be considered in relation to the patient's age; a higher percentage of degranulation after treatment with antiserum to IgE might occur in a population of cells obtained from younger patients.

We describe a means of obtaining living human respiratory mast cells coincidentally and without risk to human patients. The method has two limitations. (i) Only a small number of cells can be obtained and studied at any time. (ii) Cells can be obtained only from patients who are undergoing brush biopsies for other reasons; this limitation will remain unless the diagnostic indications for examination of mast cells are expanded in the future. It is unlikely that samples taken at different times from the same patient will be available.

The characteristics of the degranulation process of these respiratory mast cells is remarkably similar to that described for rhesus cells obtained in the same manner. Parallel studies with the latter system are appropriate, since the rhesus cells can be obtained repeatedly and under controlled conditions from the same animal (*1, 2*).

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Phosphodiesterase in Dictyostelium discoideum and the Chemotactic Response to Cyclic Adenosine Monophosphate

Abstract. *A phosphodiesterase with a low Michaelis constant for cyclic adenosine monophosphate was found in the membrane fraction of the cellular slime mold. This activity was highest during the aggregation stage. Enzyme with similar properties was also secreted by the cells. Dithiothreitol inhibited both enzymes and potentiated the cellular response to cyclic adenosine monophosphate.*

During the aggregation phase of their development, cellular slime molds are attracted to one another by a chemotactic hormone (acrasin). In *Dictyostelium discoideum*, this hormone is adenosine 3′,5′-monophosphate (cyclic AMP) (*1*). After aggregation the cells undergo multicellular differentiation, and cyclic AMP also affects the rate and possibly the outcome of this process (*2*).

Table 1. Enzyme activity and secretion during early development. Pellet activity is units of enzyme per milligram of protein; a unit is 1 nmole of cyclic AMP hydrolyzed per minute. Enzyme secretion is units secreted per milligram of cellular protein per hour. Protein was determined by the method of Lowry *et al.* (*13*).

Developmental stage	Pellet activity	Enzyme secretion
Amoeba	4.00	2.62
Early aggregation	14.7	1.46
Late aggregation	6.03	0.72

Phosphodiesterase activity—cleavage of cyclic AMP to adenosine 5′-monophosphate—has been reported in filtrates from *D. discoideum* cultures. The enzyme has a broad specificity for cyclic nucleotides, and the Michaelis constant (K_m) for cyclic AMP is high, 1 to 2 m*M* (*3*). In this report we describe a phosphodiesterase that has a much lower K_m (10 to 15 μM) for cyclic AMP (*4*). This sensitivity, together with the pattern of enzyme activity and secretion during development, suggests that this form of phosphodiesterase is involved in modulating the response of the cells to cyclic nucleotides. This interpretation is supported by our observation that inhibition of the enzyme enhances the chemotactic response to cyclic AMP.

Amoebas of *D. discoideum* were grown in the presence of *Escherichia coli* on nutrient agar sheets. The amoebas were washed free of bacteria (*5*) and plated on 2 percent agar that contained 0.01*M* phosphate buffer at *p*H 6.5, 0.001*M* ethylenediaminetetraacetic acid (EDTA), and streptomycin sulfate (500 μg/ml). At appropriate stages of development, the cells were removed by rinsing the agar with salt solution (*6*) buffered with 0.01*M* tris(hydroxymethyl)aminomethane (tris), *p*H 7.5. The suspension was centrifuged at 1500*g* for 5 minutes at 0°C, and a portion of the centrifuged cells was resuspended in an equal volume of tris buffer and frozen.

The remaining cells were suspended in 10 times their volume of salt solution that was buffered with 0.01*M* phosphate at *p*H 6.5, and the suspension was swirled on a rotating platform for 1 hour at 24°C. The cells were removed by centrifugation. To the supernatant, solid ammonium sulfate was added to 90 percent saturation. The material thus precipitated was redissolved in 0.01*M* tris, *p*H 7.5, and dialyzed overnight against the same buffer. This preparation was designated the secreted fraction.

The frozen cell suspension was thawed and centrifuged at 27,000*g* for 15 minutes, and the pellet was resuspended in tris and centrifuged again. The washed pellet was resuspended in tris and stored at −80°C; this was called the pellet fraction. The supernatants from the two centrifugations were combined and designated the intracellular fraction.

Enzyme activity was measured in a mixture that contained extract; 0.1*M* tris, *p*H 7.5; alkaline phosphatase (from *E. coli*), 25 μg/ml; adenosine deaminase (from intestinal mucosa), 0.02 μg/ml; glutamic acid dehydrogenase (from beef liver), 0.25 mg/ml; 5.5 m*M* α-ketoglutaric acid; 0.1 m*M* EDTA; 0.25 m*M* nicotinamide adenine dinucle-

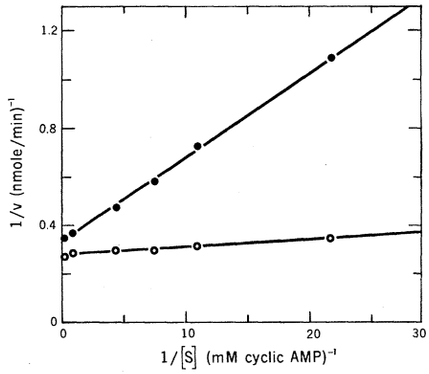

Fig. 1. Kinetic behavior of secreted enzyme in the presence (solid circles) and absence (open circles) or 1 m*M* DTT. Graph is according to the method of Lineweaver and Burk (*14*).

otide; 0.1 mM reduced nicotinamide adenine dinucleotide (NADH); and 2 mM cyclic AMP (*7*). Hydrolysis of cyclic AMP was measured as oxidation of NADH; this oxidation was monitored continuously by absorbance at 340 nm in a stoppered cuvette. The amounts of protein added were in the range in which rate was a linear function of enzyme amount. This range of protein was up to 5 μg/ml for the secreted fraction or 200 μg/ml for the pellet or intracellular fractions. Identical reaction rates were observed when these preparations were assayed by a method employing quantitative thin-layer chromatographic analysis of reaction product (*7*).

