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Exercise induced bronchospasm and the effect of ascorbic acid : a study of the possible role of prostaglandins

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EXERCISE INDUCED BRONCHOSPASM AND
ITS EFFECT ON ASSOCIATED ACTIVITY:
A STUDY OF THE POSSIBLE ROLE OF PROSTAGLANDINS

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EXERCISE INDUCED BRONCHOSPASM AND THE EFFECT OF ASCORBIC ACID: A STUDY OF THE POSSIBLE ROLE OF PROSTAGLANDINS

by

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ABSTRACT

The role of ascorbic acid in the treatment of asthma remains undefined. Ascorbic acid has previously been associated with partial protection against a number of asthmatic reactions. Since this agent is also known to modify prostaglandin synthesis a study was designed to examine the possible role of prostaglandins in exercise induced bronchospasm (EIB). Twelve subjects were recruited on the basis of a history consistent with EIB and the demonstration of a 20% decrease from baseline in MEF40% (P) during a preliminary exercise trial. On four subsequent test days the subjects ingested either: (1) 750 mg ascorbic acid, (2) 50 mg indomethacin, (3) 750 mg ascorbic acid plus 50 mg indomethacin, or (4) a placebo. These substances were given in a double-blind, randomized, crossover fashion. Exercise challenge with pulmonary function testing was performed on each drug day. For the group as a whole this study did not demonstrate the previously described attenuation of EIB by ascorbic acid. Neither indomethacin alone or in combination with ascorbic acid influenced the post-exercise bronchoconsriction seen in this group. Nevertheless, three subjects did demonstrate a consistent attenuation of EIB following treatment with ascorbic acid. The three "ascorbic acid responders" could be separated from the other subjects on the basis of a lower baseline FEV1 (expressed as percent of the predicted value). These and previous observations suggest that the effect of ascorbic acid in EIB may occur only in a subgroup of patients with EIB.

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DEDICATION:

TO MOM AND DAD

FOR ALL THEY HAVE DONE TO HELP ME GET THIS FAR

CONTENTS

LIST OF TABLES

I

EXERCISE INDUCED BRONCHOSPASM

Exercise induced bronchospasm is a phenomenon that was described as early as the first century A.D. when Aretaeus the Cappadocian noted the association between strenuous exercise and acute airway obstruction (Adams, 1856). This association, like many such early observations in medicine, was to be redescribed hundreds of years later. In 1698 Sir John Floyer not only redescribed exercise induced bronchospasm (EIB), but also pointed out that tasks having the highest levels of ventilation seemed to provoke the most severe wheezing (Floyer, 1698). Neither Floyer nor other researchers in the next two hundred and fifty years appreciated the full significance of this observation.

Henry Hyde Salter, yet another keen observer whose thoughts were not appreciated until recently, recognized in 1864 that EIB was exacerbated by exercising in a cold environment (Salter, 1864).

In 1946 Herxheimer performed the first study that objectively measured changes in lung function in response to exercise (Herxheimer, 1946). Herxheimer measured the change in vital capacity of asthmatics after exercise and postulated that hyperventilation was the key factor in EIB. A great deal of research was sparked by Herxheimer's findings. However, confusion and controversy surrounded this work on EIB.

The first point of contention arose from studies by Capel and Smart (Capel, 1959) and Engstrom (Engstrom, 1960) which, unlike previous stud-

ies, demonstrated an increase in FEV1 and lung compliance and a decrease in airway resistance in asthmatics after exercise. Jones et al believed that this apparent variability in response to exercise was due to differences in the duration and intensity of the exercise in these different studies. He showed that this was indeed the case, as asthmatic children exercising for less than two minutes had a slight increase in FEV1, while those exercising for between eight and ten minutes had a consistent decrease in FEV1 after exercise (Jones, 1962).

This initial confusion illustrates the principal sources of controversy in EIB research which for many years arose from the variability of the exercise challenges (type of exercise, duration, and intensity) and pulmonary function tests used to study EIB. These exercise challenges have included swimming (Fitch, 1971), stairclimbing (McNeill, 1966), free running (Jones, 1966), treadmill running (Sly, 1970), and cycling on a cycloergometer (Pierson, 1970). Parameters used to measure the response to exercise include FVC and TLC (Anderson, 1972), FEV1 (Jones, 1963), PEFR (Sly, 1970), maximal mid-expiratory flow rates (MMEFR) and airway resistance or conductance (Haynes, 1976), as well as flow rates at low lung volumes on MEFV and PEFV curves (Schachter, 1978a). Given the variability of the challenge from study to study, as well as differences in the severity of the asthma of the subjects studied, it is easy to see how estimates of the prevalence of EIB in asthmatics have ranged from nineteen percent (Cropp, 1975) to as high as ninety percent (Kivilloog, 1975). The difficulty of studying the mechanism of EIB under such conditions can also be appreciated.

The issue of which parameters are the most sensitive and most accurate has been addressed by Bouhuys. Bouhuys observed that the more traditional PFTs (such as FEV1 and PEFR) are not very sensitive to small changes in airway caliber and, moreover, are affected by the effort made by the subject performing the maneuver. These concepts are supported by a study by Pride which shows that subjects are able to attain an adequate PEFR even with significant obstruction if their vital capacity is adequate and ample effort is made (Pride, 1971). Other workers have also demonstrated that a deep inspiration, such as that made in generating a MEFV curve, could by itself lead to transient bronchoconstriction (Lloyd, 1963 and Nadel, 1961). Bouhuys was able to show that this difficulty could be avoided by using flow rates on PEFV curves, since the maneuver is made from only a partial inspiration (Bouhuys, 1969). Thus, Bouhuys advocated the measurement of instantaneous flow rates at lower lung volumes: MEF40% and MEF40%(P) (Bouhuys, 1976). Using such measurements in 1978 Schachter et al were able to demonstrate that in mild asthmatics the MEF40%(P) was the most sensitive parameter in detecting EIB (Schachter, 1978a). In Schachter's study, using a ten percent decrease in a parameter as a significant response, MEF40%(P) identified EIB in sixteen of nineteen asthmatics, while none of the seven nonasthmatic controls were shown to have EIB. Only seven of the nineteen asthmatics would have been identified as having EIB if a ten percent drop in FEV1 had been used as the defining parameter. Thus, parameters using PEFV curves at low lung volumes are sensitive to small degrees of bronchoconstriction.

The other type of pulmonary function testing that has been used extensively in measuring bronchoconstriction is volume displacement body plethysmography and the determination of airway resistance or specific airway conductance (Bouhuys, 1969, Bouhuys, 1970, and Haynes 1976). One problem with such measurements is that they are believed to be an indication of constriction of larger airways, while asthma (especially mild bronchosapasm) may be a disorder of the smaller, more peripheral airways. As obstruction becomes more severe in asthma, however, larger airways are more likely to become involved.

Despite the variability in the earlier studies following Herxheimer's work, the early studies described a rather consistent course for EIB (Jones, 1962, McNeill, 1966, Godfrey, 1975, and Anderson, 1975). McFadden and Ingram describe EIB stating:

> The pattern of response to exercise is characteristic. Moderate bronchodilation occurs during the early part of exercise and gives way to bronchoconstriction after work ceases. The airway obstruction is progressive, generally reaching its peak 5-10 min after exercise has stopped. The obstruction then begins to abate. Spontaneous recovery is usual, tending to be complete within 30-90 min, depending upon the severity of bronchospasm (McFadden, 1983).

Thus, by the early 1970's EIB was a better characterized phenomenon. In addition, the development of more sensitive ways to measure EIB and new pharmacological agents were inspiring further studies addressing the pathogenesis of EIB.

II

THE TRIGGER OF EIB: RESPIRATORY HEAT LOSS

A major question that interested investigators was what feature of exercise specifically triggers EIB. Three major factors were suspected: (1) hypocapnia from the hyperventilation of exercise, (2) stimulation of airway mechanoreceptors by hyperventilation, and (3) lactic acid release from skeletal muscle during exercise. Each of these hypotheses had supporting evidence, but conclusive studies awaited the development of a partial rebreathing technique that allowed the study of the effects of hypocapnia, hyperpnea, and acidosis independently of one another (McFadden, 1977a).

Support for hypocapnia as a cause of bronchoconstriction was based on three lines of evidence. First, Hafez and Crompton demonstrated that asthmatics had a decrease in FEV1 after voluntary hyperventilation that did not occur in bronchitics or control subjects. This decrease in FEV1 did not occur, however, in the subjects when hyperventilation was induced while the subjects were breathing 5% carbon dioxide (Hafez, 1968). Second, Ferguson found the arterial partial pressure of carbon dioxide to be lower after exercise in patients with EIB than in healthy controls or even in patients with diffuse lung disease. As in the first study cited above, Ferguson's asthmatics had a post-exercise decrease in FEV1 that was blocked by the addition of carbon dioxide (3% in this case) to the inspired air (Ferguson, 1969). Similarly, Fisher showed

that the administration of 6-8% carbon dioxide could prevent bronchospasm induced by running (Fisher, 1970). Of note, this EIB was not prevented by inhalation of atropine sulfate.

Several other researchers, however, were obtaining results quite different from those claiming that hypocapnia was the trigger for EIB. Chan-Yeung demonstrated a correlation between the degree of hyperventilation and the post-exercise decline in FEV1, but found a greater decline in FEV1 when subjects inspired 5.6% carbon dioxide during exercise (Chan-Yeung, 1971). Stanescu and Teculescu reported the case of a patient with EIB whose attacks were not prevented by inhaling as much as 6.5% carbon dioxide (Stanescu, 1970). These workers, as well as other groups (Simonsson, 1972a, Bar-Or, 1977, and Sheppard, 1982), found some patients who experienced less bronchoconstriction from exercise, cold air, or hyperventilation when pre-treated with atropine. These authors postulated that EIB resulted from hyperventilation stimulating airway receptors acting via a vagal reflex.

The use of a partial rebreathing technique by McFadden's group led to the conclusion that hypocapnia from hyperventilation was not the cause of EIB. This technique involved having subjects physically at rest breathing to simulate the minute ventilations reached in exercise, while varying the end-tidal carbon dioxide tensions. The bronchospasm produced by hypocapnia or hyperventilation, even with higher end-tidal carbon dioxide levels, were quite small in comparison to that produced by actual exercise (McFadden, 1977a). Hypocapnia did not adequately account for the initiation of EIB.

Another factor proposed by other researchers as the inciting stimulus in EIB was the release of lactic acid resulting from the anaerobic metabolism of exercising muscles. Several studies (Seaton, 1969, Fisher, 1970, and Vassalo, 1972) documented either a metabolic acidosis, increased lactate, or both in subjects with EIB. However, Silverman found that patients exercising on a treadmill had more severe EIB than those exercising on a bicycle, despite the subjects having higher arterial blood lactate levels during bicycling. Furthermore, the arterial blood pH was similar in the two forms of exercise (Silverman, 1972a). Strauss performed a study that convincingly eliminated acidosis as the stimulus for bronchoconstriction in exercise. The first part of this study involved measuring blood pH during exercise. The exercise challenge was then repeated while infusing enough sodium bicarbonate into each subject to prevent acidemia. All of the subjects experienced EIB of the same severity on both the control and bicarbonate days. The second part of this study involved the determination of the lowest amount of exercise and lactate level needed to precipitate EIB in each subject. This "threshold" dose of lactate was then infused into each subject at rest. None of the subjects developed bronchoconstriction with this lactic acidemia (Strauss, 1977a). This effectively ruled out a role for lactic acidemia as the trigger for EIB.

