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CLINICAL AND PHYSIOLOGICAL STUDIES ON CHRONIC BRONCHITIS

III. *Bronchial Reactivity to Inhaled Acetylcholine*^{1, 2}

By

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INTRODUCTION

Patients with a history of non-specific broncho-pulmonary disease are known to react more violently when inhaling various airborne agents than subjects without such disease (17, 45, 46, 47, 50). This so-called "bronchial hyperreactivity" seems to be profoundly important for the initiation or aggravation of respiratory symptoms during the course of disease.

Airways obstruction may be induced in man experimentally by, for example, cold air (54), cigarette smoke (33, 43), inert dust (22, 32, 55), or various chemical agents (1, 2, 4, 6, 25). Histamine and acetylcholine have long been known to provoke asthma-like attacks in patients with non-specific lung disease (17, 46, 50). Accordingly, these substances have been used in trying to assess bronchial reactivity (11, 46, 50, 51).

This paper presents studies of bronchial reactivity to acetylcholine in "healthy" subjects and in patients with different severity of chronic bronchitis.

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MATERIAL

a) Ten female and 10 male *non-bronchitic subjects* were tested. They all appeared healthy, without any chronic airways or heart disease. There were 6 laboratory assistants, 4 doctors, 6 students, 2 nurses, 1 engineer, and 1 ward-assistant. Physical characteristics and spirometric values are shown in Table 1. Personal and hereditary allergy, smoking habits, and presence of acute cold are noted in Table 2.

TABLE 1
*Physical Characteristics of Healthy Subjects Tested with
Acetylcholine Inhalation.*

Subj.	Age	Height cm	Max. FEV _{1.0}		Max. FVC		FEV _{0.0}	
			Lit. ATPS	% pred	Lit. ATPS	% pred.	Obs.	Pred.
<i>Males:</i>								
BA	28	186	4.08	94	4.56	83	90	80
SL	25	189	4.41	94	5.82	101	76	82
BO	28	184	4.44	101	5.85	106	77	81
BS	31	179	4.59	112	5.90	115	79	80
ON	25	185	3.88	84	4.90	88	79	82
IE	24	176	3.83	91	4.69	91	82	83
HAH	24	179	4.69	105	5.76	108	81	83
JE	27	180	3.93	91	5.36	101	73	82
JC	28	169	4.44	113	5.13	108	87	82
RM	32	178	3.80	94	4.49	88	85	80
<i>Females:</i>								
AGB	27	170	2.91	88	3.87	99	75	85
MES	33	167	3.01	100	3.59	97	84	84
MH	21	167	3.16	94	3.61	92	87	87
AP	27	153	2.24	79	2.60	81	86	86
SB	25	164	2.55	81	2.91	79	88	86
BJ	26	160	2.45	80	3.23	91	76	85
KG	28	169	2.62	81	3.60	92	73	85
IE _n	25	161	2.65	86	3.26	91	81	86
IE	25	168	3.93	123	4.13	106	95	86
UA	30	160	2.86	100	3.64	95	79	84
M: 10	27	181	4.21	98	5.25	99	81	81
F: 10	27	164	2.84	91	3.44	92	82	85

TABLE 2
*Effect of Healthy Subjects of Inhaling Acetylcholine—
 Relation to Allergy and Smoking.*

Subj.	Pos. Ventil. Test			Cough	Allergy		Smoking
	% ACh	% decr. FEV _{1.0}	PEF	% ACh	Familial	Personal	+ cigarettes (+) cigars
<i>Males:</i>							
BA	1%	10	18	—	—	—	—
SL	—	4	5	1%	—	—	+
BO	—	3	0	—	—	—	—
BS	—	6	1	—	—	—	—
IE	—	4	12	0.5%	—	?	+
ON	—	3	5	0.5%	—	—	+
HAH	—	6	7	—	—	—	—
JE	—	1	8	—	—	—	(+)
JC	—	3	10	0.5%	—	—	+
RM	1%	4	17	—	—	—	(+)
<i>Females:</i>							
AGB	$\left\{ \begin{array}{l} 1\% \\ 1\% \end{array} \right.$	19 15	15	1%	—	+	—
MES	0.1%	12		1%	?	+	(+)
MH	—	+1	+1	—	—	—	+
AP	—	+12		—	—	—	—
SB	$\left\{ \begin{array}{l} 1\% \\ 1\% \end{array} \right.$	33 14	6 21	1% —	+	—?	—
BJ	0.01%	11	4	—	+	+	—
KG	$\left\{ \begin{array}{l} 0.5\% \\ 1.0\% \end{array} \right.$	14 5	23 16	0.1% —	—	—	+
IE _n	—	5	8	—	—	—	—
UA	$\left\{ \begin{array}{l} - \\ - \end{array} \right.$	5 2	7 2	— 0.1%*	+	—	(+)
IE _l	—	9	2	—	—	—	—
M: 10	2			4	0	0	6
F: 10	5			5	3	3?	4

* Subj. UA had a slight cold at the second test.

All 20 subjects had an FEV% above $\mu-1$ SD except the two females AGB and BJ, who, although they were apparently healthy and symptom-free, had a FEV% just above $\mu-2$ SD (5).

