

Studies with the quantitative-inhalation challenge technique. I. Curve of dose response to acetyl-beta-methylcholine in patients with asthma of known and unknown origin, hay fever subjects, and nonatopic volunteers

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The quantitative-inhalation challenge technique has been used to study the responsiveness to Mecholyl of twenty-three subjects. Patients suffering from asthma had much greater sensitivity to inhaled Mecholyl than did hay fever patients and nonatopic subjects. The range of sensitivity of seventeen out of seventeen asthma patients extended from 2 to 68 mcg. of Mecholyl deposited in the bronchial tree, when a 30 per cent decrease in FEV₁ was achieved. Thirteen out of thirteen patients with asthma of known origin had a straight line dose-response curve (in direct proportion) to the amount of Mecholyl retained, and the response of four out of four patients with asthma of unknown origin showed irregular variations in the degree of bronchial obstruction when Mecholyl was deposited. The quantitative-inhalation technique provides reproducibility of results in a clinically usable range.

Deliberate and purposeful induction of asthma attacks is a useful procedure in the study of the pathologic physiology of asthma. With the recent development of a more precise method of measuring the amount of aerosols retained in the bronchial tree in a given range of particle size,¹⁹ it became possible to determine the exact amount of Mecholyl that could be retained in the bronchi of asthmatic and nonasthmatic subjects. Likewise, the correlation between the amount of any type of aerosolized particles retained in the bronchial tree and a coincident change in airway function could be measured. Mecholyl was chosen because it is more stable than acetylcholine.⁵ Its effects are analogous to those

From the Asthma-Allergy Department of the National Jewish Hospital at Denver.
Presented before The American Academy of Allergy, Miami, Fla., Feb. 17, 1965.
Received for publication May 10, 1965.

which follow stimulation of the parasympathetic nervous system accompanied by peripheral vasodilatation.

The major aims of this study were: (1) to determine the correlation between bronchial obstruction and the amount of Meecholy1 retained by inhalation in subjects with asthma of known and unknown origin, in subjects with hay fever, and in those without any atopy, and (2) to ascertain the range of reproducibility of results, using the quantitative-inhalation challenge technique.

It has long been known that acetylcholine and its derivatives (acetyl-beta-methylcholine and carbamylcholine) can be used to produce asthmalike episodes in subjects with bronchial asthma. Carmichael and Fraser¹ in 1933, and Fraser² in 1938 induced bronchial obstruction with acetylcholine given subcutaneously or intramuscularly. A number of investigators³⁻⁹ have used acetyl-beta-methylcholine (hereafter to be referred to as Meecholy1) by the subcutaneous and intramuscular routes to induce bronchial obstruction in susceptible individuals. Villaret and co-workers³ administered as much as 20 to 40 mg. of Meecholy1 subcutaneously; Curry and Lowell⁴ induced such episodes with as little as 1 to 6 mg. injected intramuscularly in asthma patients. Other workers⁵⁻⁸ reported a slight decrease in vital capacity in normal subjects, and Curry⁸ induced slight decreases in vital capacity in hay fever subjects with acetylcholine derivatives. Carmichael and Fraser¹ also provoked asthmalike attacks in susceptible individuals by injecting acetylcholine intravenously. Using the same route, Curry⁸ tried Meecholy1 and noted a much greater respiratory reaction than with other parenteral routes. Comroe and Starr¹⁰ produced bronchial constriction in animals, using intravenous Meecholy1. Oral doses of Meecholy1 have been given by Starr¹¹ and Starr and co-workers⁵ in doses up to 1 Gm. daily, and Starr has reported mild asthma symptoms in some of his normal subjects.

It was not until 1941 that the bronchial route for inducing asthmatic attacks with acetylcholine and its derivatives was tried. Dautrebande and Philippot¹² did extensive experiments using carbaminoylcholine aerosols. Tiffeneau and Beauvallet¹³ used a 1 per cent acetylcholine solution which provoked diminution of vital capacity in individuals with respiratory difficulty, but failed to produce any profound effect in normal individuals. Panzani,¹⁴ also using a 1 per cent solution of acetylcholine inhaled from 1 to 3 minutes, provoked decreases in vital capacity not only in asthma patients but also in others with nasal allergy and allergic bronchitis. Frank,¹⁵ giving four inhalations of a 50 per cent solution of acetylcholine, decreased the vital capacity of all of his eighteen chronic asthma subjects.

The application of Meecholy1 by inhalation has been tried more recently. Curry,⁸ in 1947, tried nebulization of a 1:40,000 solution of Meecholy1 chloride, and with as few as four inhalations produced a profound decrease in the vital capacity of his asthma subjects, one of whom required atropine and epinephrine injections for relief. He concluded that the respiratory tract was much more reactive to Meecholy1 when given by inhalation than parenterally. Lowell and Schiller,¹⁶ utilizing a solution containing 10 to 50 mg. of Meecholy1 per milliliter in buffered saline, induced asthma symptoms by inhalation in seven "intrinsic" asthma patients as readily as in younger patients with "extrinsic" asthma. Herx-

heimer^{17, 18} used a closed spirometer circuit to challenge fifteen normal individuals and thirty asthma patients with Mecholyl, and reported that the vital capacity decreased in three normal individuals and in all of his asthma patients in varying degrees.