The key to the initiation of EIB was elucidated by Deal and McFadden's group once they returned to examine the very phenomenon observed by Salter in 1864: the effect of temperature on EIB. In their initial study on the effects of temperature they found that cold air had a rather small, but statistically significant, bronchoconstrictive effect on

resting asthmatic subjects. However, inspiring cold air during exercise caused a markedly enhanced bronchospastic response. Specific conductance decreasd 85% and FEV1 decreased 100% more than when subjects exercised while breathing ambient air (Strauss, 1977b). Further studies by this group and others confirmed that the severity of EIB was determined not only by the temperature, but also by the humidity of the inspired air (Bar-Or, 1977, Chen, 1977, Strauss, 1978, Deal, 1979a, and Deal, 1979b). Cooling and drying the inspired air caused increased bronchospasm, while heating and humidifying inspired air decreases the response to exercise. McFadden summarizes how this occurs in the airways as follows:

> With exercise, as ventilation increases to meet metabolic demands, a large volume of air at ambient conditions is brought into the thorax where it must be heated to 37 degrees Centigrade and fully saturated with water vapor before it reaches the alveoli. This requires evaporation of water and the transfer of heat from the mucosal surfaces of the airways. The colder the air, the drier it is, and so more heat and water are transferred, and so the more mucosal temperature drops. The degree of obstruction that develops is directly related to the total amount of heat transferred and so to the degree of cooling that develops.... (McFadden, 1981)

This relationship can be quantified and it has been shown that respiratory heat loss varies directly with the level of ventilation and inversely with the temperature and humidity of the inspired air (Deal, 1979a and Deal, 1979b). This respiratory heat loss, in turn, correlates directly with the percent drop in FEV1 post-exercise in asthmatics (Deal, 1979b).

III

BEYOND THE TRIGGER: THE AUTONOMIC NERVOUS SYSTEM

While the theory of respiratory heat loss seems to have adequately explained the initiation of EIB, much controversy remains as to the mechanism by which airway cooling leads to bronchoconstriction.

There are two areas of speculation on the effects of cooling of the airway that are worth mentioning briefly. Some preliminary in vitro animal studies "have shown that cooling of airway smooth muscle can cause constriction, as well as diminished responsiveness to isoproterenol." (McFadden, 1981) McFadden notes, however, that while there is no data for or against this in humans, the time course seen in the in vitro model is wrong for a direct constrictor effect to be the sole explanation for EIB. He points out that as soon as exercise ends, the airways begin to rewarm and return to their baseline temperature within five minutes, while bronchospasm gets progressively worse over this same time period. Therefore, something else must be sustaining the obstruction.

Another interesting speculation that is based on animal studies is the possibility of an interconversion of alpha and beta adrenergic receptors. The idea here is that cooling of tissues leads to a conversion of beta receptors to alpha receptors, so that stimulation of smooth muscle with circulating catecholamines would produce contraction rather than relaxation (Haynes, 1976, Kunos, 1980, and McFadden, 1982). As with the direct cooling theory discussed above, there is no evidence for

this phenomenon in humans and there is still the problem with accounting for the time course of the obstruction in EIB. Furthermore, the evidence for the interconversion theory comes from submammalian species and is controversial in its own right (Benfey, 1980).

The role of the autonomic nervous system in EIB remains controversial. The adrenergic system has been implicated in asthma in many different ways. Beta adrenergic stimulation has been shown to produce bronchodilatation (believed to be through activation of adenylate cyclase and increasing cAMP levels in smooth muscle cells) and to suppress IgE dependent release of mediators from lung tissue (Orange, 1971 and Webb-Johnson, 1977). Much evidence has accumulated showing asthmatics to be hyporesponsive to beta adrenergic stimulation (Reed, 1974 and Szentivanyi, 1976). Some workers have found an exaggerated rise in circulating catecholamines in asthmatics during exercise, which they postulate is an attempt to overcome receptor hyposensitivity (Griffiths, 1972 and Beil, 1977). Others have found no differences in catecholamine levels between asthmatics and healthy controls (Anderson, 1976 and Chrys-santhopoulos, 1978). One group has even reported a decreased catecholamine response following exercise in asthmatics using a newer assay (Barnes, 1981b).

Studies using beta blockers in EIB have raised the possibility of a role for alpha adrenergic involvement in EIB. A role for alpha adrenergic hyperresponsiveness has been suggested by four lines of evidence: (1) increased EIB after beta blockade (Grieco, 1971 and Jones 1972), (2) bronchoconstriction on administration of catecholamines after beta blockade (Simonsson, 1972b, Prime, 1972, and Patel, 1973), (3) reduced

EIB in some patients after alpha blockade (Bianco, 1974, Patel, 1976, and Biel, 1978), and (4) greater response to alpha stimulation in asthmatics as measured by vascular and pupillary response (Henderson, 1979). However, there are other studies showing that asthmatics may respond to alpha blockade with bronchoconstriction rather than dilatation (Spector, 1979 and Shiner, 1983). Thus, no consistent picture emerges for an adrenergic role.

The possible role of the parasympathetic branch of the nervous system in EIB was already mentioned since the mechanoreceptor theory for EIB was postulated to involve a vagal reflex.

While heat flux, and not mechanical stimulation, is now the accepted mechanism for the initiation of EIB, it has been suggested that a vagal reflex may still mediate EIB if there are temperature sensitive neural receptors in the airways (Enright, 1979 and McNally, 1979). Studies supporting this theory have found decreased EIB after the application of a topical anesthetic to the posterior pharynx and upper airways. The authors postulate that the topical anesthesia interrupts the vagal reflex. These studies, however, did not adequately control for ventilation rate or inspired air conditions. When subsequent studies were done with appropriately controlled conditions, the initial results were not duplicated (Fanta, 1980 and Griffin, 1982).

Further confusion on the role of vagal reflexes has arisen because some investigators have shown that atropine (or other anticholinergic agents) can attenuate bronchospasm produced by exercise, hyperventilation, or cold air breathing. This was found in some asthmatics, but not in others (Stanescu, 1970, Crompton, 1968, McNeill, 1966, Simonsson,

1972a, Breslin, 1980b, Deal, 1978, McFadden, 1977b, Rasmussen, 1979, and Thomson, 1978). Different inspired air conditions have also been found to have an influence on the effectiveness of anticholinergics (Deal, 1978 and Breslin, 1980b). Alternative explanations for these variable results have been: (1) the mechanism for the obstruction has more than one determinant (Thomson, 1978, Rasmussen, 1978, and McFadden, 1980), (2) anticholinergics influence respiratory heat loss themselves (Breslin, 1980b), and (3) the possibility that inadequate doses of atropine may have been used in some studies (Sheppard, 1982).

Despite the above arguments against cholinergic mediation of EIB, cholinergic agonists are known to enhance mediator release (Kaliner, 1972 and Kaliner, 1977) and there is a positive correlation between asthmatic subjects' sensitivity to methacholine challenge and the severity of their EIB (Kiviloog, 1973). Thus, the autonomic nervous system may well play a role in EIB, though how important a role is yet to be determined. Current consensus suggests that the autonomic nervous system alone does not account for the obstruction produced by heat loss in EIB.

IV

BEYOND THE TRIGGER: MEDIATORS

The other major area of research concerning the mechanism of airway obstruction in EIB has been the role of chemical mediators. This is a field that has seen a tremendous amount of activity in the past fifteen years. Many mediators have only recently been identified in their pure biochemical form, while still others have yet to be isolated and completely characterized. Much of the work on mediators in EIB has been suggested by the findings involving mediators in allergic asthma.

Before reviewing the literature on specific mediators, it will be helpful to examine the evidence for and against mediators in EIB in general. In a study of considerable interest in 1977, Soter and Austen demonstrated mediator release from sensitized mast cells in the cooled skin of patients with cold urticaria (Soter, 1977). By analogy some authors have postulated that a similar phenomenon occurs with cooling of the airways in EIB.

The drug disodium cromoglycate (DSCG) has been shown to inhibit bronchoconstriction due to exercise and isocapnic hyperventilation, but only if given before these challenges (Silverman, 1972b, Haynes, 1976, and Breslin, 1980a). This drug does not usually affect baseline airway tone. As the main mode of action of DSCG is believed to be the stabilization of mast cell membranes, this work would explain DSCG preventing EIB by inhibiting the release of mediators stored within mast cells.

Other drugs that also prevent mast cell degranulation , such as diethylcarbamazine pamoate (Sly, 1974a), beta adrenergic agonists (Godfrey, 1976), and calcium channel blockers (Cerrina, 1981 and Patel, 1981), have also prevented EIB in some trials.

The last line of indirect evidence supporting a role for mediator release in EIB comes from the existence of a "refractory period" in EIB. If exercise challenges are repeated at short intervals, the bronchospastic response decreases (McNeill, 1966, James, 1976, and Edmunds, 1978). This has been interpreted by some as evidence for the depletion of a stored mediator that must be regenerated over a period of time.

Nevertheless, some work has cast doubt on the role of mediators in EIB. DSCG has been shown to decrease the bronchospastic response to cold air in normal subjects and to sulfur dioxide (an irritant gas) in both asthmatics and normal controls (Fanta, 1981, Harries, 1981, and Sheppard, 1981). Since neither of these situations are believed to involve mediator release, DSCG may well work by other mechanisms. Thus, the role of DSCG in EIB may involve an effect other than decreasing mediator release. Just the fact that normal individuals, who do not have sensitized mast cells, can be made to develop obstruction with exercise and other similar stimuli is cited as evidence against mediator involvement (at least mediators from mast cells) (McFadden, 1983).

This controversy over mediators extends into the literature on specific mediators. Histamine was examined first because of the known release of histamine during mast cell degranulation in allergen induced asthma (Valentine, 1976). While one earlier paper showed no increase in histamine turnover during exercise in subjects with EIB (Granerus,

1971), other workers have demonstrated increases in either arterial or venous plasma histamine levels that are correlated with decreased PFTs in EIB (Ferris, 1978, Barnes, 1981a, Anderson, 1981, and Lee, 1982a). However, Deal and McFadden's group found no increase in arterial histamine levels following challenge by isocapnic hyperventilation with cold air (a stimulus equivalent to exercise) (Deal, 1980).

Interestingly, in the same above mentioned study, Deal and McFadden failed to find an increase in neutrophil chemotactic activity (NCA) when subjects performed isocapnic hyperventilation with cold air. They took this finding to support their conclusion that mast cell derived mediators are not involved in EIB. NCA is a heat-stable, high molecular weight factor that was first described in antigen induced asthma by Atkins (Atkins, 1977). It is the same mediator that was found to be associated with the phenomenon of cold induced urticaria mentioned earlier (Wasserman, 1977). NCA has since been shown to be present in the serum of asthmatic subjects after exercise on a treadmill and has even been partially purified (Lee, 1982b). The time course for the release of NCA is quite similar to that of histamine release. Lee repeated Deal's study, but used a treadmill challenge looking for NCA release. This time the exercise was performed once while inspiring warm, humid air and on a second occasion with subjects breathing cold, dry air (Lee, 1983). This study showed that EIB and NCA both appeared together during the cold exercise challenge, while neither were produced during the hyperventilation with warm air. These results were interpreted as demonstrating that respiratory heat exchange and exercise (by some unknown additional mechanism) together cause mast cell mediator release. Lee

further postulated that isocapnic hyperventilation might cause mediator release, but at levels that are only detectable with the addition of exercise. Lee reviewed some of the literature already discussed above on vagal reflexes and suggested that different sites and mechanisms of airway obstruction may exist in different subjects. One additional study that supports this multiplicity of mechanisms involved exercising patients repeatedly at short intervals until they no longer responded with EIB. These subjects were then challenged with an allergen known to produce bronchospasm in the subjects. Six of the twelve subjects in this study remained responsive to the allergen while the other six were not (Weiler-Ravell, 1981). One would expect all of the subjects to be unable to respond to the allergen if the exercise had depleted the mediators necessary to produce bronchospasm. Thus, the subjects in Weiler-Ravell's study may represent two subgroups of of people with EIB: those with mediator-dependent EIB and those with EIB produced via another mechanism.

Slow reacting substance of anaphylaxis (SRS-A) is another important mediator of allergen (Ig E) dependent bronchoconstriction that has been investigated extensively in recent years (Orange, 1969). In contrast to histamine and NCA, SRS-A is extractable from lung cells only after immunological challenge and, thus, is not a preformed, stored mediator (Brocklehurst, 1960). While the need for an immunological stimulus for release of SRS-A seems to speak against its involvement in EIB, there are a few reasons for briefly mentioning SRS-A here. First, many modulators of EIB were first discovered in work on allergen induced asthma and there simply has not been much work to date on the role of SRS-A in

EIB. Second, there are two studies that suggest that SRS-A is involved in EIB. The drug diethylcarbamazine citrate, which has been shown to inhibit SRS-A release in rats (Orange, 1968), prevented EIB in fifteen of twenty asthmatic subjects when given before exercise challenge (Sly, 1974b). Lastly, SRS-A has been characterized as consisting of three distinct leukotrienes (LTs): LTC4, LTD4, and LTE4. These leukotrienes are derivatives of arachidonic acid and are closely related to and interact with prostaglandins (Murphy, 1979, Morris, 1980, Lewis, 1980, Corey, 1980, and Samuelsson, 1982).

