

Increased Adenosine Triphosphatase in Leukocytes of Asthmatic Children

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ABSTRACT Adenosine triphosphatase (ATPase) activities were compared in leukocytes of asthmatic and nonasthmatic children. Both Mg^{2+} - and Ca^{2+} -dependent ATPase activities were significantly elevated in two membrane fractions (59 to 66%) and in a supernatant fraction (68 to 72%) prepared from sonicated leukocytes of asthmatic subjects. Intact cell surface or ecto ATPase was also elevated (67 to 76%) in asthmatic leukocytes. Alternate day glucocorticosteroid therapy was associated with leukocyte ATPase activities intermediate between those for asthmatics not receiving steroids and for nonasthmatic control subjects. Incubation of normal leukocytes with 10^{-6} M hydrocortisone or leukocyte membranes with 10^{-4} – 10^{-3} M hydrocortisone in vitro also resulted in decreased ATPase activities. The elevated leukocyte ATPase activities appear to relate to the adrenergic imbalance in asthma previously characterized by reduced beta adrenergic responsiveness of adenylate cyclase and suggest the possibility of more than one enzymatic abnormality intrinsic to the asthmatic condition.

INTRODUCTION

In experiments to further clarify the biochemical alteration present in patients with asthma, we and others (1–5) have found that the leukocytes of these patients provide a useful source of biological material to study and have provided data supporting the beta adrenergic blockade hypothesis of asthma elucidated by Szentivanyi (6) and reviewed recently by Middleton (7). Consistent with this hypothesis, the leukocytes of asthmatic chil-

dren (1, 2) and adults (3–5) showed a diminished response of adenylate cyclase to catecholamine stimulation. Catecholamine stimulation of a number of tissues including leukocytes is associated with the potential for two pharmacologically distinct responses, the alpha and beta adrenergic. The beta adrenergic response has been linked to adenylate cyclase and the production of cyclic AMP. The alpha adrenergic response is often competitive, and it has been suggested that this results from a lowering of cyclic AMP (8). Recent studies of leukocytes of asthmatic subjects (2, 4) show that alpha adrenergic blockade with phentolamine restores normal beta adrenergic responsiveness of adenylate cyclase to catecholamine stimulation. In addition glucocorticosteroid treatment is associated with a partial restoration of normal beta adrenergic responsiveness in leukocytes of asthmatics (1, 9). Similarly glucocorticosteroids restore normal beta adrenergic responsiveness in human tracheal smooth muscle pharmacologically manipulated to simulate the asthmatic condition (10). These observations suggest that a competitive mechanism relating to enhanced alpha adrenergic sensitivity might be operant to produce apparent beta adrenergic blockade.

Belleau (11) proposed on theoretical ground that membrane adenosine triphosphatase (ATPase) would represent an ideal enzyme for alpha adrenergic modulation. We have offered support for this thesis by showing that in several cells divalent cation-dependent ATPase activities are stimulated by alpha adrenergic agonists and blocked by an alpha adrenergic blocker (12, 13). In applying this concept to asthma, a comparative study of two pairs of twins one of each pair with asthma and the other normal, showed that in addition to apparent beta adrenergic blockade the leukocytes of the asthmatic twins showed increased ATPase activity (14).

In the present study calcium- and magnesium-dependent ATPase activities were studied in the leukocytes of asthmatic children with and without steroid

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treatment and nonasthmatic controls in an effort to provide evidence for intrinsic alteration reflecting in vivo adrenergic imbalance.

METHODS

Subjects. Asthmatic children of both sexes were selected from the patient population at the National Asthma Center for these studies. They were subdivided into two groups depending on whether they were receiving steroids. Steroid therapy ranged from 5 to 50 mg prednisone orally every other day with a mean dosage of 20 mg. Blood was drawn from these subjects 48 h after the last steroid administration. Most of these subjects as well as subjects not on steroid therapy also received combination drug therapy consisting of ephedrine, theophylline, and phenobarbital. All subjects were relatively free of symptoms on the morning of the study. The patients under study with very few exceptions showed airway hyperirritability (positive methacholine inhalation challenge), obstruction to air flow, and increased mid-expiratory time and hyperinflation. IgE levels ranged from a trace to 8,250 ng/ml and peripheral blood eosinophil counts ranged from 1 to 14%. Approximately half of the patients showed positive skin test reactions to common allergens. Nonasthmatic boys and girls volunteered as control subjects. The numbers and ages of each group of subjects are provided in Table I.

Preparation of cells and membranes. 10 ml of heparinized venous blood was obtained between 8 and 9 a.m. Leukocytes were prepared after sedimentation of red cells with dextran, as described previously (1). Pellets of leukocytes were washed with saline buffered with 1 mM Tris·HCl at pH 7.0, centrifuged at room temperature, and resuspended in 4 ml saline. These suspensions typically contained fewer than 1 platelet or red blood cell per leukocyte (1). Leukocyte distributions ranged from 20 to 40% lymphocytes and 50 to 70% neutrophils, with no significant differences between asthmatics and nonasthmatics. There were no significant differences in lymphocyte counts between the asthmatic subgroups, possibly because the lymphocyte counts return to normal 48 h after steroid administration (15). Eosinophil counts were routinely higher in asthmatics than nonasthmatics: the mean percent eosinophils was 6.1 for asthmatics not receiving steroids, 5.3 for asthmatics receiving steroids, and 2.9 for nonasthmatics.