CLINICAL MATERIALS AND METHODS

Seventeen patients suffering from intermittent asthma, hospitalized at the Asthma-Allergy Department of the National Jewish Hospital at Denver, were chosen for this study. They were selected mainly on the basis of the stability of their pulmonary functions, dependability, and cooperation. There was an almost equal division between the two sexes (nine males and eight females), and their age varied considerably, between 15 and 72. All had moderate-to-severe bronchial asthma, with duration of symptoms varying from 2 to 15 years. Thirteen of them had asthma of known origin diagnosed by correlating their histories, skin and conjunctival tests, and qualitative and quantitative direct bronchial inhalational challenges. The four patients classified as having asthma of unknown origin had a negative or a remote family history of allergy, a perennial type of asthma usually aggravated by infections, negative cause-and-effect history of sensitivity to specific allergenic factors, and negative-to-small skin test results, and they were completely unresponsive to bronchial and food challenges. Antihistaminic medications were withheld 24 hours prior to challenge, and bronchodilator tablets, aerosols, suppositories, and injections were withheld at least 6 hours before bronchial challenge with Mecholyl.

The services of six hospital employee volunteers were utilized: three had hay fever without any previous history of wheezing, and three were nonatopic and served as controls.

The quantitative-inhalation challenge apparatus¹⁹ was used for all the tests in this study. A fresh solution of Mecholyl dissolved in normal saline was prepared each week. The amount of Mecholyl necessary for challenge was computed before each test. Phenol red, an innocuous dye found to be tolerated safely by asthma patients, was used as a tracer agent. Glycerine was used to stabilize particle size by reducing vapor pressure. Test materials were prepared by making a 10 ml. solution consisting of 5 ml. of distilled water with 0.08 per cent of phenol red and 5 per cent of glycerine, and 5 ml. of a known quantity of Mecholyl dissolved in normal saline. The final concentration of Mecholyl used in the Mecholyl-phenol red-glycerine mixture for challenging the asthma patients varied from 0.0125 to 1.0 mg. per milliliter; and from 0.5 to a maximum of 10 mg. per milliliter for the six volunteer subjects. For one complete test, three 5 minute runs were made. A 2 ml. quantity of the prepared concentrate was put into a Vaponefrin nebulizer for each run. One milliliter of the final solution was diluted to 100 ml. with 0.05N sodium hydroxide solution, and then read in a Coleman Junior spectrophotometer set at 550 m μ .

The concentration of Mecholyl was gradually increased step by step by a factor of one fifth to one half. Occasionally, when too high a dose was inadvertently given, the procedure was reversed and the Mecholyl concentration was gradually reduced. Patients were challenged at the same time each day and not

more than once every 24 hours. All subjects were previously checked by a placebo run during which a normal saline solution was used instead of Mecholyl. Each subject became adept at blowing into the Stead-Wells spirometer and in breathing evenly while connected to the apparatus. As much as possible, falls in FEV₁ were kept between the 15 and 60 per cent levels, for reasons of significance on one hand, and safety on the other. Only the most rapid speed (1,920 mm. per minute) of the spirometer drum was used.

Three recordings of the FEV₁ of the subject were taken before the start of and after the end of each run. A 2 ml. amount of the mixed solution was introduced into the nebulizer. A clip was placed snugly over the nose of the subject, thus allowing him to breathe only through his mouth while connected to the apparatus. In fact, through the positioning of a pair of valves, he was breathing in room air and exhaling into the machine. Simultaneously, the nebulizer was activated by compressed air flowing at a steady rate of 6 L. per minute, and the vapor particles 3 μ or less in diameter were drawn through the machine by a vacuum pump at the other end running at a constant rate of one cubic foot per minute. Brown²¹ found that particles less than 3 μ in diameter would penetrate deep in the respiratory tract. The particles over 3 μ in diameter were trapped in the dishes of the sieve impinger of the No. 1 and No. 4 stages of a model No. 0200 Andersen sampler, and particles with diameters of less than 3 μ were collected in six steel plates of a model No. 0700 Andersen sampler on the other end of the machine, near the vacuum pump (see Fig. 1).