Leukotrienes are released by human lung fragments when challenged with allergen (Orange, 1973 and Austen, 1974) and have been shown to be very potent spasmogens on isolated human bronchi (Dahlen, 1980). LTC and LTD are also potent constrictors of human airways in vivo (Holroyde, 1981 and Weiss, 1982). How leukotrienes produce these effects is the subject of much current research, with one area of interest being the interaction of leukotrienes and prostaglandins. After all, both classes of compounds are derived from arachidonic acid with the enzymes lipoxygenase and cyclooxygenase being the first step in the production pathway for leukotrienes and prostaglandins, respectively. In vitro animal studies have suggested that in some situations leukotrienes may act via the production of thromboxane, a product of the cyclooxygenase pathway (Piper, 1981, Schianterellli, 1981, and Folco, 1981). Interestingly, studies using inhibitors of various steps in arachidonic acid metabolism seem to indicate that LTD4 (at least) has both a thromboxane mediated and a direct bronchoconstrictive effect (Muccitelli, 1983 and Weichman, 1984). Further elucidation of the actions and mechanisms of leuko-

trienes will be important for understanding allergen induced asthma and possibly EIB.

V

PROSTAGLANDINS

Prostaglandins are the products of the cyclooxygenase pathway of arachidonic acid metabolism. Earlier work on prostaglandins in asthma was initially centered on prostaglandins E2 and F2α (PGE2 and PGF2α). Both are formed in high concentrations in the lung relative to the rest of the body (Karim, 1967), with lung parenchyma having much more PGF2α than PGE2, while isolated bronchi have been found to have a predominance of PGE2 (Shaw, 1975). Several lines of evidence exist to suggest the involvement of PGE2 and PGF2 in the regulation of airway tone. PGE2 is a bronchodilator in vitro (Sweatman, 1968 and Gardiner, 1975) and in vivo (Smith, 1973 and Smith, 1975a), while PGF2α is a bronchoconstrictor both in vitro (Sweatman, 1968 and Gardiner, 1975) and in vivo (Smith, 1975a, Mathe, 1973, Mathe, 1975, and Newball, 1977). Like many other potential mediators, PGF2α is a far more potent bronchoconstrictor in asthmatics than in normal subjects (Newball, 1980). The effects of these prostaglandins are not altered by antihistamines or anticholinergics, suggesting that the effects are independent of other mediators and the autonomic nervous system (Sweatman, 1968 and Shaw, 1975). Some studies suggest that PGE2 and PGF2α may act either through changing cyclic nucleotide metabolism (Shaw, 1975) or via an effect on calcium and smooth muscle contraction (Carsten, 1972, Higgins, 1972, and Shaw, 1975). Other studies have implicated an interaction with histamine, with PGs affecting

either the release of or the sensitivity to histamine (Tauber, 1973 and Walters, 1983).

As with leukotrienes, prostaglandins have been implicated in allergic asthma by studies that have shown the release of PGF2∝ from human lung fragments that had been passively sensitized and then subjected to antigen challenge (Piper, 1973). The principal metabolite of PGF2∝ has also been shown to be elevated in the plasma of subjects after an antigen inhalation challenge (Green, 1974).

Prostaglandin D2 (PGD2) and thromboxane A2 (TXA2) are two other cyclooxygenase products that have been investigated recently in a fashion similar to previous work on PGE2 and PGF2∝ . These substances are also released by sensitized lung fragments in vitro when challenged with antigen (Schulman, 1980a and Schulman, 1980b). In fact, PGD2 and TXA2 are more potent bronchoconstrictors than PGF2∝ in vitro (Hamberg, 1975 and Svensson, 1977), and PGD2 is more potent than PGF2∝ when inhaled by humans (Hardy, 1984). Furthermore, PGD2 is released in larger quantities than PGF2∝ in allergen challenged lung tissue as well (Schulman, 1981, Lewis, 1982, and Peters, 1983).

From the literature cited above it is clear that prostaglandins are involved at least in allergic asthma, though there are a lot of questions remaining to be answered. One interesting dilemna concerns nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin. These drugs are inhibitors of cyclooxygenase and, thus, they block prostaglandin synthesis (Vane, 1971 and Flower, 1972). If the generation of bronchoconstrictive prostaglandins is responsible for airway obstruction in EIB, then one might expect NSAIDs to be bronchodilators, or at least

prevent bronchospasm. Indeed, Szczeklik has reported a small group of asthmatic subjects who do have bronchodilatation in response to aspirin, another NSAID (Szczeklik, 1983). However, it is also well known that there are subjects who develop asthma attacks in response to NSAIDs (Szczeklik, 1975). Furthermore, there are studies documenting that NSAIDs have no effect (either increasing or decreasing) on the bronchoconstriction elicited by allergen (Smith, 1975b and Fish, 1981), exercise (Rudolph, 1975, Smith, 1975c, and Schachter, 1978b), or methacholine (Ogilvy, 1981) in asthmatic or normal subjects.

The literature on the role of prostaglandins in EIB is not very extensive, due in part to the difficulty of measuring prostaglandins in vivo and perhaps also to early negative studies. Allegra found PGE plasma levels to be significantly higher in asthmatics at rest than in normal controls (Allegra, 1976). He postulated that this might be a compensatory mechanism to raise cAMP in asthmatics who have beta adrenergic hyposensitivity. (This is similar to the argument discussed earlier that was put forth by those who found baseline catecholamine levels elevated in asthmatics.) Allegra's group, however, did not find a significant difference between pre- and post-exercise plasma levels of PGE and PGF2α in asthmatic subjects exercised on a treadmill (Field, 1976). Another group of workers actually found a significant decrease in the plasma level of the major metabolite of PGF2α during EIB (Anderson, 1976). This, of course, is contrary to what one would expect if EIB were caused by an increase in PGF2α. This group of workers concluded that PGF2α does not play a significant role in EIB. The other possibility suggested by Allegra's group is that systemic sampling from the pe-

ripheral plasma is just not sensitive enough to measure what may be quite significant changes in local prostaglandin concentrations within the lung tissue (Field, 1976). It may be that even very sensitive prostaglandin assays in the future will be unable to measure systemic changes if the entire process is localized and rapid. This issue is especially important in light of the finding by Newball that intravenous PGF2∝, even in doses large enough to produce uterine contractions and watery diarrhea in some subjects, produced much less bronchoconstriction than smaller doses of inhaled PGF2∝ (Newball, 1976). It should be noted that this difficulty is present to some extent where any attempt is made to measure any proposed chemical mediator in a clinical trial.

As mentioned earlier, studies showing no effect of cyclooxygenase inhibitors on EIB speak against a primary role for prostaglandins in EIB. However, there is one study by Souza and Silverman that looked at the effects of a single oral dose (25 mg) of indomethacin on EIB. They found that five of the twelve subjects were at least partially protected against EIB by the indomethacin (Souza, 1981). It must be pointed out, however, that this study used PEFR as an indicator of EIB, while two of the three negative studies alluded to earlier used the more sensitive indicators FEV1 and MEF40%(P) (Smith, 1975a and Schachter, 1978b).

Two studies have shown that DSCG does not protect against bronchospasm induced by inhalation of PGF2 (Patel, 1975 and Newball, 1977). These researchers used this data to conclude that the bronchoconstrictive action of PGF2∝ is due to a direct effect on airway smooth muscle. These studies also present indirect evidence against the role of PGF2∝ in EIB since DSCG does protect against EIB in many subjects, yet it does not prevent bronchospasm due to PGF2∝ administration.

VI

ASCORBIC ACID

The possibility that ascorbic acid might be involved in asthma was first raised in the early nineteenth century by Reisseissen, who described symptoms of asthma in patients with severe scurvy (Reisseissen, 1803). This observation suggests the following question: if ascorbic acid deficiency can produce asthma, can ascorbic acid prevent or ameliorate asthma in non-scorbutic asthmatics?

While this issue does not compare with the uproar surrounding the question of the effect of ascorbic acid on cancer or the common cold, there is controversy over the effect of ascorbic acid on asthma. The question of the protective effect of ascorbic acid in asthma has been raised in many different ways. One of the first therapeutic trials was performed by Hunt in 1938. He was unable to demonstrate any benefit from a daily oral dose of 100 mg of ascorbic acid, as measured by the number and severity of bronchospastic episodes in asthmatic subjects. Hunt also found no benefit from the intramuscular or intravenous injection of ascorbic acid (500-800 mg doses) given during acute asthmatic episodes (Hunt, 1938).

Another simple clinical trial was performed more recently in Nigeria. 41 asthmatics, all of whom had increased numbers of attacks during the country's rainy season, were studied. These patients had exacerbations of their asthma precipitated by respiratory infections (recognized

by the report of sore throat and dry cough). 22 of these subjects took one gram of ascorbic acid daily for 14 weeks, while the other 19 subjects took a placebo for the same length of time. During this trial the subjects receiving ascorbic acid had fewer asthmatic episodes and far less severe attacks than those in the placebo group. Interval measurements of plasma ascorbic acid levels confirmed a significantly greater level in the subjects assigned to the ascorbic acid group (Anah, 1980). While this study did not involve a crossover of treatment regimens, there was a marked increase in the number and severity of asthma attacks for the ascorbic acid subjects once the trial was terminated. Obviously, there are many problems with this study, including reliance on self-reporting of attacks and their severity without any objective measurement of lung function, but the most important difficulty seems to be the selection of a group of patients whose attacks were precipitated by respiratory infections. This study claims to have demonstrated a beneficial effect of ascorbic acid in asthma, but may actually have been measuring a protective effect of ascorbic acid against respiratory infections.

Other more rigorous studies have looked at the effects of ascorbic acid against bronchoconstriction produced by means other than respiratory infections. Bouhuys initially demonstrated an inhibition of histamine induced bronchoconstriction in healthy subjects following single oral doses of 500 mg of ascorbic acid (Zuskin, 1973). Workers in the same lab later were unable to document this protective effect in asthmatic subjects treated for three days with 500 mg ascorbic acid daily (Kreissman, 1977). A study by Cockroft et al also failed to show a pro-

tective effect of one gram doses of ascorbic acid against histamine induced bronchospasm in asthmatics (Cockroft, 1977). Similarly, studies of asthma induced by textile dust inhalation have demonstrated inhibition of bronchospasm by ascorbic acid (Valic, 1973 and Zuskin, 1977), while other investigators using ragweed as the antigen found no benefit from the use of ascorbic acid (Kordansky, 1977 and Kordansky, 1979). Single oral doses of one gram of ascorbic acid decreased methacholine induced bronchospasm in both healthy subjects (Ogilvy, 1981) and asthmatics (Mohsenin, 1983). One study of ascorbic acid in EIB utilized one gram of ascorbic acid given intravenously 2.5 hours prior to exercise and then measured the response to exercise by PEFR and MMEFR. In this investigation ten subjects were studied: two had a decrease in EIB, two had an increase in EIB, and six subjects had no change in the severity of EIB after infusion of ascorbic acid (Anderson, 1983). It is important to note, however, that one of the parameters used to determine the pulmonary response to exercise was the PEFR. As mentioned earler, this measurement is effort dependent and may not be reliable. Since Anderson's paper did not show specific data, it is possible that reliance on this parameter may have led to spurious conclusions.

Finally, one other study on ascorbic acid and EIB, performed by Schachter and Schlesinger, did demonstrate the beneficial effects of ascorbic acid (Schlesinger, 1980 and Schachter, 1982). Their study involved pre-treatment with 500 mg of ascorbic acid or a placebo ninety minutes prior to exercise challenge. The subjects in the study all had a history of mild asthma and documented EIB (at least a 20% drop in MEF40% or MEF40%(P) on an exercise challenge on a prior screening day).

PFTs were measured at the following times: before exercise, immediately after exercise, five minutes after exercise, and after the administration of a bronchodilator. FVC and FEV1 were the parameters that most consistently demonstrated the protective effect of ascorbic acid, although MEF40%(P) was the most sensitive indicator of bronchoconstriction.