A portion of each cell suspension was saved for measurement of intact cell surface or ecto ATPase activity. The remaining cells were sonicated at 0°C for 20 s with a Biosonic Sonifer (Bronwill Scientific, Rochester, N. Y.) at a power setting of 30. Nuclei and large cell fragments (P_1) were sedimented at 150 *g* for 10 min at 0°C. A smaller membrane fraction (P_2) consisting of mitochondria, granules, endoplasmic reticulum, and plasma membrane was collected by centrifugation of the P_1 supernatant at 44,000 *g* for 30 min at 0°C. Centrifugation of the resulting supernatant at 140,000 *g* for 60 min yielded no visible precipitate and was, therefore, not performed routinely.

ATPase. Membrane fractions P_1 or P_2 were suspended in 3 ml of 50 mM Tris·HCl, pH 7.5, by using a Dounce homogenizer; 0.4 ml of suspensions of membranes, intact cells, or supernatant was mixed with 0.05 ml of 50 mM $MgCl_2$ or $CaCl_2$ (dissolved in 50 mM Tris, pH 7.5) and incubated at 37°C. The reaction was initiated by adding 0.05 ml of 20 mM ATP (sodium salt, Sigma Chemical Co., St. Louis, Mo., dissolved in 50 mM Tris and adjusted to pH 7.5) and continued with gentle shaking for 30 min. The

TABLE I
Subjects used in ATPase studies

		Age	
		Range	Mean
Asthmatics not receiving steroids			
Number of males:	18	11.5–15.6	13.4
Number of females:	6	12.5–14.4	13.9
Total:	24	11.5–15.6	13.6
Steroid-treated asthmatics			
Number of males:	13	9.8–16.0	13.0
Number of females:	4	12.6–14.4	13.6
Total:	17	9.8–16.0	13.2
Nonasthmatics			
Number of males:	12	9.9–16.0	12.4
Number of females:	8	11.0–15.0	12.6
Total:	20	9.9–16.0	12.5

reaction was terminated with 0.05 ml of 4 N $HClO_4$ and protein was removed by centrifugation. The supernatant was neutralized with 0.05 ml of 4 N KOH, and the $KClO_4$ precipitate was removed by centrifugation. Adenosine diphosphate (ADP) or inorganic phosphate was determined in the supernatant by methods described (16), and protein was assayed according to the method of Lowry, Rosebrough, Farr, and Randall (17).

RESULTS

Optimum reaction conditions. Optimum conditions for measuring leukocyte ATPase were established with normal adult cells. The conversion of ATP to ADP and phosphate by sonicated leukocytes in the presence of Mg^{2+} was found to be linear with time up to 30 min (Fig. 1). Between 30 and 60 min the reaction rates decreased slightly. The net formation of phosphate was 95% of the formation of ADP at 30 min. The discrepancy may be due to minimal utilization of ATP by other phosphorylation reactions occurring in the whole sonicates. The close agreement between net formation of ADP and phosphate indicates that assay of either product would represent a fairly accurate measurement of ATPase activity in this system.

The conversion of ATP to ADP by Mg^{2+} - and Ca^{2+} -dependent enzymes associated with membranes was proportional to the amount of enzyme added as seen in Fig. 2. Broad pH optima were found between 7.2 and 8.0 for both Mg^{2+} - and Ca^{2+} -dependent ATPase in both P_1 and P_2 fractions of leukocytes (Fig. 3). In addition a small peak of Ca^{2+} -dependent ATPase at pH 5.2 was observed for leukocyte P_1 and P_2 fractions representing 50 and 24%, respectively, of the activities measured at pH 7.5. No additional peaks of Mg^{2+} -dependent activity were noted, suggesting that there were no significant

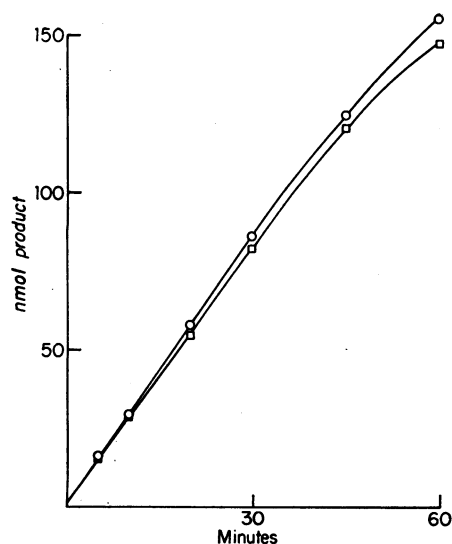


FIGURE 1 Leukocyte Mg^{2+} -ATPase as a function of time. Sonicated, unfractionated, nonasthmatic leukocytes were incubated with 2 mM ATP and 5 mM $MgCl_2$ for varying times as described in Methods. ADP (○—○) and phosphate (□—□) were measured after incubation. Each point represents the mean of three determinations.

contributions to breakdown of ATP by nonspecific phosphatases in these membrane preparations.

