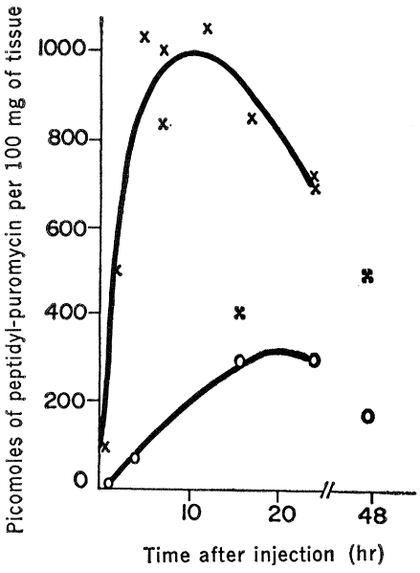

Fig. 2. Amounts of the peptidyl-puromycin mixture, precipitated by TCA, present in 100 mg of temporal cortex (wet weight) as a function of time after injections into each temporal lobe of 90 μg of puromycin alone (×) or of 120 μg each of puromycin and heximide (○).

gests that this lesion is not related to some cytotoxic effect of puromycin other than that on protein synthesis. Nor can the swelling be related solely to the effect of puromycin on the synthesis of mitochondrial protein, since there is evidence that heximide, unlike puromycin (*12–14*), has no effect on this mitochondrial activity (*14, 15*) and should not interfere with puromycin in this action.

At this time, it appears most likely that swelling of neuronal mitochondria in the presence of puromycin is due to the formation of peptidyl-puromycin. This interpretation is supported by the finding that the extent of mitochondrial swelling appears to be directly related to the amount of puromycin-peptides that are precipitated by TCA. It should be pointed out that puromycin blocks expression of memory indefinitely, whereas a mixture of puromycin and heximide has no such effect (*16*). The present findings are consistent with the possibility that the effect of puromycin on memory is due to a widespread action of peptidyl-puromycin on neuronal cytomembranes.

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Histamine Release in vitro: Inhibition by Catecholamines and Methylxanthines

Abstract. *Methylxanthines and catecholamines both inhibit antigenically induced histamine release from human leukocytes. They act synergistically to inhibit the reaction, but must be present when antigen is added; preincubation is not effective. Since both increase cellular levels of cyclic 3′,5′-adenosine monophosphate it is postulated that this compound plays a role in the regulation of allergic histamine release.*

We have previously described an in vitro system of antigen-induced histamine release from human leukocytes (*1*). The reaction follows the interaction of ragweed antigen E (a protein of molecular weight 38,000) and reaginic (IgE) antibody on the surface of leukocytes isolated from donors sensitive to ragweed (*2*). The system serves as a model of human allergy inasmuch as the sensitivity of the leukocytes to antigen E (based on the concentration of antigen required for 50 percent histamine release) reflects the clinical severity of the allergic diathesis (*3*).

The mechanism of this type of reaction, which we have called allergic histamine release, has not as yet been defined. We have shown that the cell is not injured as a result of the immune reaction, but rather that the antigen elicits an active multistep response which has some similarities to secretory phenomena. In order to release histamine the cell must be viable and the glycolytic system must be intact. Oxidative metabolism does not appear to be required (*4*).

In the course of studies on inhibitors of histamine release the action of commercial preparations of the methylxanthines (theophylline, theobromine, and caffeine) and the catecholamines (epinephrine and isoproterenol) was explored (*5*). Leukocytes were isolated from the peripheral blood of allergic donors as previously described, and suspended in a serum-free tris buffer (*1*). The inhibitory capacity of the drugs was determined by adding fractions of a cell suspension to a series of tubes containing a constant amount of antigen E and variable concentrations of the inhibitor. The reaction mixtures were then incubated at 37°C for 60 minutes, and the percentage of the total histamine released from the cells into the fluid phase was measured fluorometrically (*1, 5*). The percentage of inhibition caused by each drug 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 experimental (inhibitor-containing) tubes, respectively. Inhibition studies were carried out with the leukocytes of six to eight different allergic donors.

All of the methylxanthines inhibited histamine release; theophylline was the most active (Fig. 1). Inhibition began at 1 to 3 × $10^{-5}M$, and 50 percent inhibition was reached with concentrations about tenfold higher. Two- or threefold higher concentrations of caffeine or theobromine were required to produce an equivalent inhibitory effect. Theophylline stopped histamine release immediately, even if the reaction had begun and an appreciable percentage of the histamine had already been released. In another type of experiment cells were exposed to theophylline for 15 minutes at 37°C in the absence of antigen. They were then centrifuged, resuspended in a theophylline-free buffer, and exposed to antigen. No inhibi-

tion of histamine release was demonstrated as a result of the pretreatment with theophylline. A similar experiment, involving pretreatment with isoproterenol, led to the same result.

The catecholamines were also effective inhibitors of allergic histamine release (Fig. 1). Isoproterenol appeared to be more potent than epinephrine. Inhibition by isoproterenol began at 2 to 6 $\times$ $10^{-5}M$ and 50 percent inhibition was achieved at about 2 to 6 $\times$ $10^{-4}M$. With both groups of compounds the concentration required for 50 percent inhibition varied within the ranges noted, from day to day and cell donor to cell donor. This type of biological variation has been seen with respect to several other parameters in the leukocyte system.