In vivo animal studies and in vitro experiments have demonstrated the anti-bronchospastic actions of ascorbic acid and have been used to suggest the mechanism, or mechanisms, by which this protective effect is attained. Early work centered on the interaction of ascorbic acid and histamine, since some studies showed that ascorbic acid protects against anaphylaxis induced by antigen or histamine in various animals (Guirgis, 1965, and Dawson, 1965, 1966, and 1967). Chatterjee et al found that guinea pigs on an ascorbic acid free diet developed markedly elevated histamine levels in plasma and other tissues (Chatterjee, 1975). Further work by this group suggested that ascorbic acid had this effect by influencing the metabolism of histamine (Subramanian, 1978). Some other possible mechanisms for the effect of ascorbic acid that have not been well evaluated are reviewed by Schlesinger (Schlesinger, 1980). These include an effect on cyclic nucleotide metabolism, smooth muscle cell calcium flux, and a direct effect on airway smooth muscle related to the action of ascorbic acid as an antioxidant.

The area that has produced exciting research in recent years has been the studies of the interaction between ascorbic acid and prostaglandins. Puglisi et al have found an increased production of PGE2 (the brochodilating PG) in guinea pig tracheal tissue treated with ascorbic

acid (Puglisi, 1977). In a complementary study, this group also demonstrated that more PGF2∝ (the bronchoconstricting PG) than PGE2 was produced by tracheal tissue from scorbutic guinea pigs (Puglisi, 1976). They also discovered that in vivo treatment of guinea pigs with ascorbic acid antagonized the bronchoconstriction induced by intravenous PGF2∝ (Puglisi, 1976). This work led to the attractive hypothesis that ascorbic acid alters PG metabolism in favor of PGE2 production over PGF2∝ resulting in a net bronchodilating effect. Two clinical studies showing the protection by ascorbic acid against methacholine induced bronchospasm support this hypothesis, since the administration of the PG synthesis inhibitor indomethacin blocked the beneficial action of ascorbic acid (Ogilvy, 1981 and Mohsenin, 1983). In these studies indomethacin had no effect on methacholine induced bronchospasm when given by itself and neither ascorbic acid nor indomethacin had a significant effect on resting airway tone. Despite the lack of effect on resting tone, ascorbic acid protected against methacholine, while ascorbic acid and indomethacin given together failed to attenuate the bronchospasm induced by methacholine. Thus, these investigators postulated that ascorbic acid protects against EIB by altering active prostaglandin metabolism so that PGE2 production is predominant over PGF2∝.

Exactly how ascorbic acid alters arachidonic acid and prostaglandin metabolism is not known, but has been the subject of additional in vitro studies. It was mentioned earlier that ascorbic acid is an antioxidant and, thus, alters the redox potential of any system to which it is added. Since it had been shown that reduced glutathione increases the production of PGE2 (Lands, 1971), some authors have suggested that ascorbic

acid might increase PGE2 by maintaining glutathione in the reduced state (Lands, 1971 and Puglisi, 1977). However, two studies have shown no correlation between ascorbic acid and glutathione levels in lung tissue (Leung, 1981 and Rothberg, 1983). One of these studies, by Rothberg and Hitchcock, did demonstrate that lung microsomes from scorbutic animals did produce more PGF2∝ than microsomes from control animals when the experiment was run at low arachidonic acid concentrations (Rothberg, 1983). They suggest that, as ascorbic acid does protect against some oxidase enzymes (Danford, 1980), ascorbic acid may actually increase the conversion of PGF2∝ to PGE2.

The aim of the present study was to first confirm the existence of a protective effect of ascorbic acid against EIB. Secondly, it was expected that if the protective effect was due to an alteration in prostaglandin metabolism, then the benefit of a dose of ascorbic acid would be blocked by simultaneous adminstration of indomethacin. Thus, we postulated that we would obtain results similar to those seen in the studies that examined ascorbic acid and indomethacin in methacholine induced bronchospasm.

VII

METHODS

Asthmatic subjects were recruited for this study by advertising in the university community for persons with mild asthma. Clinic and hospitalized patients were not solicited because of their tendency to have more severe asthma. All subjects had a history of asthma as defined by the American Thoracic Society (Amer Thor Soc, 1962). None of the subjects required corticosteroid therapy at the time of the study. While subjects had asthma attacks at varying frequencies, no subject had required hospitalization for an attack. All subjects gave written informed consent as approved by the Yale University Human Investigations Committee.

Each subject completed a questionnaire detailing respiratory symptoms, allergies, medications, medical illnesses, smoking history, and history of EIB. Subjects were instructed to take no asthma medications or prostaglandin synthesis inhibitors (aspirin, ibuprofen, etc.) for twenty-four hours prior to each test day. They were also instructed to refrain from ingestion of any food or beverage containing large amounts of methylxanthines (coffee, tea, cocoa, soft drinks, etc.) or ascorbic acid (citrus fruits, juices, etc.) for twenty-four hours prior to testing. In order to be enrolled in this study each patient had to demonstrate at least a 20% reduction from their baseline MEF40%(P) on a screening exercise challenge that measured pulmonary function response

to exercise without any test drugs. A total of twelve subjects, eight male and four female, were entered into the study.

Pulmonary function testing (PFTs) consisted of forced expiratory volumes and flow rates derived from partial and maximal expiratory flow volume curves (PEFV and MEFV curves). These curves were generated by the use of a pneumotachograph integrator system and were recorded on a Gould X-Y recorder. The PEFV curve was created by having the subjects inspire to approximately two-thirds of vital capacity and then exhale forcefully to residual volume. They would then immediately inspire to total lung capacity and exhale forcefully to residual volume once again, thus generating the MEFV curve. The parameters obtained from these curves were the following:

1. forced vital capacity (FVC),
2. forced expiratory volume in one second (FEV1),
3. peak expiratory flow rate (PEFR),
4. flow rate after expiration of 50% of the volume of a maximal inspiration (Vmax50%), and
5. flow rates at 60% of baseline vital capacity below total lung capacity on both MEFV and PEFV curves (MEF40% and MEF40%(P)).

Exercise testing was performed in an air-conditioned laboratory where the room temperature and humidity were relatively constant: temperature = 68 +/- 2°F (range = 62-74°) and humidity = 59 +/- 7% (range = 50-71%).

Subjects exercised on a cycloergometer (Monark). Subjects began pedaling at a speed of 20 km/hr against a zero workload. At the end of each one minute interval the subjects' heart rate was measured with an

electrocardiograph (Hewlett-Packard) and minute ventilation was measured by directing exhaled air through a calibrated gas meter. Once these values were recorded the workload was increased by 150 kilopond-meters/minute with subjects continuing to pedal at a rate of 20 km/hr. Subjects continued this constant rate against increasing workloads until either the heart rate exceeded 170 beats/minute or the subject was too fatigued to go on.

While exercising the subjects inspired through a mouthpiece connected to an Otis-McKerrow valve. A length of tubing connected the intake portion of the valve and mouthpiece to a column filled with calcium sulfate granules (Drierite) with the other end of the column open to room air. The outflow from the mouthpiece was directed through more tubing to the calibrated gas flow meter. Subjects wore a nose clip so that they would only be breathing the dried air through the mouth, bypassing the warming and humidifying effects of the nasopharynx. This kind of experimental apparatus produces an average partial pressure of water vapor of 3 mm of Hg in the inspired air (Schachter, 1984).

As mentioned above, subjects refrained from the use of asthma medications and substances containing methylxanthines or ascorbic acid for twenty-four hours prior to each test day. Each subject was tested at approximately the same time of day (+/- 2 hrs.) on all trial days to avoid any diurnal variation. Whenever PFTs were measured, subjects performed the PEFV and MEFV maneuvers three times, with one minute intervals between each pair of curves. Thus, the values for any PFT parameter at a given time point were actually taken to be the average of the values from the three sets of curves.

On the initial screening day a baseline set of PFTs was obtained. Subjects then exercised according to the protocol described above. Three pairs of PEFV and MEFV curves were then obtained at the following times after completion of exercise:

1. one minute,
2. five minutes,
3. fifteen minutes,
4. thirty minutes, and
5. sixty minutes.

After the sixty minute PFTs each subject received two metered doses (0.65 mg each) of metaproterenol sulfate (Alupent) inhaler. Ten minutes after the administration of this bronchodilator a final set of PFTs was measured (post-alupent curves). A subject had to experience at least a 20% reduction in MEF40%(P) from pre-exercise values at some point after exercise to have sufficient EIB to go on to the rest of the study.

The remainder of the study consisted of four trial days. On each day a pre-drug baseline set of PFTs was obtained. Subjects then received two capsules that contained one of the following combinations:

1. two placebos (lactose),
2. one placebo plus 750 mg of ascorbic acid,
3. one placebo plus 50 mg of indomethacin, or
4. 750 mg of ascorbic acid plus 50 mg of indomethacin.

These were given in a double-blind, randomized, crossover fashion such that each subject received all four combinations, just in different orders. After ingestion of the two capsules there was a two hour waiting period before beginning the exercise challenge. During this two hour

period subjects were allowed to leave the laboratory, but were asked not to exercise or eat. After the waiting period the exercise challenge was performed in identical fashion to the screening day, with PFTs before exercise, after exercise, and after alupent.

All PFT raw data points were analyzed after being converted to a number reflecting the change from that day's baseline PFTs. The change from baseline was expressed in two ways:

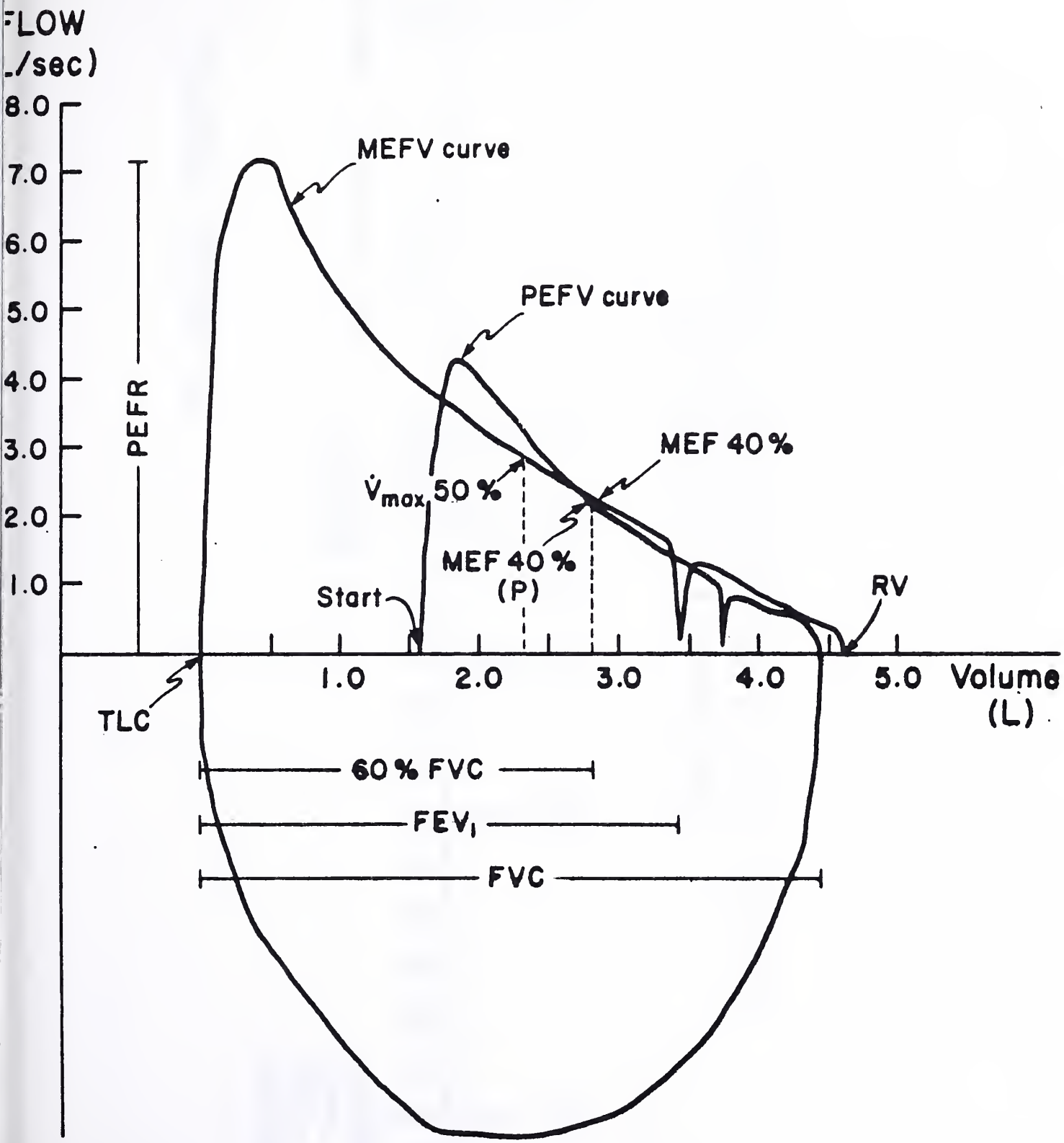
1. The absolute change from baseline (simply the difference between a PFT at a given time and that PFT prior to drug ingestion on that day) and
2. The percentage change from baseline (calculated by taking the absolute difference, dividing it by the baseline value and multiplying by 100).