Optimum levels of Mg^{2+} and Ca^{2+} were found to be 5 mM for ATPase in both membrane fractions of leukocytes (data not shown) when 2 mM ATP was used. The following reaction conditions, similar to those

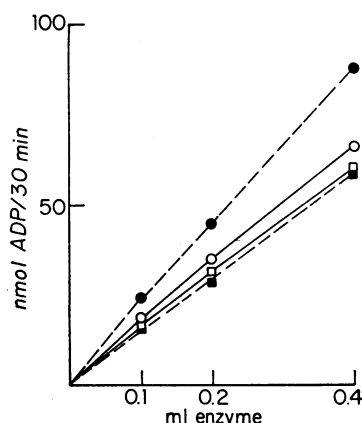


FIGURE 2 Leukocyte membrane ATPase as a function of enzyme. Membrane fractions prepared from nonasthmatic leukocytes were incubated with 2 mM ATP and 5 mM divalent cation for 30 min and the product ADP determined as described in Methods. Activities are plotted for varying amounts of Mg^{2+} -ATPase in fraction P_1 (○—○), Ca^{2+} -ATPase in fraction P_1 (□—□), Mg^{2+} -ATPase in fraction P_2 (●—●), and Ca^{2+} -ATPase in fraction P_2 (■—■) in a total volume of 0.5 ml. Each point represents the mean of three determinations.

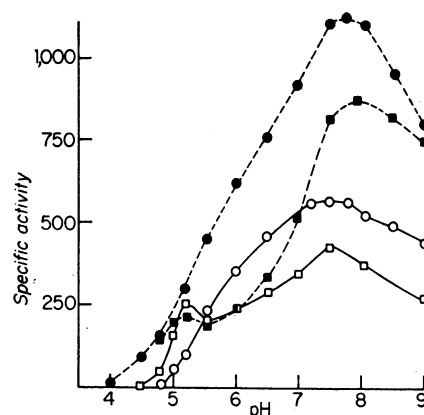


FIGURE 3 Leukocyte membrane ATPase as a function of pH. The experiment was conducted as described for Fig. 2 with 50 mM Tris·HCl buffered at various pH values. The pH was measured in duplicate tubes at 37°C after the 30-min incubation. Each point represents the mean of three determinations.

used previously for lymphocyte ATPase (16) and for leukocyte ATPase (18), were, therefore, routinely used: 5 mM divalent cation, 2 mM ATP, 50 mM Tris at pH 7.5, and 20–100 μ g leukocyte protein in a final volume of 0.5 ml, incubated at 37°C for 30 min. ADP was routinely measured and specific activity expressed as nmol ADP formed in 30 min per milligram protein.

Comparison of ATPase activity in leukocytes of asthmatic and nonasthmatic subjects. Specific activities of Mg^{2+} - and Ca^{2+} -dependent ATPase in fractions P_1 and P_2 of leukocytes from asthmatic children not on steroid therapy, asthmatics receiving steroid therapy, and nonasthmatic children are compared in Fig. 4. For

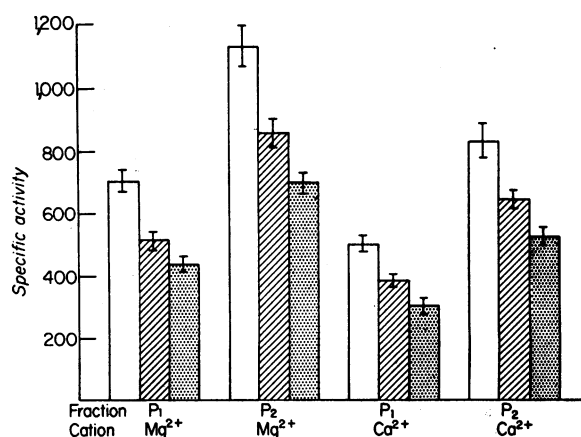


FIGURE 4 Membrane ATPase activities. The mean ATPase specific activities (nmol ADP/30 min/mg protein) are plotted for membrane fractions P_1 and P_2 of leukocytes from 24 asthmatic children not receiving steroids (clear bars), 17 asthmatic children receiving steroid therapy (hatched bars), and 20 nonasthmatic children (dotted bars). Error bars indicate SEM.

each group of subjects the mean of the Mg^{2+} -ATPase was greater than the Ca^{2+} -ATPase, and the specific activities in fraction P_2 were greater than those in P_1 . Specific activities of the ecto ATPase and supernatant fraction ATPase are compared for the three groups in Fig. 5. The specific activity of the Mg^{2+} -dependent component of ecto ATPase was greater than the Ca^{2+} -dependent component while the reverse was true for the supernatant activities.

For each enzyme measured, the activity was greatest for asthmatics not receiving steroid therapy, intermediate for asthmatics receiving steroids, and lowest for the nonasthmatic subjects. Considerable variation in all activities was observed within any group of subjects, while variations less than 20% were recorded in repeated experiments with the same individual. Data for all cell fractions is summarized in Table II as ratios of specific activity for the three groups of subjects. The ratio of activities for asthmatics to nonasthmatics ranged from 1.59 to 1.76, and the ratio for asthmatics to steroid-treated asthmatics ranged from 1.30 to 1.49. Differences between the steroid-treated group and the nonasthmatics were smaller and were not significant for ecto and supernatant ATPase activities.