The catecholamines and methylxanthines appear to act synergistically in the inhibition of histamine release. Thus, very low concentrations of epinephrine (as low as $10^{-7}M$) enhanced the inhibiting capacity of theophylline. In experiments such as that tabulated in Table 1, epinephrine and theophylline were added to cells in concentrations below the level that caused inhibition. If the two were combined, however, very definite inhibition was observed.

It is well appreciated from the work of Sutherland and others that both classes of compounds considered in this report interact with the cyclic 3',5'-adenosine monophosphate (3',5'-AMP) system (*6*). In many tissues the catecholamines enhance the action of adenyl cyclase, the enzyme which converts adenosine triphosphate to cyclic 3',5'-AMP, and thereby increase the rate of cyclic 3',5'-AMP formation (*7*). The methylxanthines are competitive inhibitors of a phosphodiesterase that inactivates cyclic 3',5'-AMP, and they thereby also increase the tissue concentrations of this compound (*8*). These actions are felt to provide an important mechanism by which the catecholamines and methylxanthines exert their action. The cyclic 3',5'-AMP system has been found in all nucleated mammalian cells thus far studied, and Sutherland has demonstrated its presence in human leukocytes (*9*). Thus, it seems reasonable to suggest that these drugs are able to inhibit allergic histamine release through their ability to increase the intracellular level of cyclic 3',5'-AMP. This supposition is supported by the synergistic action of theophylline and epinephrine. In preliminary experiments dibutyryl cyclic 3',5'-AMP was found to inhibit histamine release directly; 50 percent inhibition occurred at about $10^{-3}M$.

The effect of the cyclic 3',5'-AMP system has been explored primarily in its relationship to metabolic processes (*6*) but it has been recently suggested that it has a role in the control of insulin secretion by the pancreas (*10*). In describing the parameters of allergic histamine release we have noted the similarities between this process and secretion. It is important to note, however, that an increase in the intracellular level of cyclic 3',5'-AMP has generally led to a stimulation of enzymatic or secretory activity. Thus, epinephrine and theophylline enhance these reactions rather than cause inhibition as they do in the present system (*6*). However, the activity of at least one well-studied enzyme, glycogen synthetase, may be inhibited by cyclic 3',5'-AMP (*11*). Of particular interest is the observation that theophylline and isoproterenol inhibit only if added to cells when antigen is present. They have no effect if removed from the cellular environment before antigen exposure. This suggests that they operate on a system which is antigen-activated and thus central to the mechanism of the allergic reaction. In another context, the action of isoproterenol, epinephrine, and theophylline in vitro may perhaps be useful in clarifying the role of these drugs in the treatment of asthma.

Table 1. Inhibition of histamine release: the synergistic effect of theophylline and epinephrine.

Inhibitor added		Inhibition (%)
Theophylline (molar)	Epinephrine (molar)	
3×10^{-4}		29
1×10^{-4}		0
3×10^{-5}		0
	1×10^{-6}	0
3×10^{-4}	1×10^{-6}	64
1×10^{-4}	1×10^{-6}	36
3×10^{-5}	1×10^{-6}	20

Note added in proof: Since submitting this report, we have learned that 30 years ago Schild reported that epinephrine inhibits "anaphylactic" histamine release from guinea pig lung (*12*).

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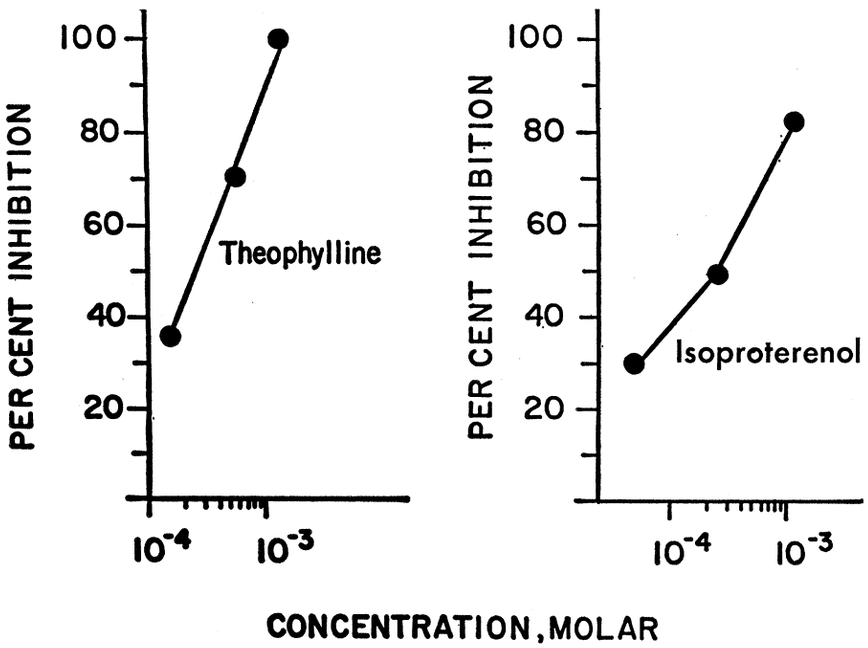

Fig. 1. The inhibition of allergic histamine release by theophylline and isoproterenol.

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