brane (*9*). Without any mechanical agitation, the fragmented slugs were loosened at the periphery, and gradually they fell into single cells. After 3 hours only the central parts of the pieces were intact (Fig. 1a), which eventually became also completely dissociated (Fig. 1, b and c). The tips of the slugs were dissociated last. This corresponds to the observation that the presumptive stalk cells that form this part of the slug adhere more strongly to each other than the presumptive spores (*6*). No dissociation was observed in controls containing 32 mg of Fab per milliliter from nonimmunized rabbits (Fig. 1d).

The slow dissociation rate indicates that at a given time only a small portion of the Fab receptor sites is accessible to being blocked by Fab. It remains to be seen whether Fab can break already established bonds between associated cells, or whether it seizes only newly synthesized or detached groups, thus hindering them from contributing to cell adhesion. In this case cell dissociation by Fab would depend on turnover or exchange of its receptor sites.

The dissociated cells were actively motile and began to reaggregate approximately 5 hours after the removal of unbound Fab; they also aggregated in the Fab solution about 12 hours after the beginning of incubation.

For the organism used in this study, application of univalent antibody fragments provides a gentle and efficient method of cell dissociation. This method may be advantageous also in other systems, especially for experiments in which degradation of the cell surface has to be avoided or when the dissociated cells should be kept for longer periods in the single cell state.

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Histamine Augments Leukocyte Adenosine 3′,5′-Monophosphate and Blocks Antigenic Histamine Release

Abstract. *Extracellular histamine stimulates accumulation of adenosine 3′,5′-monophosphate in human leukocytes and prevents antigenic release of histamine from cells of allergic donors. Both effects occur at histamine concentrations that can be achieved by antigenic release of the amine in vitro.*

Leukocytes from human donors allergic to ragweed release histamine after exposure to ragweed antigen E (*1*). This effect, mediated by cell-bound reaginic (IgE) antibody, serves as a useful model in vitro for human allergy, inasmuch as the sensitivity of the leukocytes to antigen E reflects the clinical severity of the allergic diathesis (*2*). Inferential evidence, based on the effects of catecholamines and methylxanthines, suggests that elevated intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) inhibit IgE-mediated release of histamine from human leukocytes and human or primate lung tissue (*3*). Histamine itself stimulates production of cyclic AMP in some tissues, such as brain (*4*). We have examined the possibility that histamine might stimulate production of the cyclic nucleotide in leukocytes as well. If so, extracellular histamine should prevent the release of intracellular histamine triggered by exposure to specific antigen. The experiments reported here were designed to test this hypothesis.

Human leukocytes were isolated and suspended in an isotonic, tris-buffered artificial medium (tris-A). In some instances, 0.6 m*M* Ca^{2+} and 1.0 m*M* Mg^{2+} were added (tris-ACM) (*1*).

Synthesis of cyclic AMP by intact leukocytes was measured as described elsewhere (*5*). Cells suspended in tris-A were incubated with [^{3}H]adenine (6 c/mmole, 1 μc per 10^7 cells) for 40 minutes at 37°C, centrifuged, and resuspended in either tris-A or tris-ACM containing appropriate concentrations of histamine and theophylline, $10^{-2}M$ (*6*), and incubated for a further 10 minutes at 37°C. Histamine caused a dose-related increase in accumulation of cyclic [^{3}H]AMP in leukocytes (Fig. 1), usually maximal at $10^{-4}M$ and detectable at about $10^{-6}M$. The maximal effect, expressed as percentage of increase over basal [^{3}H]nucleotide accumulation, varied considerably from subject to subject. In other experiments (not shown), histamine stimulated adenyl cyclase activity in broken leukocytes, as measured by the method of Krishna *et al.* (*7*).

Histamine release from leukocytes of ragweed-sensitive donors was measured by adding fractions of a cell suspension in tris-ACM to a series of tubes containing a constant amount of antigen E and variable concentrations of exogenous histamine. The reaction mixtures were then incubated at 37°C for 60 minutes, and the percentage of the total cellular histamine released into the fluid phase was determined fluorimetrically (*1*, *3*). The percentage of inhibition caused by histamine was calculated from the formula $[(C-E)/C] \times 100$, where C and E stand for the percentage of histamine release in the control and histamine-containing tubes, respectively.