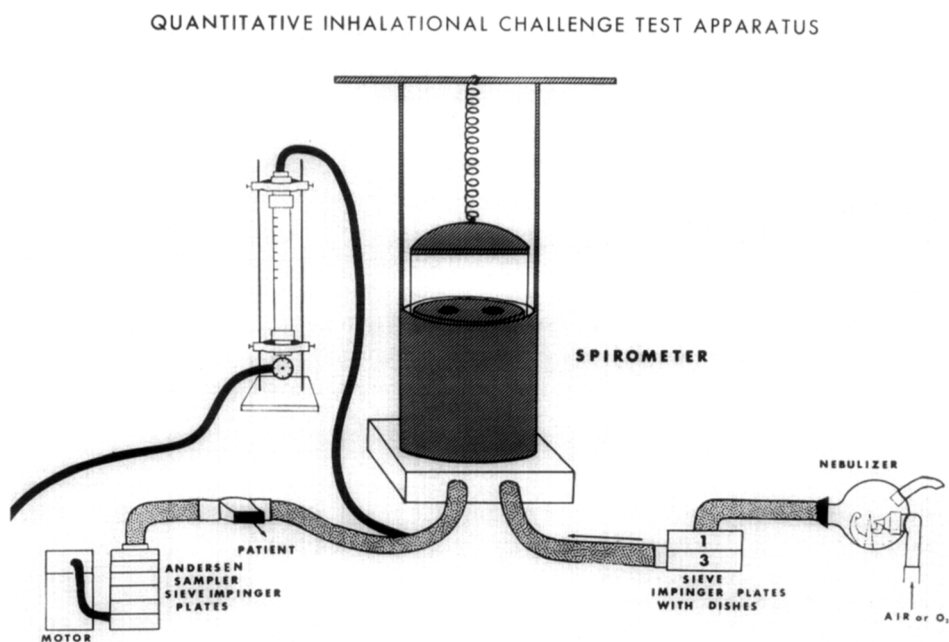


Fig. 1

Diagram of apparatus.

The three FEV₁ measurements taken after the end of the first 5 minute run were recorded. If no decrease* in function occurred, the test proceeded as follows: the distribution of the phenol red-Mecholyl mixture was determined in each of the six plates, the dishes, the tubing, and the nebulizer. The total amount of dye recovered was determined. (The dye was recovered by means of 10 to 20 ml. of 0.05N sodium hydroxide solution and read in the Coleman Junior spectrophotometer, making the necessary adjustments for computation purposes. The machine was then washed with warm tap water and dried.) A second 5 minute run was made, with the patient breathing in room air as he did in the first run. After the run, three FEV₁ measurements were taken again, and if the FEV₁ remained stable, the distribution of dye in the apparatus was determined again. Only if the variation in the distribution of the particles of the dye was less than 5 per cent between the first two runs was the final run undertaken, when the patient would breathe in the solution containing Mecholyl. The final run was similar to the first two runs, except that in the former the position of the valves was so arranged that the subject breathed in from the apparatus vapor stream and exhaled into the machine. Enough compressed air from another source was added to compensate for the room air previously inhaled by the patient during the first two runs. Immediately after the end of the final 5 minute run, three FEV₁ measurements were taken, followed by similar measurements 2 minutes later, and then every 5 minutes thereafter until the FEV₁ began to rise. The induced airway obstruction, if present, was then promptly terminated by inhalation of a nebulized solution of 0.50 per cent isoproterenol. The distribution of the dye in the apparatus was measured once more, and the total amount of dye recovered was again determined. The optical density of the material found in the six plates at the end of the final run was subtracted from the mean of the optical density of the material of the six plates after the first two runs. The optical density of the material retained was converted into micrograms of Mecholyl retained in the bronchial tree by using the formula:

$$\frac{\text{†O.D. retained by subject}}{\text{Total †O.D. in nebulizer}} = \frac{\text{X (amount of Mecholyl retained)}}{\text{Total amount of Mecholyl in nebulizer}}$$

The percentage fall in FEV₁ was calculated by using this formula:

$$\frac{\text{Highest FEV}_1 \text{ before final run} - \text{Highest FEV}_1 \text{ of lowest series after final run}}{\text{Highest FEV}_1 \text{ before final run}}$$

Epinephrine, aminophylline, atropine, and oxygen were always at hand to deal with any prolonged or severe descent in FEV₁. When indicated, isoproterenol by nebulization was used to terminate the attack as soon as the pulmonary function began to improve. All tests were performed in the presence of a physician. Graphs were prepared using the percentage fall in FEV₁ as the ordinate, and the amount of Mecholyl retained in the bronchial tree (converted into micrograms) as the abscissa. Each dot represents a single experiment of

*Less than 10 per cent.

†Optical density.

three runs, separated by at least twenty-four hours from another experiment with the same patient.

RESULTS

1. Asthmatic patients

Linear dose-response curves to Mecholyl could be plotted for thirteen of the seventeen asthma patients who were included in this study (Fig. 2, Table I). There was almost invariably, in these thirteen patients, a direct relation between the amount of Mecholyl retained in the bronchial tree and the resulting decrease in the FEV_1 ; and all had asthma of known origin, as supported by history, skin tests, conjunctival tests, and qualitative and quantitative challenges to inhalant allergens.

At the level of an FEV_1 decrease of 30 per cent, the amount of Mecholyl retained in the bronchi ranged from 6 mcg. in the most sensitive to 68 mcg. in the least sensitive patient, with the mean recorded at 29.5 mcg.; at the level of an FEV_1 decrease of 50 per cent, the amount of Mecholyl retained in the bronchi ranged from 15 to 97 mcg., with a mean recorded at 47.5 mcg. A narrower range of sensitivity as depicted by a steeper slope was found in those who were more sensitive to Mecholyl.