To test the effects of drugs commonly used for the treatment of asthma on ATPase activities, three nonasthmatic adult males volunteered to take tablets containing ephedrine, theophylline, and phenobarbital for several days. No significant changes in any of the ATPase activities were noted after this regimen, which ranged from 5 days for two subjects to 8 days for the third subject (Table III).

In further attempts to explain the different ATPase activities among the three groups of subjects we compared the distribution of protein and total enzyme activity among the cell fractions (Table IV). A range

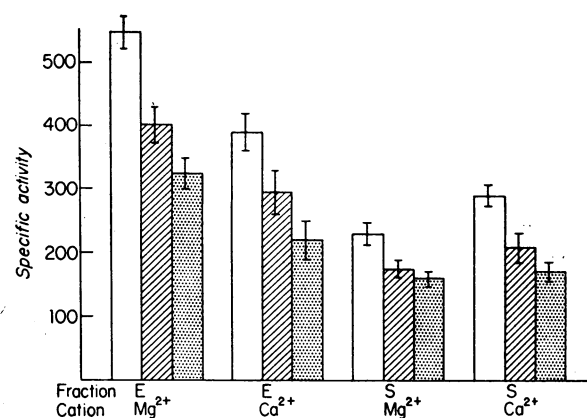


FIGURE 5 Supernatant and ecto ATPase activities. The mean ATPase specific activities (nmol ADP/30 min/mg protein) are plotted for ecto (E) and supernatant (S) fractions of leukocytes from 24 asthmatic children not receiving steroids (clear bars), 17 asthmatic children receiving steroid therapy (hatched bars), and 20 nonasthmatic children (dotted bars). Error bars indicate SEM.

of 30–38% of total leukocyte protein was found in the P_1 fraction, 21–25% in the P_2 fraction, and 40–45% in the supernatant. These values were variable, possibly due to minor differences in the extent of cell disruption, but no significant differences were found among the means for the groups of subjects. Also, no significant differences were noted between males and females within any group of subjects when comparing protein distribution or ATPase activities.

Effects of hydrocortisone in vitro. Two types of experiments were performed in an effort to duplicate in vitro the effect of corticosteroid therapy on leukocyte ATPase activity. First (Table V), hydrocortisone was added directly to membranes of leukocytes and incubated for 10 min at 37°C before addition of ATP. The

TABLE II
Ratios of ATPase Specific Activity

Cell fraction	Cation	A/N	P	A/A + S	P	A + S/N	P
P_1	Mg	1.62	<0.01	1.37	<0.01	1.18	NS
P_1	Ca	1.66	<0.01	1.31	<0.01	1.27	<0.02
P_2	Mg	1.62	<0.01	1.32	<0.01	1.23	<0.02
P_2	Ca	1.59	<0.01	1.30	<0.01	1.22	<0.02
E	Mg	1.67	<0.01	1.35	<0.01	1.24	NS
E	Ca	1.76	<0.01	1.31	<0.05	1.35	NS
S	Mg	1.68	<0.01	1.34	<0.05	1.26	NS
S	Ca	1.72	<0.01	1.49	<0.01	1.23	NS

Ratios of ATPase specific activity (nmol ADP per mg protein per 30 min) are indicated for the groups of subjects: A, asthmatics not receiving steroids ($n = 24$); A + S, asthmatics receiving steroid therapy ($n = 17$); and N, normal children ($n = 20$). P values were calculated from the individual specific activity data by Student's *t* test; NS indicates no significant difference (*P* not less than 0.05).

TABLE III
Effect of Bronchodilator Drugs on Leukocyte ATPase

Subject	(days)	Fraction	Mg ²⁺ -ATPase		Ca ²⁺ -ATPase	
			Before	After	Before	After
K. M.	(5)	P ₁	328±11	333±15	219±14	234±7
		P ₂	529±23	553±31	381±28	421±19
		E	246±10	255±13	159±12	172±5
		S	103±4	107±7	123±11	133±9
R. G.	(6)	P ₁	550±8	503±11	350±9	342±5
		P ₂	889±16	727±23	665±7	636±9
		E	389±15	343±12	254±6	246±12
		S	170±13	148±6	210±11	215±4
D. C.	(8)	P ₁	417±18	395±19	275±14	286±5
		P ₂	680±31	649±25	473±38	501±3
		E	320±15	302±16	191±12	200±9
		S	129±7	125±9	149±8	143±6

Leukocyte ATPase activities were determined by ADP assay in triplicate as described in Methods for three nonasthmatic adult males before and after taking bronchodilator medication (Verequad, Knoll Pharmaceutical Company, Orange, N. J., consisting of ephedrine, theophylline, and phenobarbital) four times a day for the indicated number of days. Specific activities (nmol ADP per 30 min per mg protein) ±SD are given for leukocyte enzyme preparations P₁, P₂, E (ecto), and S (supernatant) before and after taking medication. No significant differences occurred in any of the activities due to the drugs.

Mg²⁺-ATPase activity was reduced in a dose-dependent manner in three experiments by hydrocortisone ranging from 10⁻⁶ to 10⁻³ M. To achieve the highest hydrocortisone concentrations, hydrocortisone sodium succinate was used because of its greater water solubility.