Exogenous histamine caused a dose-dependent inhibition of antigenic histamine release from the cells of allergic donors (Fig. 2, left). Extracellular histamine at concentrations ranging from 10^{-7} to $10^{-6}M$ caused 50 percent inhibition of release of intracellular histamine in all subjects tested. These

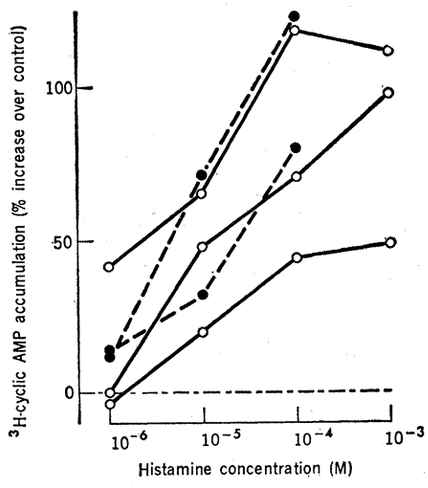

Fig. 1. Conversion of [^{3}H]adenine to intracellular cyclic [^{3}H]AMP by intact leukocytes (ordinate) as a function of extracellular histamine concentration (abscissa). Each line represents a single subject. Incubation medium was either tris-ACM (solid circles) or tris-A (open circles). Accumulation of cyclic [^{3}H]AMP is represented as percentage of increase over basal accumulation (in the absence of extracellular histamine), which averaged 0.28 $\pm$ 0.14 percent of [^{3}H]adenine added per 10^5 leukocytes per 10 minutes of incubation (*5*).

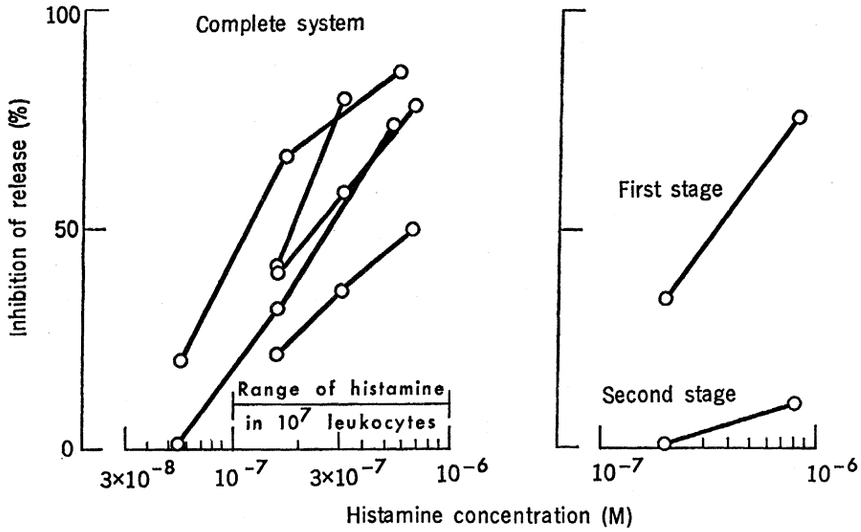

Fig. 2. Inhibition of antigenic release of histamine from human leukocytes as a function of extracellular histamine concentration. In the complete system (left) antigen and histamine were added simultaneously to cells incubated in tris-ACM. Each curve represents the inhibition of histamine release from cells of a single subject. Histamine release was also separated into two separate stages, as described in the text, and inhibition by exogenous histamine was studied in cells from the same subject (right).

concentrations are equivalent to the amount of histamine ordinarily contained in the cells used in these experiments (10^7 per tube).

The release of histamine following exposure to antigen in this "complete" system (containing Ca^{2+}, Mg^{2+}, and antigen) can be divided into two separate phases (*8*): The first or "activation" phase involves exposure of cells to antigen in a medium devoid of divalent cations (tris-A). No actual release of histamine occurs, because the release process is dependent on divalent cations (*1*). When these cells are washed free of fluid phase antigen, however, and resuspended in tris-ACM, release of histamine proceeds immediately, without the lag (1 to 5 minutes) characteristic of release in the complete system. This second, Ca^{2+}-Mg^{2+}–dependent, phase is designated the "release" phase. The time allowed for the activation phase is critical (*8*): a period of 2 minutes, which causes maximal release of histamine subsequently, was employed in the experiments reported here. The time allowed for the release phase was 60 minutes, as in the complete system. The inhibitory effect of variable concentrations of exogenous histamine on cells from five donors was tested in both the activation and release phases.