Four patients, all of whom had asthma of unknown origin, did not give a straight line response to Mecholyl (Fig. 3). The decrease in FEV_1 could not be predicted from the amount of Mecholyl retained by inhalation. Except for one patient who was more sensitive than any with asthma of known origin, all had sensitivity to Mecholyl within the same range as the latter.

In the overwhelming majority of the tests performed, the maximum fall in FEV_1 occurred 2 minutes after the end of the 5 minute challenge. Almost invariably, the FEV_1 started to rise 5 to 10 minutes after the end of the challenge. A feeling of tightness of the chest and/or the presence of audible wheeze was

Table I. *Thirteen patients with asthma of known origin: comparison of minimum with maximum significant fall in FEV_1 and the amount of bronchial retention of Mecholyl in micrograms*

<i>Name, sex, age</i>	<i>Lowest significant fall in FEV_1 (%)</i>	<i>Amount of Mecholyl retained (mcg.)</i>	<i>Highest significant fall in FEV_1 (%)</i>	<i>Amount of Mecholyl retained (mcg.)</i>
To, M, 16	23	3	65	22
Ro, M, 16	17	5	60	30
Dn, M, 35	23	8	52	56
Se, F, 15	24	9	60	40
Sa, F, 18	22	9	56	17
Ne, M, 19	26	8	69	40
Ho, M, 21	25	12	60	29
Ca, F, 72	17	37	59	55
At, M, 15	16	22	61	85
Dv, M, 18	15	15	44	67
Jo, M, 52	15	36	42	58
Cr, F, 17	28	57	62	95
Li, F, 16	14	53	53	105-115

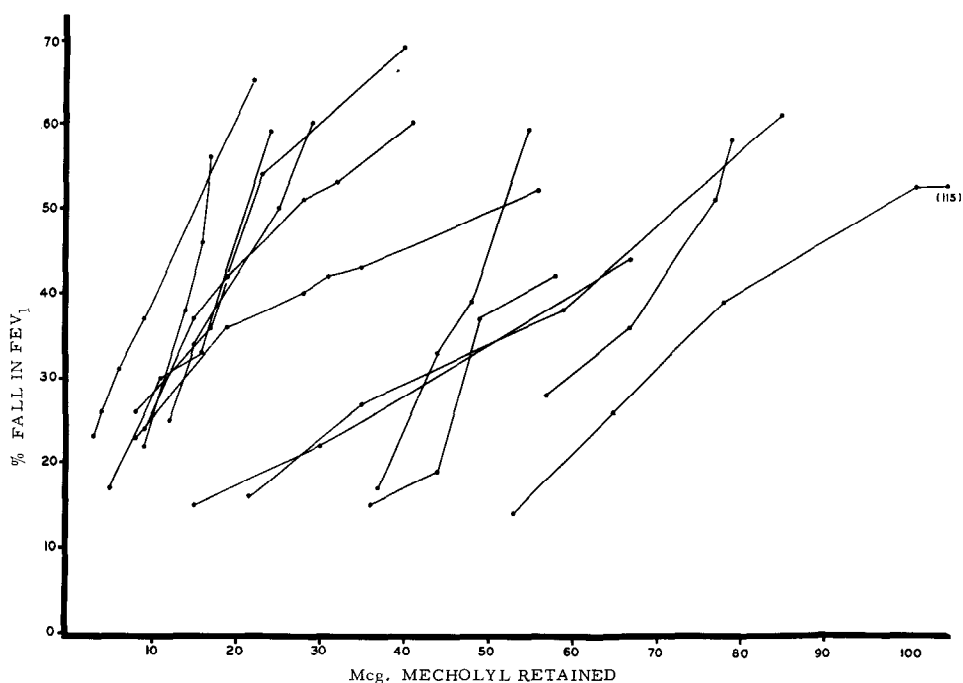


Fig. 2

Mecholyl dose responses on 13 patients with asthma of known origin.

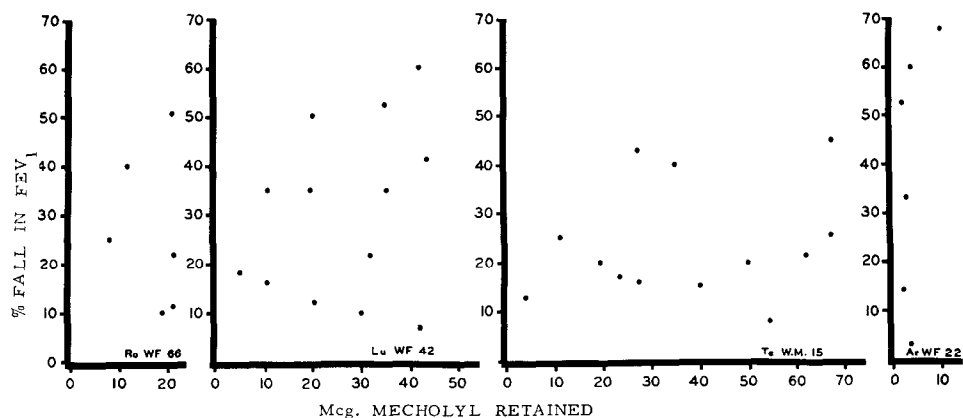


Fig. 3

Mecholyl dose responses on 4 patients with asthma of unknown origin.

noted in most patients when the first second vital capacity was reduced by 30 per cent or more. Isoproterenol was used to terminate the attack, when needed, but in no case was epinephrine injection or oxygen inhalation necessary. No systemic symptoms were noted in any of the asthma subjects. There seemed to be

no significant difference, from the clinical viewpoint of severity of asthma (as judged by therapeutic indications), between the more sensitive and the less sensitive patients with asthma of known or of unknown origin.