In comparing the effects of one drug combination to another absolute changes on one day were compared to absolute changes on another day and percentage changes from baseline were likewise compared. Thus, the calculations for comparing ascorbic acid to placebo, for example, are actually differences of differences. All comparisons were made using paired Student's t-tests (Snedecor and Cochran, 1967).

The percentage change from baseline was assumed to more accurately reflect changes in the subjects because of the wide range of baseline values. Clearly, a drop in MEF40%(P) of 0.3 l/sec is much more significant, statistically and clinically, for a subject with a baseline MEF40%(P) of 1.0 l/sec than for a subject with a baseline MEF40%(P) of 3.0 l/sec.

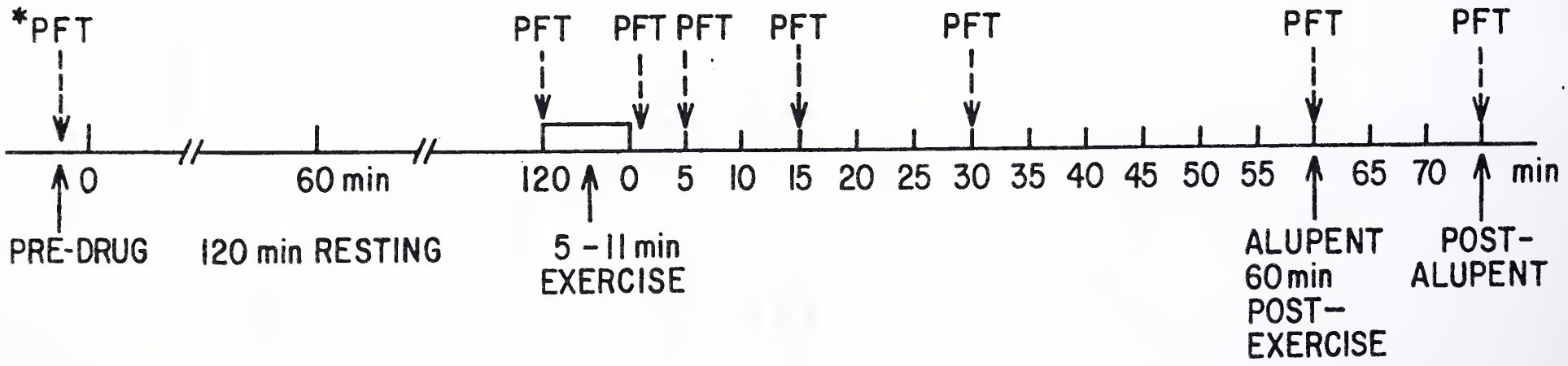
FIGURE 1

EXAMPLE OF FLOW-VOLUME CURVES



SCHEMATIC REPRESENTATION OF DAILY ROUTINE OF VITAMIN C-EIB PROTOCOL

FIGURE 2



*PULMONARY FUNCTION TEST

VIII

RESULTS

Tables 1 and 2 below give a summary of the anthropometric data obtained from the twelve subjects. (Values are the mean +/- one standard deviation.)

TABLE 1

Anthropometric Data Summary

Subjects	Age (yrs.)	Height (cm)	Weight (kg)
12	22.2+/- 2.6 (18-27)	171.2+/-7.8 (157-180)	62.1+/-22.4 (52-100)

Sex	Race
4 Female	10 White
8 Male	1 Black
	1 Oriental

TABLE 2

History from questionnaire

Taking Asthma Medications	=	6
History of Chronic Bronchitis	=	0
History of Smoking	=	1 (infrequent pipe)
History of EIB	=	12
worse EIB in cold weather	=	9
worse EIB with URI	=	10
Frequency of Wheezing:		
> once/week	=	6
¬ once/month	=	4
< once/month	=	2
Wheezing Exacerbated by:		
Animals	=	9
Dusts	=	11
Pollens	=	10
Cigarette smoke	=	7
Cold weather	=	10
Exercise	=	12
History of Allergy :		
Dust, Pollen, Mold	=	9
Hives	=	3
Eczema	=	4
Foods	=	4
Hayfever	=	10

8.1 RESPONSE TO EXERCISE WITHOUT DRUGS

The initial screening day determined whether or not a subject qualified for the remainder of the study and also allowed for the characterization of the subjects' response to exercise. Tables 3 and 4 give the raw data for the response to exercise as measured by the FEV1 and MEF40%(P). All parameters showed a significant brochospastic effect at five and fifteen minutes post-exercise ($P < .05$), except for FVC, which showed a significant decrease only at the five minute point. Again all parameters, except FVC, demonstrated a significant bronchodilatation in response to the metaproterenol one hour after exercise ($P < .05$). These values were significant using both the absolute and percentage changes from baseline.

TABLE 3

FEV1 Screening Day

Subject	Base	1min	5min	15min	30min	60min	post-alupent
1	3.9	3.9	3.5	3.5	3.5	3.6	3.5
2	1.9	2.3	1.8	1.8	1.8	2.2	2.7
3	2.2	1.8	1.6	1.7	1.8	2.0	2.5
4	3.0	2.6	3.0	3.1	2.8	2.9	3.3
5	2.9	2.9	2.7	2.8	2.8	3.0	3.4
6	3.2	3.4	3.3	3.4	3.5	3.5	3.9
7	2.3	2.2	2.2	2.2	2.2	2.3	2.4
8	3.5	3.4	3.2	2.9	3.1	3.5	3.8
9	3.2	2.8	2.7	2.7	2.8	2.9	3.5
10	3.9	3.8	3.7	3.7	3.9	3.9	4.0
11	4.1	4.0	4.0	3.9	3.5	4.1	4.0
12	4.2	4.4	4.2	4.2	4.2	4.1	4.2
Mean	3.2	3.1	3.0	3.0	3.0	3.2	3.4
S.D.	0.8	0.8	0.8	0.8	0.8	0.8	0.6

TABLE 4

MEF40%(P) Screening Day

Subject	Base	1min	5min	15min	30min	60min	post-alupent
1	2.6	2.5	2.0	2.5	2.8	3.9	3.9
2	0.9	1.1	0.7	0.8	1.0	1.1	2.0
3	1.4	0.7	0.4	0.3	0.5	0.4	1.6
4	1.6	0.9	0.8	1.0	1.3	2.1	3.5
5	1.5	1.4	1.0	1.0	1.4	1.2	2.1
6	2.1	1.6	2.0	2.3	2.9	2.9	4.6
7	0.8	0.6	0.5	0.5	0.7	0.8	1.3
8	2.1	2.0	1.4	1.2	1.6	2.1	2.9
9	2.7	0.9	0.8	1.0	1.5	1.8	3.6
10	2.8	1.9	2.0	2.1	2.3	2.6	3.8
11	2.9	2.1	2.2	2.5	2.9	3.7	3.6
12	4.1	4.0	3.2	3.1	3.7	3.3	5.2
Mean	2.1	1.6	1.4	1.5	1.9	2.2	3.2
S.D.	1.0	1.0	0.9	0.9	1.0	1.2	1.2

Of note, these subjects did not demonstrate the typical course of EIB entirely. While they did have bronchospasm that was worst at five to fifteen minutes post-exercise which improved over the next hour, these subjects did not experience bronchodilatation immediately after exercise. In fact, both the PEFR and MEF40%(P) were decreased at one

minute post-exercise ($P < .05$), indicating brochospasm already at this early point.

The twelve subjects admitted to the drug portion of the study were selected on the basis of having a decrease in MEF40%(P) of at least 20% from baseline at some point after exercising. The mean maximal decrement in MEF40%(P) was 39% (+/- 19%), with a range of 22-79%. The mean maximal decrease in FEV1 was only 10% (+/- 8%). If a decrease in FEV1 of at least 10% had been used as the selection criterion, only five of the twelve subjects would have been enrolled in the drug trial.

8.2 RESPONSE TO EXERCISE WITH DRUGS

Tables 5 and 6 show the baseline PFTs for all subjects before exercise on the screening day. This table also expresses the PFTs in terms of the predicted values for an individual of the same height, weight, and race.

Tables 7 and 8 then compare the FEV1 and MEF40%(P) baseline values (pre-exercise) on all of the study days. This data demonstrates that the subjects were starting in essentially the same state each day before taking any of the drug combinations ($P > .05$ for comparing any PFT between any two days).

TABLE 5

Baseline Pulmonary Function Testing

	FVC		FEV1		PEFR	
Subject	l	%pred	l/sec	%pred	l/sec	%pred
1	4.3	101	3.9	112	9.2	132
2	3.3	95	1.9	62	3.5	61
3	3.3	95	2.2	74	4.9	88
4	4.0	114	3.0	100	5.6	100
5	5.1	107	2.9	72	5.7	73
6	4.4	93	3.2	82	6.8	89
7	3.4	104	2.3	81	4.8	87
8	4.8	101	3.5	88	6.9	88
9	4.5	80	3.2	71	5.6	68
10	4.9	113	3.9	108	8.6	115
11	5.0	111	4.1	110	6.8	91
12	4.6	114	4.2	126	9.2	134
Mean	4.3	102	3.2	90	6.5	94
S.D.	0.7	10	0.8	20	1.8	23

TABLE 6

Baseline Pulmonary Function Testing

	Vmax50%		MEF40%	MEF40%(P)
Subject	l/sec	%pred	l/sec	l/sec
1	5.7	129	4.3	2.6
2	1.3	31	1.0	0.9
3	2.0	50	1.4	1.4
4	3.2	79	2.4	1.6
5	2.0	38	1.6	1.5
6	3.3	67	2.4	2.1
7	1.8	45	1.4	0.8
8	2.9	57	2.4	2.1
9	2.7	51	2.2	2.7
10	4.1	83	3.0	2.8
11	4.3	83	3.6	2.9
12	4.9	107	4.1	4.1
Mean	3.2	69	2.5	2.1
S.D.	1.4	30	1.1	1.0

TABLE 6

Baseline Pulmonary Function Data

TABLE 7

Comparison of Baseline FEV1

Subject	Screen	Placebo	Vit C	Indocin	C + Indo
1	3.9	3.5	3.8	3.5	3.7
2	1.9	2.0	2.1	2.1	2.2
3	2.2	2.0	2.0	2.2	2.0
4	3.0	2.9	3.1	2.9	3.0
5	2.9	2.5	2.5	2.6	2.5
6	3.2	3.6	3.6	3.5	3.6
7	2.3	2.1	2.0	2.1	2.1
8	3.5	3.8	3.8	3.9	3.7
9	3.2	2.9	2.8	3.0	3.4
10	3.9	3.6	3.8	3.6	3.8
11	4.1	3.9	4.1	4.1	3.8
12	4.2	4.1	4.3	4.1	3.9
Mean	3.2	3.1	3.2	3.1	3.1
S.D.	0.8	0.8	0.8	0.8	0.7

TABLE 8

Comparison of Baseline MEF40%(P)

Subject	Screen	Placebo	Vit C	Indocin	C + Indo
1	2.6	4.1	4.4	4.2	4.4
2	0.9	0.8	0.8	1.0	1.0
3	1.4	0.9	0.5	0.9	0.5
4	1.6	1.1	1.3	1.6	1.2
5	1.5	1.0	1.1	0.9	1.0
6	2.1	2.6	2.8	2.2	1.5
7	0.8	0.5	0.5	0.4	0.4
8	2.1	1.9	1.9	2.0	2.1
9	2.7	1.6	1.2	1.4	1.9
10	2.8	2.1	2.5	2.6	2.1
11	2.9	4.2	3.3	4.5	4.3
12	4.1	2.7	2.6	2.8	2.0
Mean	2.1	2.0	1.9	2.0	1.9
S.D.	1.0	1.2	1.2	1.3	1.3

Tables 9 through 16 display the raw data for FEV1 and MEF40%(P) for all time points on all five of the study days. (The times given are the number of minutes after the completion of the exercise challenge. The other values are as follows: "Base" = value prior to drug ingestion, "Rest" = value two hours after drug ingestion and prior to the exercise

challenge, and "P.A." = post-alupent, value after subjects received metaproterenol upon completing the exercise challenge.)