TABLE 3

Grouping According to FEV% Values from the Control Series in the ACh Test Related to Normal Predicted.

Parameters		Grouping according to FEV%								
		I $\geq \mu - 1SD$			II $< \mu - 1SD \geq \mu - 2SD$			III $< \mu - 2SD$		
		μ	-1SD	Obs.	μ	-1SD	Obs.	μ	-1SD	Obs.
FEV%	Males	71	64	71	71	64	59	72	64	44
	Females	77	72	79	81	76	72	80	75	56
	Mean	75	69	76	76	70	66	74	67	48
FEV _{1.0}	Males	3.23	2.73	3.03	2.96	2.46	2.06	3.03	2.53	1.35
	Females	2.35	2.00	2.30	2.69	2.35	2.14	2.58	2.23	1.68
	Mean	2.73	2.31	2.62	2.82	2.40	2.10	2.88	2.43	1.45
FVC	Males	4.49	3.99	4.32	4.26	3.76	3.63	4.32	3.82	3.07
	Females	2.96	2.56	2.91	3.34	2.94	2.92	3.25	2.82	2.97
	Mean	3.62	3.18	3.52	3.77	3.32	3.25	3.97	3.32	3.04
AGE	Males			53			56			57
	Females			53			44			47
	Mean			53			50			54
No. of Subj.	Males			10			7			17
	Females			13			8			8
Total				23			15			25

b) The *patient* group consists of 28 female and 30 male subjects with chronic bronchitis. Their physical characteristics were given in Paper No. 1. In the present study they were grouped according to their FEV% values (max. FEV_{1.0}/actual FVC $\times$ 100) as recorded in the control series of 3-4 forced expirograms made immediately before inhaling acetylcholine. The FEV% values so obtained were related to predicted normal values and arranged in three groups with the following characteristics:

- I. FEV% larger or equal to $\mu - 1$ SD.
- II. FEV% less than $\mu - 1$ SD, but larger than or equal to $\mu - 2$ SD.
- III. FEV% less than $\mu - 2$ SD.

TABLE 4

Effect of Inhaling Increasing Concentrations of Acetylcholine and later Isoprenaline. Patients with Different Degree of Airways Obstruction.

Parameters mean values		Groups according to pred. FEV ⁰ / ₀		
		I	II	III
		Change in per cent of control value		
FEV _{1.0}	Control, lit.	2.72	2.17	1.43
	0.01 % ACh	—0.4	—1.9	—3.9*
	0.1	—2.8	—2.7	—11.5§
	0.5	—6.2§	—13.4§	—19.5§
	1.0	—8.8§	—14.0§	—21.7§
	Isoprenaline	—3.7	+0.2	+3.3
FVC	Control, lit.	3.67	3.31	3.08
	0.01	—1.2	—0.7	—5.1*
	0.1	—2.9	—5.5	—10.3§
	0.5	—6.8§	—11.2§	—18.6§
	1.0	—8.7§	—11.9§	—19.2§
	Isoprenaline	—4	—2.9	+4.6
FEV%	Control	75	65	47
	0.01	75	63	48
	0.1	75	64	47
	0.5	75	57	47
	1.0	73	63	45
	Isoprenaline	75	65	50
PEF	Control, lit./min.	408	326	215
	0.01	—2.9*	—0.1	—7.8§
	0.1	—4.5§	—4.3	—14.5§
	0.5	—10.4§	—10.5	—18.7§
	1.0	—11.4§	—14.7§	—27.1§
	Isoprenaline	—3.3	+9.1	+13.6
Number of	Control†	23	15	25
	0.01	21	10	19
FEV _{1.0}	0.1	21	12	19
FVC	0.5	21	11	17
FEV%	1.0	19	12	15
	Isoprenaline	21	10	14

* = $P < 0.05$.

§ = $P < 0.01$.

† = Number of patients.

TABLE 5

Effect of Acetylcholine Inhalation in Relation to History of Bronchial Irritability, Smoking and Allergy.

(Reaction to the lowest concentration of ACh from one or several tests was recorded).

			FEV ₀ groups			
			I 23 (10M, 13F)	II 15 (6M, 5F)	III 25 (17M, 8F)	Total
Acetylcholine Tests	Ventila- tory effect	0.01	0	0	3	3
		0.1	4	2	11 (1)	17 (1)
	at resp.	0.5	7	3	4	14
	ACh	1.0	2 (2) §	4 (2)	(4)	6 (8)
	conc. %	Neg.	8	4	2	14
	Cough elicited	0.01	6	3	9	18
		0.1	6	5	4	14
	at ACh	0.5	7	3	2	12
	conc. %	1.0	1	(1)	1 (4)	2 (5)
		Neg.	3	3	5	11
History of bronchial irritability			17	11	12	40
			3	2	4	9
			9	3	12	24
Questions No.*			5	3	7	15
Smokers & ex-smokers			20	10	20	50
History of Allergy	Fam.		11/23	8/13	10/21	29/57
	Pers.		5/23	4/13	10/21	19/57
Allergy Tests	Cut.		7/20	5/13	3/21	15/54
	Inhal.		2/19	1/11	5/19	7/49
Eosinophils	Blood or Sputum		7/22	5/13	6/21	18/56
Subj. Appearing in Diff. Groups due to Diff. Basal Values			Nos. 8, 111 in Group I and II	Nos. 5, 10, 25, 114 in Groups II and III		6

* Cough and dyspnoea worse at: Q1: Bad weather (damp, fog, cold). - Q2: Temp. changes. - Q3: Smoke, dust, odors. - Q4: Movements, exertion.