2. Subjects without asthma

Of the three hay fever subjects, an almost linear response to Mecholyl was obtained in two, but at much higher concentrations of Mecholyl than in the least sensitive patient with asthma of known origin: one about fifteen times and the other about fifty-two times more at the level of 30 per cent decrease in FEV₁. One of the hay fever subjects had a maximum fall of 42 per cent, the second 51 per cent, and the third only 16 per cent (Figs. 4 and 5; Table II). The decrease of FEV₁ in the subject with the greatest reduction seemed to be maximal, since further increase in dose could no longer increase the bronchial obstruction.

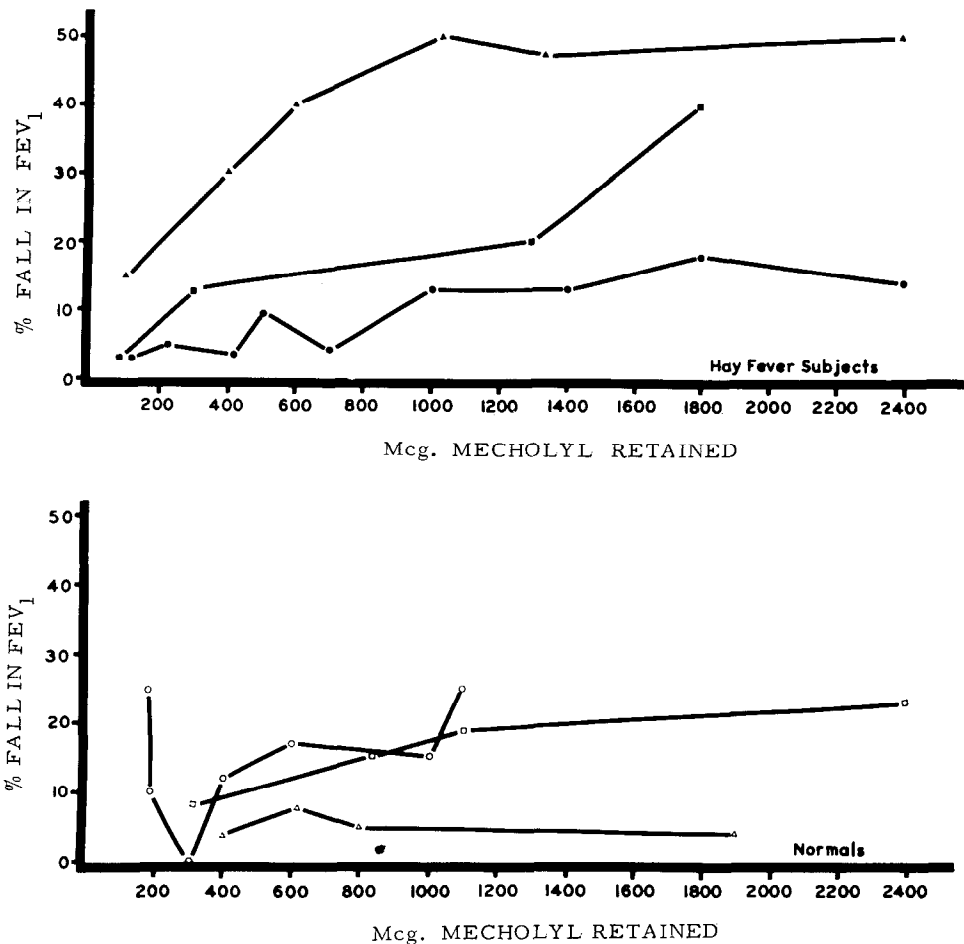


Fig. 4

Mecholyl dose responses on 3 hay fever subjects and 3 normal subjects.