The mean values for the group are plotted over time in Figures 3 and 4. These figures graphically demonstrate the course of EIB in these subjects and how there was very little variation produced by any of the drugs. There was still significant bronchospasm at five and fifteen minutes post-exercise (using both the absolute and percent changes from baseline) for almost all parameters as on the screening day ($P < .05$). The only data that varied much from the screening day was the FVC on the day that the subjects received ascorbic acid and indomethacin together. On that particular day, the FVC was decreased significantly ($P < .05$) from baseline at all time points from one to sixty minutes post-exercise (absolute and percent changes). This is markedly different from the screening day where only the five minute point achieved statistical significance.

FIGURE 3

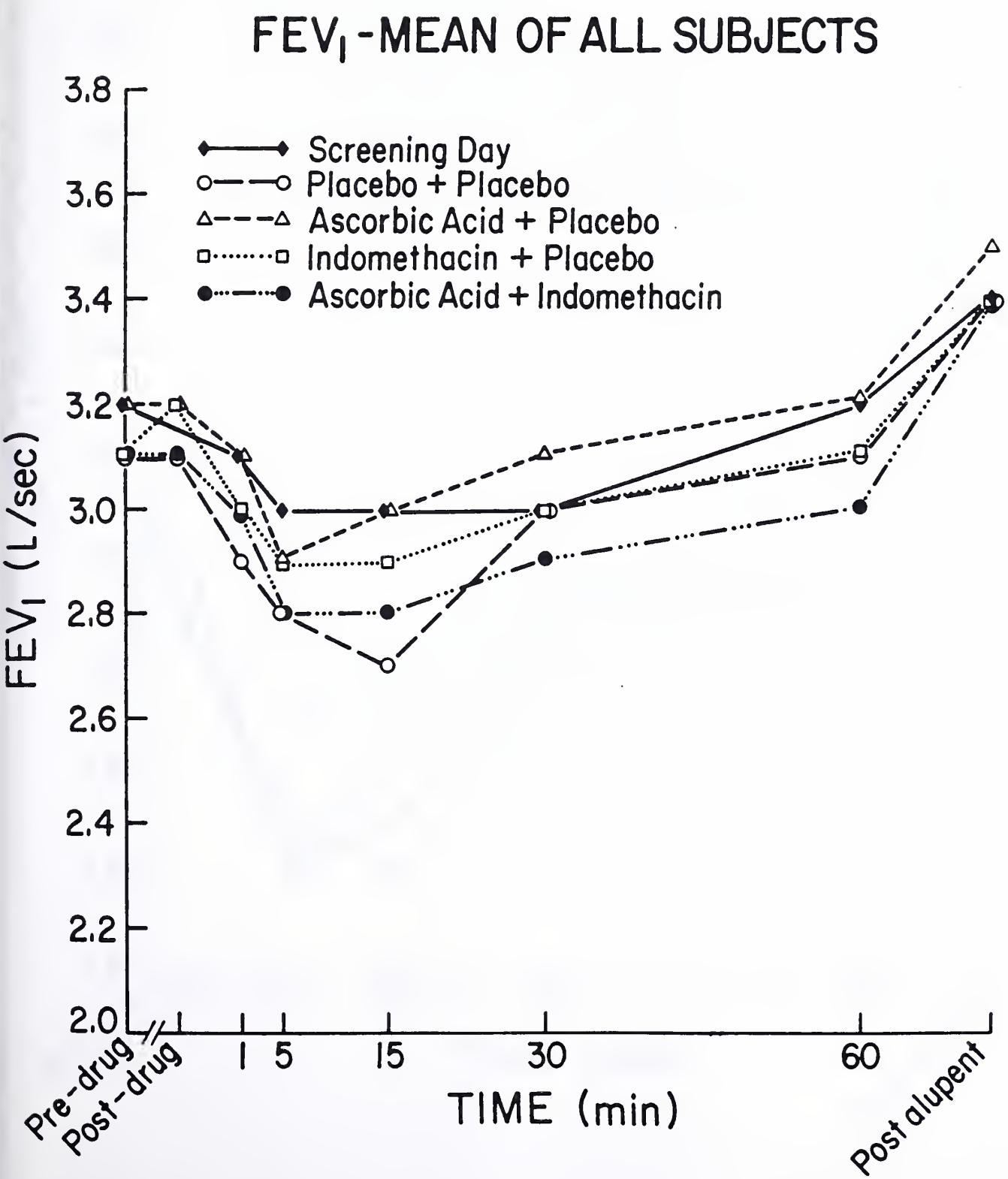

FEV1 - MEAN OF ALL SUBJECTS
Screening Day
Placebo + Placebo
Ascorbic Acid + Placebo
Indomethacin + Placebo
Ascorbic Acid + Indomethacin
FEV1 (L/sec)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Pre-drug
Post-drug
1
5
15
30
60
Post alupent
TIME (min)

FIGURE 4

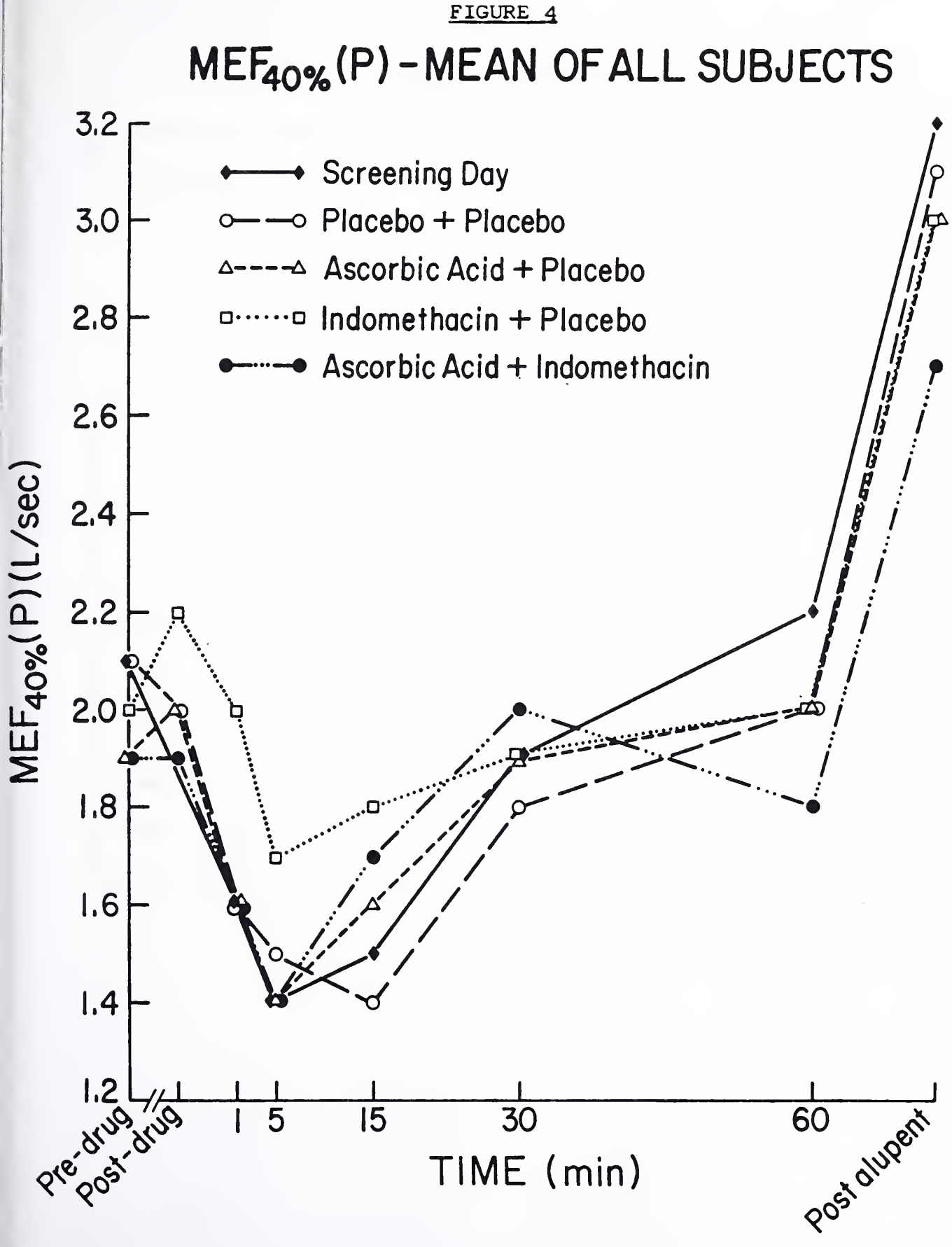

MEF40%(P) - MEAN OF ALL SUBJECTS
Screening Day
Placebo + Placebo
Ascorbic Acid + Placebo
Indomethacin + Placebo
Ascorbic Acid + Indomethacin
MEF40%(P)(L/sec)
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
Pre-drug
Post-drug
1
5
15
30
60
Post alupent
TIME (min)

TABLE 9

FEV1 - Placebo Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A
1	3.5	3.4	3.7	3.5	3.5	3.5	3.5	3.4
2	2.0	2.2	2.2	1.8	1.8	2.1	2.1	2.6
3	2.0	2.1	1.9	1.2	1.1	1.7	2.0	2.5
4	2.9	3.0	2.8	2.8	2.8	3.0	2.8	3.2
5	2.5	2.7	2.2	2.3	2.2	2.7	2.5	3.2
6	3.6	3.4	2.8	2.6	2.5	3.2	3.5	3.9
7	2.1	2.1	2.0	2.0	2.0	2.2	2.2	2.4
8	3.8	3.7	4.0	3.6	3.6	3.7	3.7	3.9
9	2.9	3.0	2.3	2.3	2.3	2.7	3.0	3.6
10	3.6	3.5	3.3	3.3	3.4	3.5	3.7	4.0
11	3.9	3.8	4.0	3.8	3.7	3.8	3.9	4.0
12	4.1	4.1	4.1	4.2	4.0	4.0	4.0	4.0
Mean	3.1	3.1	2.9	2.8	2.7	3.0	3.1	3.4
S.D.	0.8	0.7	0.8	0.9	0.9	0.7	0.7	0.6

TABLE 10

FEV1 - Placebo + Vitamin C Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	3.8	3.5	3.9	3.8	3.8	3.7	3.6	3.6
2	2.1	2.0	2.1	1.7	1.7	2.0	2.2	2.6
3	2.0	2.0	1.5	1.1	1.0	1.3	1.7	2.4
4	3.1	3.0	3.1	2.9	2.9	3.0	3.1	3.2
5	2.5	2.9	2.5	2.2	2.6	2.9	2.8	3.3
6	3.6	3.7	3.6	3.6	3.5	3.6	3.7	3.9
7	2.0	2.1	2.0	1.9	2.0	2.1	2.1	2.3
8	3.8	3.9	3.8	3.8	3.9	4.0	4.0	4.2
9	2.8	3.2	2.5	2.4	2.8	2.6	3.1	3.8
10	3.8	3.7	3.5	3.5	3.6	3.7	3.7	4.1
11	4.1	3.9	4.1	3.9	3.9	3.7	3.9	4.0
12	4.3	4.1	4.3	4.2	4.2	4.2	4.1	4.2
Mean	3.2	3.2	3.1	2.9	3.0	3.1	3.2	3.5
S.D.	0.8	0.8	0.9	1.0	1.0	0.9	0.8	0.7