TABLE IV
Total Protein and ATPase Units in Leukocytes

Fraction:	P ₁	P ₂	S
Protein: percent found in subcellular fractions			
Asthmatics not on steroids	38	22	40
Asthmatics receiving steroids	35	21	44
Nonasthmatics	30	25	45
ATPase: total units per 100 mg leukocyte protein*			
Mg ²⁺ -ATPase			
Asthmatics not on steroids	2,700	2,500	920
Asthmatics receiving steroids	1,800	1,810	750
Nonasthmatics	1,310	1,750	612
Ca ²⁺ -ATPase			
Asthmatics not on steroids	1,910	1,840	1,160
Asthmatics receiving steroids	1,340	1,350	915
Nonasthmatics	910	1,320	760

* Approximately 70 μg protein per million leukocytes were measured in HC10₄ precipitates by the technique of Lowry et al. (17).

In controls with 5 × 10⁻³ M sodium succinate, the ATPase activity was not affected, indicating that inhibition by hydrocortisone sodium succinate was due to the steroid.

Second (Table VI), intact leukocytes were preincubated with 10⁻⁶ M hydrocortisone at 37°C for varying times before the preparation of membranes. In the first experiment progressive inhibition was observed for preincubation times ranging from 5 to 120 min. In the second experiment puromycin was added to the cells in one set of tubes during a 60-min preincubation with 10⁻⁶ M hydrocortisone. The puromycin caused a 16% decrease in Mg²⁺-ATPase specific activity measured subsequently in the membranes, but did not alter the percent decrease due to hydrocortisone. This suggests that inhibition by hydrocortisone is not mediated by effects on protein synthesis.

DISCUSSION

The beta adrenergic blockade hypothesis of asthma advanced by Szentivanyi (6) has prompted a search for abnormalities of function and sensitivity in the adenylate cyclase-cyclic AMP system of various cells and tissues of patients with asthma. A number of studies with isolated leukocytes by ourselves (1, 2, 14), Parker and coworkers (3, 9, 19), Alston, Patel, and Kerr (4) and Gillespie, Valentine, and Lichtenstein (5) have provided evidence indicating that in asthma this system

manifests a reduced sensitivity to beta adrenergic stimulation:

- (a) Basal activity of adenylate cyclase is depressed (2, 3, 5, 9, 19).
- (b) Beta adrenergic stimulation of adenylate cyclase is depressed (1-5); however, prostaglandin E₁ stimulation is normal (19).
- (c) Corticosteroid treatment of patients in vivo and of cells in vitro restores basal levels of adenylate cyclase (9), and restores normal beta adrenergic sensitivity (1, 9).
- (d) Alpha adrenergic blockade in vitro restores normal beta adrenergic sensitivity of adenylate cyclase (2, 4).

The accumulated evidence, while not explaining why, indicates that this system is suppressed and most specifically to beta adrenergic stimulation. The restoration of normal responsiveness with steroids and with alpha adrenergic blockade indicates that the reduced responsiveness might result from enhanced alpha adrenergic sensitivity or reduced steroid sensitivity.

These possibilities provided the rationale for examining other enzyme systems for abnormality of function and sensitivity. In many cells there appears to be a reciprocal relationship between alpha and beta adrenergic responses (7, 20, 21) and between ATPase and adenylate cyclase activities (22, 23). This may be due in part to competition by the two enzymes for substrate (8, 16) and in part to direct inhibition of ATPase by cyclic AMP (23, 24). Our experiments indicated that membrane ATPase activity of leukocytes and other hematopoietic cells can be stimulated by catecholamines through an alpha adrenergic mechanism (12, 13). Others have reported stimulation by catecholamines of Mg²⁺-ATPase in brain (25) and erythrocytes (26), and of Na⁺, K⁺, Mg²⁺-ATPase of brain (27) and liver (24).

TABLE V
Hydrocortisone Inhibition of Mg²⁺-ATPase

	Hydrocortisone	Hydrocortisone Na succinate	
	10 ⁻⁶ M	10 ⁻⁴ M	10 ⁻³ M
Exp. 1	98	93	81*
Exp. 2	85*	74*	50*
Exp. 3	94	80*	68*

Data is given as percent of control Mg²⁺-ATPase activity measured in nonasthmatic leukocyte membranes (P₁ + P₂) in replicates of six as described in Methods. Standard deviations (not shown) were less than 5% in all cases. Hydrocortisone or hydrocortisone sodium succinate (Sigma Chemical Co., St. Louis, Mo.) was added in 0.1 ml aliquots of Tris buffer, pH 7.5, at 5 times the indicated final concentration and incubated with membranes at 37°C for 10 min before adding ATP.

* Significant inhibition occurred.

TABLE VI
Preincubation of Leukocytes with Hydrocortisone

	Preincubation minutes	Control activity	Percent of control
Exp. 1	5	552	80
1	45	552	65
1	120	552	51
Exp. 2	60	423	87
2*	60	397	85

Leukocytes from nonasthmatic subjects were preincubated at 37°C in Hank's balanced salt solution for the indicated time with or without 10⁻⁶ M hydrocortisone (Sigma Chemical Co., St. Louis, Mo.) in replicates of six. Cells were then washed twice with saline and sonicated. Mg²⁺-ATPase of membranes (P₁ + P₂) was determined in triplicate for each preincubation tube. Control activity is expressed as nmol ADP per mg protein per 30 min. Inhibition by preincubation with hydrocortisone was significant (*P* < 0.01) in every case.

* Puromycin was present at 100 µg/ml in the preincubation mixture.