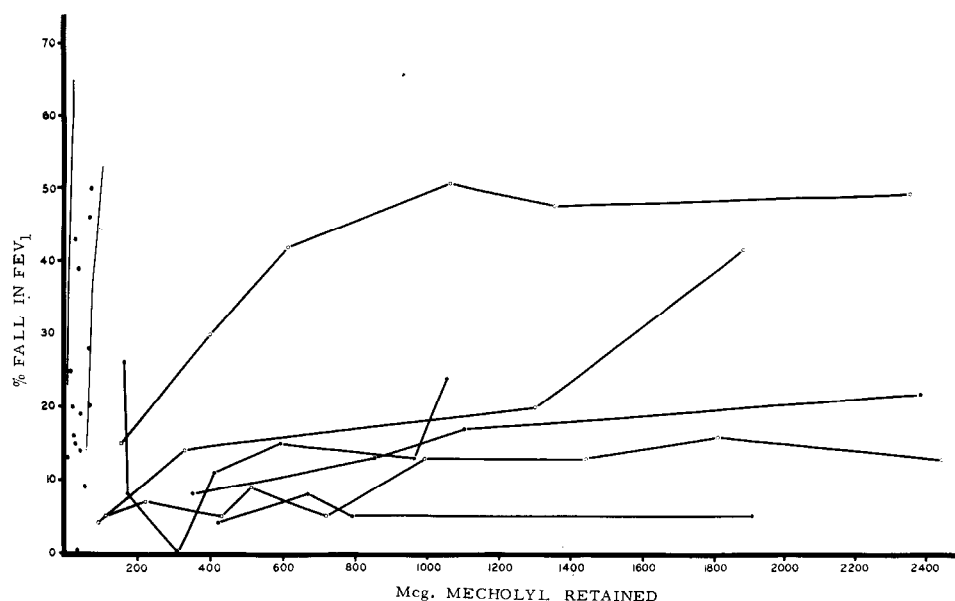


Fig. 5

Composite Mecholyl dose responses on the most sensitive and the least sensitive patients with asthma of known origin, the least sensitive patient with asthma of unknown origin, the hay fever subjects, and the normal subjects. Key: ● ● ●, patient with asthma of unknown origin; —, patients with asthma of known origin; ● — ●, normal subjects; ○ — ○, hay fever subjects.

Table II. Six subjects without bronchial asthma, showing their greatest fall in FEV_1 and the greatest amount of bronchial retention of Mecholyl*

Subject, sex, age	Highest per cent fall in FEV_1 (%)	Greatest amount of bronchial retention (mcg.)
Al, M, 28	9	1,920
Mk, M, 30	23	2,400
Sr, F, 25	26	950
An,† F, 21	16	2,440
El,† F, 32	42	1,900
Mg,† F, 22	51	2,350

*Greatest fall in FEV_1 does not necessarily correspond to the greatest amount of bronchial retention of Mecholyl.

†Hay fever.

The FEV_1 of none of the three nonatopic subjects could be made to fall as much as 30 per cent, even though deposition of Mecholyl reached up to 2.4 mg. (Figs. 4 and 5; Table II). Higher doses could not be given to any of the non-asthmatic subjects because of severe side-effects, such as coughing, substernal constriction, pharyngeal itching, flushing of the face and neck, profuse salivation, and occasionally urinary urgency and throbbing headache, particularly after 1 mg. or more of drug had accumulated in the bronchial tubes. The amount of deposited Mecholyl was similar for both hay fever and normal subjects.

Fig. 5 is a composite of all the results obtained in the most sensitive patient

and least sensitive patient with asthma of known cause, the least sensitive patient with asthma of unknown cause, the hay fever subjects, and the nonatopic subjects.

DISCUSSION

Grollman states that "Many potent pharmacologic agents, such as acetylcholine or epinephrine, act in such small amounts that the maximum amount of the drug reacting with specific receptors in the cells does not alter the concentration of the drug in the surrounding fluid, and the drug is destroyed rapidly with immediate recovery of the cell."²² "Many investigators have found that this relationship is usually typified by an S-shaped dosage-response curve."²³ This presupposes a threshold dose below which the drug does not act, and a maximum dose in which there is a limit of responsiveness, so that further increase in dose no longer gives an increase in response, since all the receptors have already been acted upon.²² We observed this latter phenomenon in our least sensitive patient with asthma of known origin (Fig. 2) and in one of the hay fever subjects (Fig. 4).

Each of our asthma subjects, regardless of whether the cause of his asthma was known or not, suffered a 30 per cent decrease in FEV₁ upon retention within the bronchial tree of 68 mcg. or less of Mecholyl. No other condition is known at the present time to cause more exquisite sensitivity to this substance. It may thus be possible to use this fact in differentiating asthma from other diseases of bronchial obstruction, especially when supported by history and other laboratory evidence. Indeed, for purposes of absolute confirmation of the scientific validity of the above statement, the range of sensitivity to Mecholyl should be determined in patients concerning whom there is no question of the absence of asthma, but who are known to have other conditions.