When exogenous histamine was present in medium during the activation phase and washed off (along with antigen) before the cells were resuspended in tris-ACM, the subsequent release of intracellular histamine was consistently inhibited (Fig. 2, right). The inhibition was comparable to that observed in the complete system (Fig. 2, left) both in extent and in the dose of exogenous histamine required. By contrast, when the cells had already been exposed to antigen in tris-A, the addition of exogenous histamine to the medium during the release phase (tris-ACM) produced little or no inhibition of release.

These results suggest that extracellular histamine inhibits leukocyte histamine release by interfering with some intracellular event during the activation phase, a process not dependent on extracellular Ca^{2+} or Mg^{2+}. The possibility that histamine interferes with the Ca^{2+}-Mg^{2+}–dependent release reaction (by analogy to a chemical "mass-action" effect) is ruled out by the amine's failure to inhibit during the release phase, after exposure of cells to antigen has already occurred.

In other experiments (results not shown), histamine was added to the complete (tris-ACM) medium at varying times after antigen. The inhibitory effect of $1 \times 10^{-6} M$ exogenous histamine decreased rapidly with increasing time after exposure to antigen: if histamine and antigen were added together, histamine release was inhibited by 80 to 90 percent. If histamine was added 2 minutes after antigen (at a time when no histamine release had occurred), the inhibitory effect had decreased (20 to 40 percent). When histamine was added 7 minutes after addition of antigen (at a time when release was about half complete), no inhibition whatever could be detected.

It appears likely that histamine's inhibitory effect on antigen-induced histamine release was mediated by elevated intracellular concentrations of cyclic AMP. Isoproterenol, which stimulates leukocyte adenyl cyclase (*9*), and methylxanthines, which inhibit cyclic AMP degradation in many tissues (*10*), including leukocytes (*5*), both inhibit allergic histamine release. Dibutyryl cyclic AMP, a lipid-soluble analog of the endogenous nucleotide, also inhibits histamine release (*3*). All three agents, like histamine, inhibit release in the complete system (tris-ACM) and in the activation phase. All three are inactive (or nearly so) if added during the release phase, when exposure of cells to antigen has already occurred.

The conclusion that histamine acts on leukocytes through an effect on adenyl cyclase is only tentative, however; theophylline, which potentiates the effect of isoproterenol on histamine release (*3*), failed to potentiate the effect of histamine (preliminary results not shown). The methylxanthines inhibit a specific phosphodiesterase which metabolizes cyclic AMP, and should potentiate the effect of a drug which acts by increasing synthesis of the nucleotide. Such potentiation is a useful but probably not absolute criterion for establishing the role of cyclic AMP in mediating the effect of a drug (see *10*).

Another qualification deserves emphasis: most of the histamine in leukocytes is confined to the basophils, which make up a small fraction of the mixed leukocyte populations in which we measured cyclic AMP accumulation. Our conclusions, then, depend on the assumption that cyclic AMP accumulation in basophils responds to histamine in the same way as the mixed populations.

In summary, these experiments represent a positive test of the hypothesis that cyclic AMP mediates the effects of a number of drugs that inhibit allergic histamine release. Histamine was found to increase accumulation of newly synthesized cyclic AMP in leukocytes. Like the catecholamines, methylxanthines, and dibutyryl cyclic AMP, histamine also interferes with IgE-medi-

ated histamine release at an early stage after exposure to antigen, a stage that is not dependent on divalent cations.

Unlike the other drugs listed, histamine is an endogenous compound which may be present at high concentration in the extracellular fluid of an inflammatory reaction, of which it may actually be a chemical mediator. It is possible, however, that histamine plays an additional role: by inhibiting release of additional histamine from leukocytes newly arrived at the scene of an allergic reaction, histamine may act as a negative modulator of the intensity of inflammation. If such a concept proves to be correct, cyclic AMP may act in leukocytes as an inhibitor of processes which are ordinarily necessary for host defenses, but which may become so intense that they endanger the host.