These observations supporting the theory (11) of alpha adrenergic modulation of ATPase prompted us to examine the leukocytes of asthmatics and controls for altered basal levels of ATPase activity which might reflect in vivo adrenergic imbalance. Intact cells were examined for ecto ATPase activity. The membrane ATPase activities of disrupted cells were separated by rate-zonal centrifugation to yield crude fractions representing nuclei and large fragments of plasma membrane (P₁) and smaller fragments of membranes corresponding to plasma, mitochondrial, and ribosomal constituents (P₂). These fractions represent the major subcellular components containing ATPase activities (over 80% of the total) and were assayed in the presence of magnesium or calcium ions to optimize those conditions under which alpha adrenergic stimulation had been previously observed (12, 13). These fractions while admittedly crude correspond to the magnesium- and calcium-dependent ATPase activities associated by others with cellular functions of magnesium (28) and calcium (29, 30) transport, passive permeability (31), nutrient transport (16, 32, 33), and locomotive (34) and secretory functions (35).

In each of the experimental conditions the leukocytes of asthmatic patients showed greater ATPase activity than those of age-sex matched subjects without asthma. In addition, asthmatic patients treated with corticosteroids showed an intermediate ATPase activity consistent with their clinical improvement on the steroid therapy. This increased ATPase activity manifest in the leukocytes of asthmatics could not be attributed to treatment with other bronchodilator therapy since these

medications showed no effect on the leukocyte ATPase activity of normal subjects so treated. Similarly, no real differences in component cell populations studied existed to explain the differences in ATPase activity observed. The increased leukocyte ATPase activity would appear then to be intrinsic to the asthmatic state.

Corticosteroid treatment of both the patient and the isolated leukocytes was associated with a reduction of the increase in basal ATPase activity of the cells. The effect of hydrocortisone at physiologic concentrations to reduce ATPase activity in a puromycin-resistant manner, in conjunction with its direct effect at somewhat higher concentrations to reduce ATPase activity in isolated membrane fractions, offers evidence for a direct membrane action of hydrocortisone independent of the more generally accepted action to modify nuclear metabolism and protein synthesis (36). This effect of corticosteroids to decrease ATPase activity has been observed with in vivo treatment followed by isolation of various tissues (37-39) and with in vitro treatment of a cultured cell line (40). The biochemical basis of this action remains to be clarified.

The interpretation of the present findings in relation to previous conceptions concerning beta adrenergic blockade, although tentative at this time, raises important questions concerning the relationship of the adenylate cyclase-cyclic AMP system to other hormone responsive enzyme systems involved in cellular regulatory function. Based upon experiments indicating that beta adrenergic stimulation is associated with depressed allergic mediator release from mast cells and leukocytes (41, 42) and with bronchodilation (6), it may be concluded that the asthmatic patient being defective in response to this stimulation is prone to enhanced immunologically induced mediator release and subsequent bronchoconstriction. Both alpha adrenergic and cholinergic mechanisms have recently been implicated in both enhanced allergic mediator release (43) and in bronchoconstrictive mechanisms (7, 44). Insofar as these mechanisms appear to be enhanced in their activity in asthma, it seems reasonable to speculate that the enzyme systems mediating these influences will be found to be increased in their activity. The alpha adrenergic-ATPase relationship is suggested by the present studies. Similarly, cholinergic-cyclic GMP relationships have been observed in a number of tissues (45) including lymphocytes (45-47) and lung (45), and the cyclic GMP second messenger system has been proposed as an opposing influence to the cyclic AMP system (45, 48). The suggestion that cyclic GMP may be increased in the tissues of asthmatics, thus facilitating bronchoconstriction and mediator release, was made by Polson, Krzanowski, and Szentivanyi (49), who have shown that manipulations producing an asthma-

like condition in rodents are associated with an increase in the levels of cyclic GMP in their lungs. We predict, then, that the beta adrenergic defect observed in the cells of asthmatics may well result from more primary imbalances in the membrane ATPase and guanylate cyclase-cyclic GMP systems.

The therapeutic approach to patients with asthma has been to stimulate their adenylate cyclase-cyclic AMP system through the combined use of beta adrenergic agonists (isoproterenol and epinephrine), cyclic AMP phosphodiesterase inhibitors (theophylline and caffeine), and glucocorticosteroids. This approach is pharmacologically sound and is usually associated with symptomatic improvement. The present findings concerning ATPase suggest that the adrenergic imbalance present in asthma is associated with increased activity of other enzyme systems and raises the possibility that were these systems to be better understood, a more primary defect might emerge, which would allow the development of a more effective patient therapy.