In previous studies on Mecholyl sensitivity, no attempt has been made to separate asthma of known from that of unknown etiology, and this may explain why a linear response curve was not found. Thus Curry⁸ found with low doses of Mecholyl given intravenously that the decrease in vital capacity did not change even when the dose was varied; however, larger concentrations given parenterally provoked proportionally greater decreases in the vital capacity of his asthma subjects. Again, Curry observed that, in some of his patients who had been given the same amount of Mecholyl several times, variable responses in reduction in vital capacity were achieved. He attributed this to variability in the degree of drug absorption due to slight changes at the site of injection. However, the patients in our study with asthma of unknown origin also showed marked irregularity in bronchial response to Mecholyl, although the route and type of administration (bronchial inhalation of measured particles) was the same. It would be interesting to know whether the patients of Curry who manifested variable bronchial response to the same dose could have been classified as suffering from asthma of unknown origin. Samter²⁴ advanced the hypothesis that "intrinsic" bronchial asthma could be a cholinergic disease, causing symptoms by excessive release or by incomplete inactivation of acetylcholine. On the other hand, variability of bronchial response to Mecholyl may also be evidence

of an unstable parasympathetic nervous system in patients with asthma of unknown origin, a possibility which may lend some support to the old "pathologic vagotonia" theory of asthma of Eppinger and Hess,²⁵ which might apply only to such patients. It may be that the normal response is a straight line and that increased sensitivity of response to Meeholyl in patients with asthma of known origin is due to a different pathologic mechanism from that in patients with asthma of unknown cause (parasympathetic lability).

It is tempting to speculate that a dose response curve with Meeholyl might be useful as a means of differentiating asthma of allergic cause from that of unknown cause. One patient (Ca) was given a provisional diagnosis of asthma of unknown origin after the usual studies for allergy were done. We obtained a straight line dose-response curve with Meeholyl and were thus alerted to seek further for an allergic cause. Subsequently, extracts of dust from her own home were made and were used to provoke positive skin reactions and inhalational responses. Of course, a larger number of patients should be studied before this hypothesis can be confirmed.

It was our observation that the more nearly perpendicular the curve of response was to the abscissa (the amount of Meeholyl retained), the more sensitive the bronchial tree of the patient tended to be to inhaled allergens. It is possible that Meeholyl sensitivity develops concomitantly with sensitivity to inhaled allergens, perhaps making it worthwhile to rechallenge the same subjects with Meeholyl after several years of allergen hyposensitization. Makino²⁶ found no direct or inverse relationship between sensitivity to acetylcholine and hyposensitization injections. The duration of his study, between six months and a year, may have been too short to evoke any changes.

None of our nonatopic subjects achieved a 30 per cent decrease in FEV₁, although huge amounts (up to 2.4 mg.) of Meeholyl were retained in the bronchial tree. We were able to decrease the FEV₁ of two of the three hay fever subjects, by 42 per cent and 51 per cent respectively, with doses of Meeholyl as high as those administered to the nonatopic subjects. The finding of Meeholyl sensitivity greater in some hay fever subjects than that in normal controls is in agreement with the observations of previous workers.^{8, 27} It may be desirable to recheck the Meeholyl sensitivity of hay fever patients from time to time (such as once a year), particularly if spontaneous wheezing develops.

The occurrence among our nonasthmatic subjects of systemic manifestations after bronchial challenge with Meeholyl proves not only that Meeholyl was retained, but also that it was absorbed via the bronchial route. The manifestations were in the form of vasodilatation of the blush areas, increased salivary secretion, and, occasionally, urgency of micturition and throbbing headache.

The straight line dose-response curves between the 25 and 50 per cent levels of decrease in FEV₁ obtained by the study of our patients with asthma of known origin confirms the reproducibility and precision of the quantitative-inhalation technique as a means of measuring quantities of aerosol retained in the bronchial tree. In some of the patients who were still available after several months, the same amount of Meeholyl retained produced a decrease in FEV₁ within 10 per cent of the original decrease. With this method it was possible to predict for

each patient with asthma of known cause the degree of airway obstruction to a known quantity of Mecholyl. We have not been as successful in reproducing predicted decreases in FEV₁ with extracts of inhaled allergens measured in protein nitrogen units. Mecholyl has a narrower range of reproducibility than allergenic extracts because, being a chemical, it is easily standardized, compared with allergenic extracts which are more susceptible to deterioration and are more difficult to standardize. Furthermore, the metabolic action of Mecholyl is short lived, so that within 24 hours the condition of the patient returns to the level before challenge; this is unlike the case of pollen extracts, in which it is not known how long the effects or even the antigen itself will remain.

Thus far, this technique is limited by the time required to do the test, and the selection of cooperative patients at least temporarily free of symptoms, with stability of airway function.

