of the distributions is produced, in contrast to the broad unimodal distribution resulting from Lee's assumption that the distributions are all log normal.