TABLE 11

FEV1 - Placebo + Indomethacin Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	3.5	3.4	3.6	3.4	3.4	3.3	3.3	3.4
2	2.1	2.2	2.6	2.0	2.0	2.1	2.1	2.6
3	2.2	1.9	1.5	1.1	1.0	1.4	1.7	2.4
4	2.9	3.0	3.0	3.0	3.0	3.2	3.0	3.3
5	2.6	3.0	2.7	2.4	2.5	2.7	2.6	3.4
6	3.5	3.7	3.2	3.1	3.0	3.2	3.4	3.8
7	2.1	2.1	2.1	2.0	2.1	2.1	2.2	2.3
8	3.9	3.9	4.1	4.0	3.7	4.1	4.1	4.2
9	3.0	3.1	2.2	2.2	2.3	2.6	3.1	3.8
10	3.6	3.8	3.5	3.3	3.6	3.5	3.6	4.0
11	4.1	3.7	3.9	3.8	4.0	3.8	3.7	4.1
12	4.1	4.0	4.2	4.2	4.2	4.2	4.2	4.1
Mean	3.1	3.2	3.0	2.9	2.9	3.0	3.1	3.4
S.D.	0.8	0.7	0.9	0.9	0.9	0.9	0.8	0.7

TABLE 12

FEV1 - Vitamin C + Indomethacin Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	3.7	3.6	3.6	3.5	3.4	3.4	3.6	3.6
2	2.2	2.1	2.2	1.7	1.6	1.9	1.9	2.3
3	2.0	1.9	1.8	1.3	1.2	1.5	1.7	2.5
4	3.0	3.0	2.8	2.7	2.6	2.8	2.7	3.2
5	2.5	2.7	2.1	1.7	2.0	2.1	2.4	2.9
6	3.6	3.5	3.1	2.6	2.3	3.0	3.4	3.8
7	2.1	2.2	2.1	1.8	2.0	2.0	2.1	2.3
8	3.7	3.7	4.1	4.0	3.8	3.9	4.0	4.0
9	3.4	3.4	3.0	3.0	2.8	3.0	3.0	3.7
10	3.8	3.7	3.4	3.4	3.5	3.6	3.6	4.1
11	3.8	3.8	3.8	3.7	4.0	3.8	3.8	3.9
12	3.9	4.0	4.2	4.1	4.0	4.2	4.2	4.4
Mean	3.1	3.1	3.0	2.8	2.8	2.9	3.0	3.4
S.D.	0.7	0.7	0.8	1.0	1.0	0.9	0.9	0.7

TABLE 13

MEF40%(P) - Placebo Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	4.1	3.4	3.5	3.7	3.3	3.4	3.4	3.9
2	0.8	1.0	1.1	0.6	0.6	0.8	1.2	2.0
3	0.9	1.1	0.6	0.2	0.2	0.4	0.9	1.8
4	1.1	1.6	1.2	0.9	1.2	1.5	1.3	2.4
5	1.0	1.3	0.7	0.7	0.8	1.4	0.9	1.9
6	2.6	2.0	0.9	0.6	0.6	1.6	2.4	3.9
7	0.5	0.5	0.4	0.4	0.3	0.5	0.6	1.0
8	1.9	2.0	2.5	1.8	1.7	1.9	2.2	2.7
9	1.6	2.0	0.4	0.4	0.6	1.1	2.0	3.5
10	2.1	1.9	1.2	1.2	1.7	2.1	2.3	4.5
11	4.2	4.5	4.1	4.2	3.6	4.2	4.1	4.6
12	2.7	3.1	2.9	2.9	2.8	3.0	3.0	4.8
Mean	2.1	2.0	1.6	1.5	1.4	1.8	2.0	3.1
S.D.	1.0	1.1	1.3	1.4	1.2	1.2	1.1	1.3

TABLE 14

MEF40%(P) - Placebo + Vitamin C Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	4.4	3.7	3.7	2.9	3.3	3.5	3.7	5.0
2	0.8	1.0	0.9	0.3	0.4	0.6	0.9	1.0
3	0.5	0.2	0.2	0.1	0.0	0.1	0.3	1.4
4	1.3	1.6	1.6	1.1	1.2	1.5	1.7	2.3
5	1.1	1.6	1.0	0.7	1.1	1.5	1.2	2.0
6	2.8	2.9	1.7	1.8	1.8	2.7	2.7	4.0
7	0.5	0.9	0.4	0.3	0.5	1.0	0.8	1.7
8	1.9	2.0	2.1	2.0	1.8	2.1	2.2	2.8
9	1.2	1.9	0.6	0.6	1.0	1.2	1.4	3.1
10	2.5	2.3	1.5	1.7	2.2	2.3	2.3	4.1
11	3.3	3.5	3.3	3.2	3.6	3.6	3.6	4.3
12	2.6	2.4	2.8	2.3	2.6	2.5	2.6	3.7
Mean	1.9	2.0	1.6	1.4	1.6	1.9	2.0	3.0
S.D.	1.2	1.0	1.1	1.1	1.1	1.1	1.1	1.3

TABLE 15

MEF40%(P) - Placebo + Indomethacin Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	4.2	4.4	3.9	3.1	3.3	3.2	3.7	5.0
2	1.0	0.9	1.8	0.9	0.8	1.2	0.9	1.3
3	0.9	0.6	0.3	0.2	0.2	0.3	0.5	1.0
4	1.6	1.5	1.8	1.5	1.7	2.1	1.9	3.4
5	0.9	1.4	1.1	0.7	0.8	1.0	0.9	2.2
6	2.2	2.6	1.3	0.8	1.1	1.5	1.8	3.9
7	0.4	1.0	0.5	0.3	0.6	0.8	0.7	1.5
8	2.0	1.9	2.4	1.9	1.8	2.4	2.4	2.9
9	1.4	1.9	0.2	0.2	0.4	0.7	1.3	2.9
10	2.6	2.8	2.2	1.7	2.2	1.8	2.7	4.0
11	4.5	4.5	4.4	4.3	4.2	4.3	4.3	4.6
12	2.8	3.3	3.5	3.2	3.3	3.3	3.3	3.3
Mean	2.0	2.2	2.0	1.7	1.8	1.9	2.0	3.0
S.D.	1.3	1.3	1.4	1.3	1.3	1.2	1.3	1.3

TABLE 16

MEF40%(P) - Vitamin C + Indomethacin Day

Subject	Base	Rest	1min	5min	15min	30min	60min	P.A.
1	4.4	3.6	3.3	2.5	2.8	2.8	3.1	4.4
2	1.0	0.8	0.8	0.4	0.3	0.4	0.7	1.4
3	0.5	0.6	0.3	0.2	0.2	0.3	0.3	1.4
4	1.2	1.4	1.0	0.9	0.9	1.2	1.0	2.2
5	1.0	1.1	0.7	0.3	0.5	0.6	0.9	1.5
6	1.5	1.4	0.7	0.4	0.3	0.9	1.5	3.1
7	0.4	0.5	0.4	0.2	0.3	0.6	0.5	1.1
8	2.1	2.2	3.1	2.6	2.5	2.8	2.7	3.2
9	1.9	2.3	1.0	1.1	1.2	1.5	1.9	3.0
10	2.1	2.0	1.3	1.4	1.3	1.5	1.6	2.8
11	4.3	4.4	4.4	4.5	4.6	4.4	4.4	4.8
12	2.0	2.3	2.7	2.6	2.6	2.8	2.7	4.0
Mean	1.9	1.9	1.6	1.4	1.7	2.0	1.8	2.7
S.D.	1.3	1.2	1.4	1.4	1.4	1.8	1.2	1.2

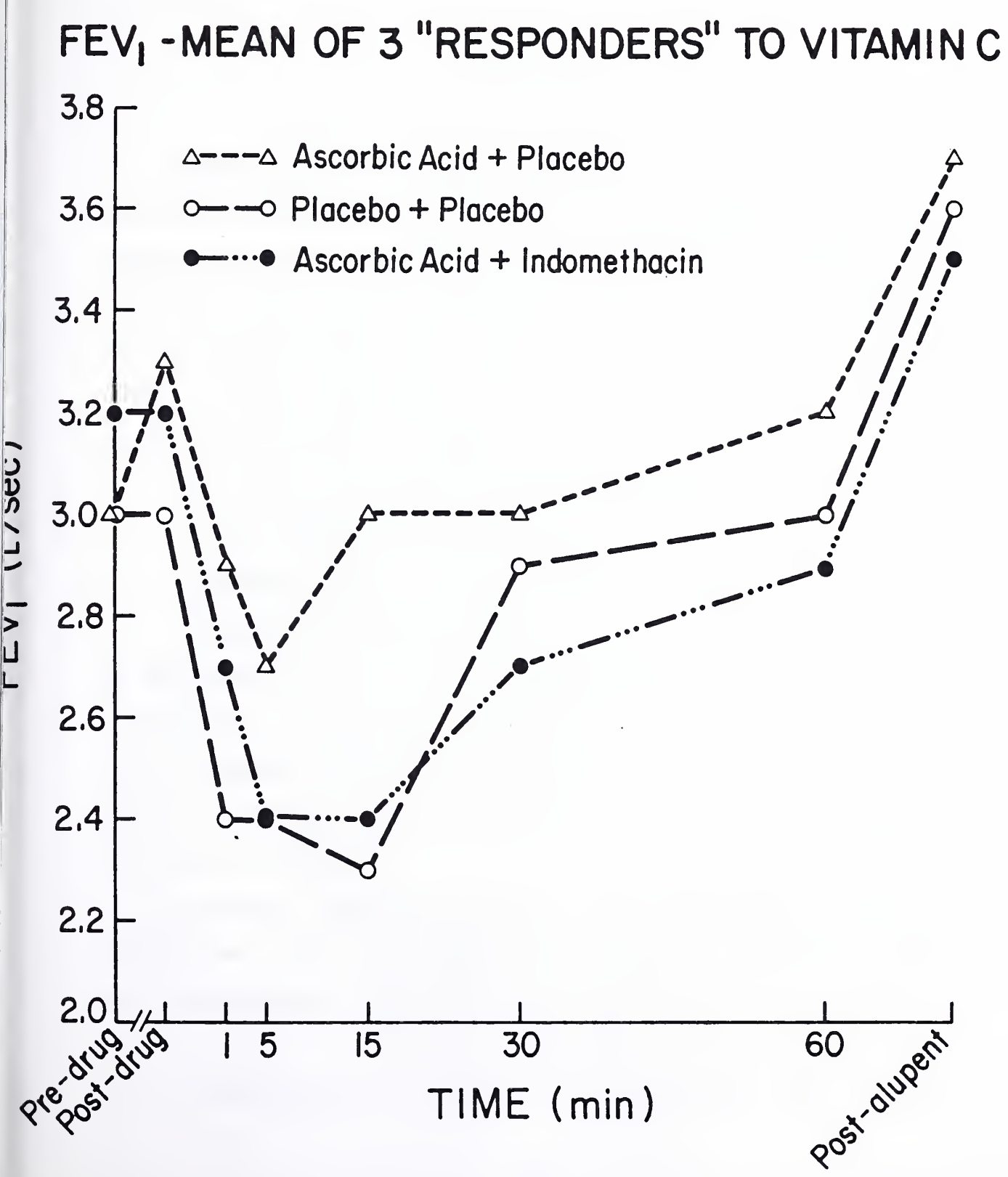
Comparisons between the drug days were made as described in the Methods section. There were no significant differences between the effects of ascorbic acid and placebo on the response to exercise ($P > .05$) at any of the time points. Likewise, there were no significant differences between the effects of indomethacin and placebo. However, the combination of ascorbic acid and indomethacin did produce some small, yet statistically significant, differences from placebo. This drug combination produced a decrement in the FVC that was 0.2 l/sec (or approximately 5%) lower than the placebo values at 30 and 60 minutes post-exercise and after the metaproterenol. Despite this, comparing the ascorbic acid and indomethacin combination to ascorbic acid alone did not demonstrate any statistically significant differences.