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REFERENCES

1. Logsdon, P. J., E. Middleton, Jr., and R. G. Coffey. 1972. Stimulation of leukocyte adenylyl cyclase by hydrocortisone and isoproterenol in asthmatic and nonasthmatic subjects. *J. Allergy Clin. Immunol.* **50**: 45-56.
2. Logsdon, P. J., D. V. Carnright, E. Middleton, Jr., and R. G. Coffey. 1973. The effect of phentolamine on adenylate cyclase and on isoproterenol stimulation in leukocytes from asthmatic and nonasthmatic subjects. *J. Allergy Clin. Immunol.* **52**: 148-157.
3. Parker, C. W., and J. W. Smith. 1973. Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. I. Leukocyte responsiveness to β -adrenergic agents. *J. Clin. Invest.* **52**: 48-59.
4. Alston, W. C., K. R. Patel, and J. W. Kerr. 1974. Response of leukocyte adenylyl cyclase to isoprenaline and effect of alpha-blocking drugs in extrinsic bronchial asthma. *Br. Med. J.* **1**: 90-93.
5. Gillespie, E., M. Valentine, and L. M. Lichtenstein. 1974. Cyclic AMP metabolism in asthma: studies with leukocytes and lymphocytes. *J. Allergy Clin. Immunol.* **53**: 27-33.
6. Szentivanyi, A. 1968. The beta adrenergic theory of the atopic abnormality in bronchial asthma. *J. Allergy.* **42**: 203-232.
7. Middleton, E., Jr. 1972. Autonomic imbalance in asthma with special reference to beta adrenergic blockade. *Adv. Inter. Med.* **18**: 177-197.
8. Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Cyclic AMP. Academic Press, Inc., New York. 223-228.

9. Parker, C. W., M. G. Huber, and M. L. Baumann. 1973. Alterations in cyclic AMP metabolism in human bronchial asthma. III. Leukocyte and lymphocyte responses to steroids. *J. Clin. Invest.* **52**: 1342-1348.
10. Townley, R. G., T. Honrath, and H. M. Guirgis. 1972. The inhibitory effect of hydrocortisone on the alpha adrenergic responses of human and guinea pig isolated respiratory smooth muscle. *J. Allergy Clin. Immunol.* **49**: 88. (Abstr.).
11. Belleau, B. 1967. Stereochemistry of adrenergic receptors: newer concepts of the molecular mechanism of action of catecholamines and antiadrenergic drugs at the receptor level. *Ann. N. Y. Acad. Sci.* **139**: 580-605.
12. Coffey, R. G., J. W. Hadden, E. M. Hadden, and E. Middleton, Jr. 1971. Stimulation of ATPase by norepinephrine: an alpha-adrenergic receptor mechanism. *Fed. Proc.* **30**: 497. (Abstr.).
13. Coffey, R. G., and E. Middleton, Jr. 1973. Release of histamine from rat mast cells by lysosomal cationic proteins. Possible involvement of adenylate cyclase and adenosine triphosphatase in pharmacologic regulation. *Int. Arch. Allergy Appl. Immunol.* **45**: 593-611.
14. Falliers, C. J., R. R. de A. Cardoso, H. N. Bane, R. Coffey, and E. Middleton, Jr. 1971. Discordant allergic manifestations in monozygotic twins: Genetic identity vs. clinical, physiologic, and biochemical differences. *J. Allergy.* **47**: 207-219.
15. Chai, H., and A. Gilbert. 1973. The effect of alternate-day prednisone on the white blood count in children with chronic asthma. *J. Allergy Clin. Immunol.* **51**: 65-70.
16. Hadden, J. W., E. M. Hadden, E. E. Wilson, R. A. Good, and R. G. Coffey. 1972. Direct action of insulin on plasma membrane ATPase activity in human lymphocytes. *Nat. New Biol.* **235**: 174-176.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
18. Block, J. B., and S. L. Bonting. 1964. Sodium-potassium activated adenosinetriphosphatase and cation transport in normal and leukemic human leukocytes. *Enzymol. Biol. Clin.* **4**: 183-198.
19. Parker, C. W., M. L. Baumann, and M. G. Huber. 1973. Alterations in cyclic AMP metabolism in human bronchial asthma. II. Leukocyte and lymphocyte responses to prostaglandins. *J. Clin. Invest.* **52**: 1336-1341.
20. Watlington, C. O. 1969. α -Adrenergic inhibition of sodium ion transport: the interaction of vasopressin and 3',5'-AMP. *Biochim. Biophys. Acta.* **193**: 394-402.
21. Turtle, J. R., and D. M. Kipnis. 1967. An adrenergic receptor mechanism for the control of cyclic 3',5'-adenosine monophosphate synthesis in tissues. *Biochem. Biophys. Res. Commun.* **28**: 797-802.
22. Mosinger, B., and M. Vaughan. 1969. Adenosine 3',5'-monophosphate and regulation of lipolysis in rat adipose tissue. *Adv. Exp. Med. Biol.* **3**: 63-74.
23. Mózsik, G. Y. 1970. Direct inhibitory effect of adenosine monophosphates on Na⁺-K⁺-dependent ATPase prepared from human gastric mucosa. *Eur. J. Pharmacol.* **9**: 207-210.
24. Luly, P., O. Barnabei, and E. Tria. 1972. Hormonal control in vitro of plasma membrane bound (Na⁺-K⁺)-ATPase of rat liver. *Biochim. Biophys. Acta.* **282**: 447-452.
25. Kaack, B. 1972. Influence of acetylcholine and adrenaline on ATPase activity. *Comp. Gen. Pharmacol.* **3**: 296-298.
26. Mirčevová, L., and A. Šimonová. 1969. Effect of adrenaline, noradrenaline, and insulin on Mg⁺⁺-dependent ATPase. *Experientia (Basel).* **25**: 1028-1029.
27. Yoshimura, K. 1973. Activation of Na-K activated ATPase in rat brain by catecholamine. *J. Biochem.* **74**: 389-391.
28. Beauchamp, R. S., S. Silver, and J. D. Hopkins. 1971. Uptake of Mg²⁺ by KB cells. *Biochim. Biophys. Acta.* **225**: 71-76.
29. Whittam, R., and K. P. Wheeler. 1970. Transport across cell membranes. *Annu. Rev. Physiol.* **32**: 21-60.
30. Schatzman, H. J., and G. L. Rossi. 1971. The (Ca²⁺ + Mg²⁺)-activated membrane ATPase in human red cells and their possible relations to cation transport. *Biochim. Biophys. Acta.* **241**: 379-392.
31. Bowler, K., and C. J. Duncan. 1967. Evidence implicating a membrane ATPase in the control of passive permeability of excitable cells. *J. Cell Physiol.* **70**: 121-126.
32. Hadden, J. W., E. M. Hadden, and R. G. Coffey. 1971. Lymphocyte responses to hormones: stimulation of glucose uptake and membrane ATPase by insulin and norepinephrine. *Fed. Proc.* **30**: 687. (Abstr.).
33. Hadden, J. W., E. M. Hadden, and R. A. Good. 1971. Alpha adrenergic stimulation of glucose uptake in human erythrocyte, lymphocyte, and lymphoblast. *Exp. Cell Res.* **68**: 217-219.
34. Huxley, H. E. 1973. Muscular contraction and cell motility. *Nature (Lond.)*. **243**: 445-449.
35. Stormorken, H. 1969. The platelet release reaction. Its General aspects and relation to phagocytosis/pinocytosis. *Scand. J. Clin. Lab. Invest. Suppl.* **107**: 115.
36. O'Malley, B. W. 1971. Mechanisms of action of steroid hormones. *N. Engl. J. Med.* **284**: 370-377.
37. Takagi, I., and K. Yamamoto. 1969. Effects of thyroidectomy, adrenalectomy, thyroid hormones, and glucocorticoids on Na⁺, K⁺-activated ATPase of rat anterior pituitary. *Jap. J. Physiol.* **19**: 465.
38. Ribo, J. M., and F. Ponz. 1969. Magnesium-activated ATPase in the sodium- and potassium-dependent enzyme system from rat small intestine. *Rev. Esp. Fisiol.* **25**: 257-266.
39. Hellerström, C., I. Täljedal, and B. Hellman. 1965. Quantitative studies on isolated pancreatic islets of mammals. 5' Nucleotidase and adenosine triphosphatase activities in normal and cortisone-treated rats. *Endocrinology.* **76**: 315-322.
40. Melnykovych, G., M. A. Swayze, C. F. Bishop, and C. Costlow. 1969. Effects of prednisolone on esterases in heteroploid cells of human origin. *Biochim. Biophys. Acta.* **184**: 672-674.
41. Orange, R. P., M. A. Kaliner, P. J. LaRaia, and K. F. Austen. 1971. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. II. Influence of cellular levels of cyclic AMP. *Fed. Proc.* **30**: 1725-1729.
42. Lichtenstein, L. M., and R. DeBernardo. 1971. Immediate allergic response: in vitro action of cyclic AMP-active and other drugs on the two stages of histamine release. *J. Immunol.* **107**: 1131-1136.
43. Kaliner, M., R. P. Orange, and K. F. Austen. 1972. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV. En-