The secretion of enzyme was highest before aggregation, as is shown in Table 1 for a typical experiment. Activity in the pellet fraction reached its peak later, when the cells entered the multicellular state (aggregation). Comparable activities in the pellet fraction were observed when cells were lysed in a French pressure cell. Activity of pellet enzyme was completely solubilized by the addition of detergents such as digitonin or Triton, results indicating that the enzyme was bound to a membrane fraction. Enzyme activity within the cell (the pellet and intracellular fractions) increased during the incubation of suspended cells; thus, secretion of enzyme did not result merely from loss of an existing pool but involved synthesis or activation of additional enzyme.

As is shown in Fig. 1, the apparent K_m of the secreted enzyme was much less than 2 mM cyclic AMP. Although the method used was not sufficiently sensitive for use with a substrate concentration less than 40 μM, the apparent K_m was in the range of 10 μM cyclic AMP. A similar value was obtained with pellet enzyme. In contrast to the enzyme form with high K_m, the activity of the form with low K_m was not stimulated by added divalent metal ion. As Fig. 1 also shows, 1 mM dithiothreitol (DTT) inhibited hydrolysis of cyclic AMP in a mixed but largely competitive manner.

The chemotactic response of *D. discoideum* amoebas to cyclic AMP was observed by use of the bioassay procedure developed by Konijn (*8*). Addition of 1 mM DTT to the source of cyclic AMP enhanced the cellular response (Fig. 2). Both the extent of the response to a given concentration of cyclic AMP (Fig. 2) and the sensitivity

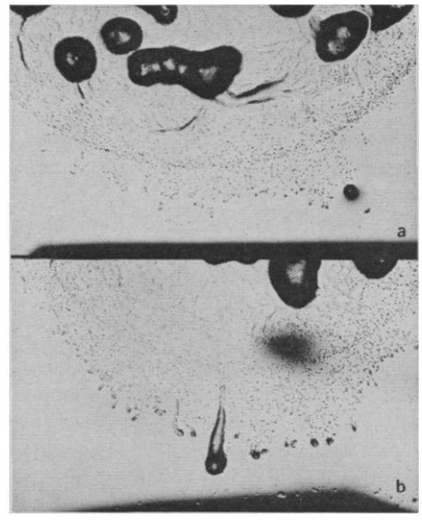

Fig. 2. Stimulation of the chemotactic response to cyclic AMP by DTT. A drop of cell suspension was allowed to dry into an agar surface and formed a disk of cells. A block that had been soaked in a solution containing (a) 2.5 μM cyclic AMP or (b) 2.5 μM cyclic AMP and 1 mM DTT was placed next to the edge of the disk (*8*). The portion of this disk nearest the block is seen at the top of these pictures, and the block is at the bottom. The dark areas within the disks are cell aggregates. Cells have left the disk and have moved toward the block in both (a) and (b), but the response is greater in (b), where the block contains DTT.

of the response were increased in the presence of DTT, results suggesting that the effective concentration of cyclic AMP was increased by the inhibition of phosphodiesterase. When it was used alone, DTT did not attract the cells.

Chassy (*9*) observed interconversion of two forms—one with high K_m, the other with low K_m—of purified extracellular phosphodiesterase. This process appears to involve sulfhydryl compounds. The much lower rate and the direction of this interconversion process (DTT promotes formation of the low K_m form) indicate that it is distinct from the inhibition we observe.

We suggest that this low K_m form of phosphodiesterase is the physiologically significant form during the aggregation process. The K_m of this enzyme is in a range that allows maximum flexibility in enzyme rate at those concentrations of cyclic AMP which produce a chemotactic response. The reported lower limit of the cells' sensitivity is about $10^{-8}M$ cyclic AMP (*10*), a value well below the K_m of the enzyme, and we observed (*7*) that the upper limit of cyclic AMP concentrations that elicit a chemotactic response (around 100 μM) corresponds to the concentration at which the phosphodiesterase is saturated.

The location of the enzyme during development is also consistent with an involvement in the chemotactic response. Prior to aggregation, when the individual cells are acting on one another at a distance, the enzyme is secreted into the medium; but during aggregation, when the cells are in close contact, the enzyme is found in the membrane fraction.

Finally, the observation that an inhibitor of the enzyme potentiates the cellular response to cyclic AMP is consistent with a modulating role for phosphodiesterase in this response and further suggests that natural inhibitors of the enzyme could regulate the effects of cyclic AMP in this organism. Sussman reported fractionation of active preparations of acrasin into several components, one of which stimulated the response of the cells to another component (*11*). We observed that a number of sulfhydryl compounds both inhibit the enzyme and potentiate the cellular response (*7*). Lenhoff (*12*) proposed that a sulfhydryl compound, reduced glutathione, is involved in the control of another cellular movement, the feeding response of *Hydra littoralis*.

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