In addition to looking at the effects of the drugs on EIB, this study was also able to assess their effects on resting airway tone. This was accomplished by comparing the pre-drug baseline ("Base" in the tables) with the post-drug, resting PFT's done before the exercise challenge ("Rest" in the tables). None of the drug combinations had a significant effect on resting lung function (all P values > .05).

Figure 5 illustrates the FEV1 curves for three subjects in the group who had less EIB on the days when ascorbic acid was given. In these three subjects the suggested "protective action" of ascorbic acid appeared to be blocked by the addition of indomethacin. Three other subjects appeared to have worse EIB after treatment with ascorbic acid, while six subjects appeared to have EIB of the same severity with ascorbic acid. Indomethacin alone did not have a significant effect on any of the subjects, regardless of their response to ascorbic acid. The

three subjects who responded favorably to ascorbic acid did not differ from the other subjects in terms of any of the following parameters: age, sex, history of allergies, asthma, or EIB, or severity or pattern of EIB response to screening exercise challenge. However, these three subjects did have more severe asthma as measured by their baseline FEV1 as percent of predicted. The ascorbic acid responders had an FEV1 that was, on average, 75% of predicted. The other nine subjects had an average FEV1 that was 96% of predicted. Similarly, the three ascorbic acid responders had lower percent predicted values than the other subjects for FVC, PEFR, and Vmax50%. The three subjects who appeared to have worse EIB after ascorbic acid treatment could not be distinguished from the six non-responders using any of the above parameters.

FIGURE 5


FEV1 -MEAN OF 3 "RESPONDERS" TO VITAMIN C
Ascorbic Acid + Placebo
Placebo + Placebo
Ascorbic Acid + Indomethacin
FEV1 (L/sec)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Pre-drug
Post-drug
1
5
15
30
60
Post-alupent
TIME (min)

IX

DISCUSSION

9.1 RESPONSE TO EXERCISE WITHOUT DRUGS

Data from the initial screening day of the study documented EIB in all twelve subjects on the basis of at least a 20% drop in MEF40%(P) from baseline at some point after exercise. These subjects may be separated into two groups based on the criterion of Schachter et al (Schachter, 1978a). It was in that study that the researchers were able to separate subjects with EIB into two groups:

1. One group with decreased air flow using measures of small airway status (MEF40% and MEF40%(P)) and
2. A second group with decreased flow according to both small and large (FEV1) airway parameters.

In the present study six subjects had post-exercise decreases in FEV1 of at least 10% on the screening challenge, while the other six subjects did not decrease their FEV1 by that much. This study, therefore, confirms the observation that two distinct patterns of airway obstruction in EIB can be described.

One somewhat unexpected finding in the screening data was that the subjects as a group showed significant bronchospasm as early as one minute after exercise, with MEF40%(P) decreasing from 2.1 +/- 1.0 l/sec at baseline to 1.6 +/- 1.0 l/sec at one minute. EIB has been described with bronchoconstriction beginning about five minutes after exercise,

preceded by bronchodilatation immediately post-exercise (McFadden, 1983). However, one must recall that each time point in this study is actually the mean of three PEFV and MEFV curve maneuvers done one minute apart. Thus, the value for the one minute post-exercise point is really the mean of three values obtained at one, two, and three minutes post-exercise. Suspecting that taking the mean of these three measurements might have obscured the initial bronchodilatation because of subsequent, progressive bronchoconstriction in the later values, the MEF40%(P) was re-examined. This was done using the data from the screening day, looking at the individual curves that made up each time point. Indeed, the three curves did demonstrate a progressive decline in MEF40%(P), with the individual values being:

1. One minute post-exercise = 1.8 +/- 1.0 l/sec,
2. Two minutes post-exercise = 1.6 +/- 1.1 l/sec, and
3. Three minutes post-exercise = 1.4 +/- 0.9 l/sec.

Even the first value, however respresents a 0.3 l/sec decrease from baseline, but this did not achieve statistical significance ($P > .10$). It may be that the post-exercise bronchodilatation is quite short-lived and was not picked up in this study because the first value was not obtained until a full minute after exercise had ceased. Two studies using exercise challenges and PFTs the most similar to this study also did not demonstrate significant bronchodilatation immediately post-exercise (Schachter, 1978a and Schachter, 1982). In fact, in the latter of these studies, the only value reaching statistical significance immediately after exercise was a decrease in FVC.

All PFTs demonstrated a bronchodilating response to metaproterenol (except FVC) as expected.

9.2 RESPONSE TO EXERCISE WITH DRUGS

This study was designed to confirm the protective effect of ascorbic acid in EIB and to examine the mechanism of such an effect. Our working hypothesis was that this effect was due to an alteration in prostaglandin metabolism. It was expected that this protection would be blocked by the simultaneous administration of indomethacin, as had been shown to be the case with methacholine induced bronchospasm (Ogilvy, 1981 and Mohsenin, 1983). However, the present study failed to confirm the existence of a protective effect of ascorbic acid against EIB. There were some trends in the data suggesting a possible benefit at fifteen minutes post-exercise, with the FEV1 5% and MEF40%(P) 9% better, respectively, with ascorbic acid than with placebo. Neither of these values, however, achieved statistical significance (P = .14 and P = .11, respectively). The FVC was only 0.5% better for ascorbic acid than placebo at the same time point. These findings are in contrast to Schachter and Schlesinger (Schachter, 1982) who found the protective effect trend in all parameters and at all times measured (immediate post-exercise, five minutes post-exercise, and after metaproterenol). The values that achieved significance in their study were those reflecting a protective effect on the bronchoconstriction of larger airways (FEV1 and FVC). Our work does not support these earlier results.

It should be noted that when individual subjects were examined in this earlier study only eight out of the twelve subjects showed less EIB

when ascorbic acid was administered. Of these eight subjects, five individuals had more significant benefit from the ascorbic acid (greater than a 10% improvement in FEV1 at five minutes post-exercise when compared to the response to placebo). These five subjects, like the ascorbic acid responders in our study, had a lower percent predicted of FEV1 than the other subjects in the study (63% and 83% of predicted, respectively). Thus, it may be that those subjects with a lower percent predicted of FEV1 are more likely to derive benefit from treatment with ascorbic acid. Furthermore, a possible explanation for the differences between our results and those of the earlier study may be the greater number of "ascorbic acid responders" in Schachter and Schlesinger's group.

The five subjects in Schachter and Schlesinger's study who had the greatest response to ascorbic acid were also distinguishable from the other subjects by their more severe EIB on the screening exercise challenge. This characteristic was apparent in the PFTs that reflect both large and small airway status. The ascorbic acid responders had mean FEV1 and MEF40%(P) of 78% and 42% of their baseline values, respectively, at five minutes post-exercise. The remaining subjects had mean FEV1 and MEF40%(P) of 89% and 62%, respectively, at the same time point. This difference between ascorbic acid responders and non-responders was not evident in the present study. In our study the responders had five minute FEV1 and MEF40%(P) values of 93% and 64%, while the non-responders had values that were 93% and 65% of baseline. Thus, both studies suggest that ascorbic acid responders may be those individuals with a lower baseline FEV1 (as percent of predicted value). However, this

study does not confirm the finding in the earlier investigation that ascorbic acid responders are also those who experience greater bronchospasm in response to exercise.

There are really very few differences between this protocol and the one used by Schachter and Schlesinger. This study looked at additional drug combinations and carried the measurement of PFTs out for a longer period of time after exercise ended. The exercise challenges and PFTs themselves were carried out in virtually the same fashion in the two studies. This study had subjects wait two hours between drug ingestion and exercise challenge, while the earlier study utilized only a 90 minute waiting period. This is probably not a significant difference, since our study gave subjects 750 mg of ascorbic acid, while Schachter and Schlesinger's subjects ingested only 500 mg.

The next thing to examine for differences between these two studies is the subjects themselves. Already mentioned above was the apparent difference in the number of "ascorbic acid responders" between the two studies and the difference in the severity of EIB between the "responders" in these investigations. The difference in number of "responders" may also be reflected in this study group appearing to have a slightly healthier baseline than the previous study group, with FVC, FEV1, and PEFR close to predicted values (102%, 90%, and 94%, respectively), as opposed to the earlier group where these parameters were 87%, 75%, and 78% of predicted. The baseline MEF40%(P) for the two cohorts was almost identical: 2.1 +/- 1.0 and 2.1 +/- 1.1 l/sec. Many of the other measurements are also quite similar. There are no strong factors distinguishing these two study populations.

Another possible explanation for the different results in the studies lies in a phenomenon alluded to earlier: two different patterns of airway obstruction in EIB, possibly defining two distinct subgroups of asthmatics. The study by Schachter et al describing these two patterns of airway response to exercise (Schachter, 1978a) is not the only work that has revealed such a dichotomy in EIB. Studies looking at the effects of various pharmacological agents on EIB have demonstrated that some subjects have the predominant site of obstruction in their peripheral airways. This group of subjects had EIB attenuated by DSCG, but did not derive any benefit from anticholinergic agents (McFadden, 1977b and Thomson, 1978). Conversely, a small group of subjects are protected from EIB by atropine and not by DSCG (Godfrey, 1976). There is yet a third subpopulation whose main site of obstruction is in the larger airways, yet they have less EIB when given either DSCG or ipratropium bromide (another anticholinergic agent) (McFadden, 1977b and Thomson, 1978). Further evidence for dual mechanisms in EIB is provided by a study where ipratropium provided protection against bronchospasm induced by isocapnic hyperventilation in subjects who normally experienced bronchospasm after many such challenges repeated over a short interval without this drug. Subjects who did not continue to have bronchospasm after repeated challenges (analogous to the refractory period in EIB) did not benefit from ipratropium (Wilson, 1982). The hypothesis that emerges from this work is one where EIB actually may develop by two different mechanisms in different subjects:

1. A peripheral airway response that is from mediator release and responds to DSCG and

2. A central airway response that is mediated by reflexes and responds to anticholinergic medications.

The existence of such heterogeneity may help explain why studies have produced conflicting results when they have examined only one mechanism or the other at any single time.

In the present study we were unable to demonstrate a therapeutic effect of ascorbic acid for the group of subjects as a whole. While not statistically significant, there was a subgroup of three subjects who did appear to have their EIB attenuated by ascorbic acid, as shown in Figure 5. In this group the response to indomethacin was consistent with our hypothesis. This raises the possibility that the response to ascorbic acid may be another factor that can distinguish different types of asthmatics, possibly related to the different proposed mechanisms for EIB. In this small group of responders to ascorbic acid, however, there were no clear trends in small or large airway patterns identified that would suggest where ascorbic acid might be having its effect. Schachter and Schlesinger's data supports an effect of ascorbic acid on large airways because FEV1 and FVC were the parameters that indicated a protective effect on a statistically significant level (Schachter, 1982). This would be consistent with a role for ascorbic acid in the vagally mediated type of larger airway bronchospasm, and is further supported by work on the effect of ascorbic acid on methacholine induced bronchospasm and Mohsenin, 1983). Since the protection of ascorbic acid against methacholine is blocked by indomethacin, one would have to postulate an even more complex interaction between ascorbic acid, prostaglandins, and the cholinergic nervous system. (Of course, this might be even more

complicated given the trends in our study showing some effect of ascorbic acid on smaller airway parameters as well.)

Our results demonstrating no effect of either ascorbic acid or indomethacin on baseline airway tone agree with the earlier studies (Ogilvy, 1981, Schachter, 1982, and Mohsenin, 1983). This supports the notion that if ascorbic acid has a therapeutic effect, it probably works on a dynamic process in EIB, such as prostaglandin generation.

What is clear from the above discussion is the need for studies utilizing a larger number of subjects to look at ascorbic acid and EIB and the identification of a subgroup of asthmatics that are responsive to ascorbic acid. The magnitude of the effect of ascorbic acid in such a subgroup remains to be determined, but the experience in our study and the previous study of Schachter and Schlesinger suggest that it is not very great.

Another promising direction in this research is the development of the inhibitors of the various steps in arachidonic acid metabolism (Van Wauwe, 1983 and Weichman, 1984). Once such agents are adapted for use in animal studies and then human clinical trials, one will be better able to tease apart the relative contributions of leukotrienes and prostaglandins to EIB and how ascorbic acid may influence these pathways.

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