It is interesting that most effects so far attributed to cyclic AMP in either leukocytes or platelets are inhibitory: antigenic release (presumably from mast cells) of histamine from primate lung (*3*) or of slow-reacting substance of anaphylaxis into rat peritoneum (*11*), neutrophil candidacidal activity (*5*), lymphocyte transformation (*12*), platelet aggregation (*13*), and phagocytic release of lysosomal enzymes (*14*) are all thought to be inhibited by intracellular cyclic AMP. It would be of interest to test the ability of histamine to interfere with these processes.

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Cyclic 3′,5′-Nucleotide Phosphodiesterase: Cytochemical Localization in Cerebral Cortex

Abstract. *The distribution of cyclic 3′,5′-nucleotide phosphodiesterase activity in rat cerebral cortex was determined by cytochemical methods. The detectable phosphodiesterase activity was localized in postsynaptic (dendritic) nerve endings, most of it immediately adjacent to the synaptic membrane. Most of the postsynaptic nerve endings showed phosphodiesterase activity.*

An increasing amount of evidence suggests that adenosine 3′,5′-monophosphate (cyclic AMP) may be involved in the regulation of metabolism and function in nervous tissue (*1*). Certain of the data suggest that cyclic AMP may be intimately associated with the molecular events underlying the process of synaptic transmission. For instance, evidence has been presented that exogenous cyclic AMP can mimic the inhibitory effect of norepinephrine on cerebellar Purkinje cells (*2*). Moreover, brief periods of synaptic activity in autonomic ganglia caused a severalfold increase in the amount of cyclic AMP in the ganglia, which occurred largely, if not entirely, in postsynaptic cells (*3*). This increase in cyclic AMP may modify the excitability of certain postsynaptic cells by the following chain of events (*3, 4*): (i) activation of a protein kinase by cyclic AMP, (ii) phosphorylation of the synaptic membrane by the kinase, (iii) change of ionic permeability of the membrane, (iv) change of membrane potential, and (v) change of excitability. This scheme provides a plausible molecular mechanism for prolonged modulation of synaptic transmission either by facilitation or by inhibition.

An important factor limiting the magnitude and duration of action of cyclic AMP in tissues is the rate of enzymatic destruction of the nucleotide by cyclic 3′,5′-nucleotide phosphodiesterase. Of all mammalian tissues studied, the brain has the highest phosphodiesterase activity (*5*). A knowledge of the precise location of phosphodiesterase activity within the cells should be valuable in clarifying the function of cyclic AMP in nervous tissue. We have, therefore, undertaken a cytochemical study of the localization of phosphodiesterase in brain tissue. In our initial investigations, the parietal lobe of the rat cerebral cortex has been used.

Cyclic 3′,5′-nucleotide phosphodiesterase hydrolyzes cyclic AMP to 5′-AMP. The basis of our cytochemical procedure was to convert this 5′-AMP to adenosine and inorganic phosphate with an excess of 5′-nucleotidase, and to precipitate the newly formed inorganic phosphate with lead ions, thus forming an electron-opaque product at or near the site of phosphodiesterase activity.

Adult male albino rats (160 to 180 g) were anesthetized by intraperitoneal injection of 3.1 percent chloral hydrate (1 ml per 100 g of body weight) and then perfused through the left ventricle with 400 ml of 2 percent glutaraldehyde in 0.05*M* cacodylate-nitrate buffer (*p*H 7.4) containing 0.25*M* dextrose. The cerebral hemispheres were removed and washed overnight in the same buffer. The hemispheres were then cut into strips 2 to 3 mm thick, from which 50-μm slices of tissue were prepared in a Smith-Farquhar tissue chopper. The slices were first incubated for 30 minutes at room temperature in a solution containing 60 m*M* tris maleate buffer (*p*H 7.4), 2 m*M* $MgCl_2$, and 0.25*M* sucrose (TMS buffer) containing 5′-nucleotidase (*6*). The slices were next incubated for 30 minutes at 37°C in TMS buffer containing 2 m*M* lead nitrate, 5′-nucleotidase (3 mg/ml), and 3 m*M* cyclic AMP, either in the ab-