REFERENCES

1. Carmichael, E. A., and Fraser, F. R.: The Effects of Acetylcholine in Man, *Heart* **16**: 263, 1933.
2. Fraser, F. R.: The Clinical Aspects of the Transmission of the Effects of Nervous Impulses by Acetylcholine. Lecture I: Physiology of Acetylcholine, *Brit. M. J.* **1**: 1249, 1938.
3. Villaret, M., Vallery-Radot, P., Justin-Besançon, L., and Claude, F.: Recherches préliminaires sur les crises provoquées chez les asthmatiques par certains esters de la choline, *Compt. rend. Soc. biol.* **116**: 1343, 1934.
4. Curry, J. J., and Lowell, F. C.: Measurement of Vital Capacity in Asthmatic Subjects Receiving Histamine and Acetyl-Beta-Methyl Choline, A Clinical Study, *J. ALLERGY* **19**: 9, 1948.
5. Starr, I., Jr., Elsom, K. A., Reisinger, J. A., and Richards, A. N.: Acetyl-beta-methylcholine. I. The Action on Normal Persons, With a Note on the Action of the Ethyl Ether of Beta-Methylcholine, *Am. J. M. Sc.* **186**: 313, 1933.
6. Hurtado, A., and Kaltreider, N. L.: Studies of Total Pulmonary Capacity and Its Subdivisions. VII. Observations During the Acute Respiratory Distress of Bronchial Asthma and Following the Administration of Epinephrine, *J. Clin. Invest.* **13**: 1053, 1934.
7. Moll, H. H.: The Action of Parasympathetic-mimetic Drugs in Asthma, *Quart. J. Med.* **9**: 229, 1940.
8. Curry, J. J.: Comparative Action of Acetyl- β -methyl Choline and Histamine on the Respiratory Tract in Normals, Patients With Hay Fever, and Subjects With Bronchial Asthma, *J. Clin. Invest.* **26**: 430, 1947.
9. Bresnick, E., Beakey, J. F., Levinson, L., and Segal, M. S.: Evaluation of Therapeutic Substances Employed for the Relief of Bronchospasm. V. Adrenergic Agents, *J. Clin. Invest.* **28**: 1182, 1949.
10. Comroe, J. H., Jr., and Starr, I., Jr.: Further Studies on the Pharmacology of Acetyl- β -methylcholine and the Ethyl Ether of β -methylcholine, *J. Pharmacol. & Exper. Therap.* **49**: 283, 1933.
11. Starr, I., Jr.: Acetyl-beta-methylcholine. III. Its Action on Paroxysmal Tachycardia and Peripheral Vascular Disease, With a Discussion of Its Action in Other Conditions, *Am. J. M. Sc.* **186**: 330, 1933.
12. Dautrebande, L., and Philippot, E.: Crise d'asthme expérimental par aérosols de carbaminoylcholine chez l'homme traitée par dispersat de phénylaminopropane. Etude de l'action sur la respiration de ces substances par la détermination de volume respiratoire utile, *Presse. méd.* **49**: 942, 1941.
13. Tiffeneau, R., and Beauvallet, M.: Epreuve de bronchoconstriction et de bronchodilatation par aérosols. Emploi pour le dépistage, la mesure et le contrôle des insuffisances respiratoires chroniques, *Bull. Acad. Med., Paris* **129**: 165, 1945.
14. Panzani, R.: Test de la dyspnée provoquée par l'acétylcholine et diagnostic de l'allergie respiratoire, *Internat. Arch. Allergy* **7**: 25, 1955.
15. Frank, D. E.: The Effect of Drugs in Acetylcholine-Induced Bronchial Obstruction, *Ann. Allergy* **17**: 200, 1959.
16. Lowell, F. C., and Schiller, I. W.: The Induction of Asthmalike Attacks in Subjects With "Idiopathic" Asthma, *J. ALLERGY* **19**: 172, 1948.
17. Herxheimer, H.: Bronchial Obstruction Induced by Allergens, Histamine, and Acetyl-beta-methylcholine Chloride, *Internat. Arch. Allergy* **2**: 27, 1951.
18. Herxheimer, H.: Induced Asthma in Man, *Lancet* **1**: 1337, 1951.

19. Itkin, I. H., Anand, S., Yau, M., and Middlebrook, G.: Quantitative Inhalation Challenge in Allergic Asthma, *J. ALLERGY* **34**: 97, 1963.
20. Middlebrook, G.: Immunological Aspects of Airborne Infection: Reactions to Inhaled Antigens, *Bact. Rev.* **25**: 331, 1961.
21. Brown, J. H., Cook, K. M., Ney, F. G., and Hatch, T.: Influence of Particle Size Upon the Retention of Particulate Matter in the Human Lung, *Amer. J. Public Health* **40**: 450, 480, 1950.
22. Grollman, A., editor: *Pharmacology and Therapeutics*, ed. 5, Philadelphia, 1962, Lea & Febiger, pp. 40-42.
23. Martin, F. W., and Cook, E. F., editors: *Remington's Practice of Pharmacy*, ed. 11, Easton, Pa., 1956, The Mack Publishing Co., p. 1368.
24. Samter, M.: Bronchial Asthma; Inventory and Outlook, *Postgrad. Med.* **37**: 319, 1965.
25. Eppinger, H., and Hess, L.: *Vagotonia, a Clinical Study in Vegetative Neurology*, New York, 1915, Nervous and Mental Disease Publishing Co., pp. 28-30, 46-48.
26. Makino, S.: Studies on Inhalative Tests in Bronchial Asthma (II)—With Special Emphasis Upon Inhalative Acetylcholine Test, *Jap. J. Allergy* **13**: 32, 1964.
27. Townley, R. G., Dennis, M., and Itkin, I. H.: Comparative Action of Acetyl-beta-methylcholine, Histamine, and Pollen Antigens in Subjects With Hay Fever and Patients With Bronchial Asthma, *J. ALLERGY* **36**: 121, 1965.