- hancement by cholinergic and α -adrenergic stimulation. *J. Exp. Med.* **136**: 556-567.
44. Reed, C. E. 1974. Abnormal autonomic mechanisms in asthma. *J. Allergy Clin. Immunol.* **53**: 34-41.
 45. Goldberg, N. D., M. K. Haddox, D. K. Hartle, and J. W. Hadden. 1973. The biological role of cyclic 3',5'-guanosine monophosphate. *In* Pharmacology and the Future of Man. Proceedings of the 5th International Congress of Pharmacology. Karger, Basel. **5**: 146.
 46. Hadden J. W., E. M. Hadden, and N. D. Goldberg. Cyclic GMP and Cyclic AMP in lymphocyte metabolism and proliferation. *In* Cyclic AMP, Immune Response and Tumor Growth. W. Braun, L. M. Lichtenstein, and C. W. Parker, editors. Springer Verlag Inc., New York. In press.
 47. Strom, T. B., C. B. Carpenter, M. R. Garovoy, K. F. Austen, J. P. Merrill, and M. Kaliner. 1973. The modulating influence of cyclic nucleotides upon lymphocyte-mediated cytotoxicity. *J. Exp. Med.* **138**: 381-393.
 48. Goldberg, N. D., M. K. Haddox, E. Dunham, C. Lopez, and J. W. Hadden. 1974. The Yin Yang hypothesis of biological control: opposing influences of cyclic GMP and cyclic AMP in the regulation of cell proliferation and other biological processes. *In* Cold Spring Harbor Conferences on Cell Proliferation. Vol. 1. Control of Proliferation in Animal Cells. B. Clarkson and R. Baserga, editors. 609-626.
 49. Polson, J. B., J. J. Krzanowski, and A. Szentivanyi. 1974. The effect of histamine (H) on the pulmonary levels of cyclic nucleotides in normal mice and under conditions of pharmacological or bacterial sensitization. *J. Allergy Clin. Immunol.* **53**: 100. (Abstr.).