§ Figures within brackets denotes the only conc. tested.

The grouping was thus somewhat different from that reported in Paper II. The corresponding values for $FEV_{1.0}$ and FVC are also included in Table 3.

Group I consisted of 10 males and 13 females; average age was 53 (28–66) years. Seven males and 8 females constituted Group II; the mean age was 50 (36–66) years. The last group, III, included 17 male and 8 female subjects with an average age of 54 (37–66) years; the males were somewhat older than the females.

In 6 instances of 25 repeated tests, different basal $FEV\%$ values were found, which meant that these 6 patients were referred to different groups on different test occasions. The results in these patients only show the lowest acetylcholine chloride concentration at which positive tests were recorded. The 6 patients were only recorded in the lowest group in question concerning history of irritability and allergy (Table 5).

METHODS

Drugs. The *acetylcholine chloride* (ACh) used was manufactured by B. Hoffman-La Roche & Co. According to the manufacturer the temperature is the main factor in keeping a solution potent. A 1 per cent solution was reported to be hydrolized by 1.9 per cent after storage for 25 days in $+10^{\circ}\text{C}$. Thus a 1 per cent solution may be stored in a refrigerator for at most a couple of days; lower concentrations were never stored.

0.1 g ACh was dissolved in 10 ml saline = 1 per cent solution, part of which was diluted to the double volume (= 0.5 per cent ACh). The 1 per cent solution was diluted 10 times (= 0.1 per cent), and part of the latter solution was further diluted 10 times (= 0.01 per cent ACh). For nebulization, 0.5 ml solution was used, thus constituting 5, 2.5, 0.5 and 0.05 mg in the respective solutions.

A plastic nebulizer (Vaponefrin Co.) giving a mean particle size of 2.6 microns was used with an air flow of 3 lit./min.

Wearing a nose-clip, the subjects breathed for $1-1\frac{1}{2}$ minutes through the nebulizer until the solution was finished. This means that at least 50 per cent of the solution was exhaled through the nebulizer. *Isoprenaline* was given with the Medihaler® device; 2–3 inhalations were made.

Procedure. Basal values were obtained by making 4–5 forced expiratory spirometers on a low-resistance spirometer (40). Peak flow measurements were performed in alternation with the spirometers (41). After inhalation of the 0.01 per cent ACh solution, forced expirograms and peak flow measurements were

made at about 1, 3, 5 and, sometimes, 7 minutes afterwards. In the same way, 0.5 ml of the next concentration, 0.1 per cent acetylcholine, was inhaled and another series of 3-4 forced expirograms and PEF were done. The same scheme was followed for solutions of 0.5 and 1.0 per cent ACh. After the last series of spirograms, 2-3 inhalations of Isoprenaline (0.12-0.18 mg Isoproterenol) were given, after which 3-4 FVC and PEF were recorded.

Analysis. From the spirograms were calculated the forced expiratory one-second volume ($FEV_{1.0}$), the forced vital capacity (FVC), and $FEV_{1.0}$ in per cent of actual FVC ($= FEV\%$). The *mean values* for each series were calculated and compared with the mean value of respective preceding control values. The difference in per cent of the control value was recorded for each parameter except for $FEV\%$, which was given in absolute value.

In subjects with normal $FEV\%$ ($\geq \mu-1$ SD), a test was considered positive when there was a decrease of average control $FEV_{1.0}$ or FVC of ≥ 10 per cent, or ≥ 15 per cent of mean control PEF. In subjects with an $FEV\% < \mu-1$ SD, the reduction of $FEV_{1.0}$ and FVC had to be 15 per cent and that of PEF 20 per cent to be considered significant (40, 41).

RESULTS

A. Healthy Subjects.

Seven subjects (5 females and 2 males) had positive ventilatory tests, one each on ACh concentration 0.01, 0.1 and 0.5 per cent, and 4 on 1 per cent. Four of the females were tested twice, and reacted positively on both occasions. Nobody coughed after inhaling 0.01 per cent ACh; 5/18 coughed first at 0.5 per cent, and 8/19 first at 1 per cent.

When the individual results were compared with respective history of allergy and smoking habits, some interesting connections were found (Table 2):

a) In the 7 subjects with positive ventilatory test, 4 subjects, all females, revealed some allergy or heredity for allergy (AGB, BJ, SB, MES). Two subjects with familial allergy but without personal allergy were smokers (KG, RM), one was a smoker with allergy (MES). According to χ^2 tests with Yates correction for small numbers (16), there was a significantly larger frequency of positive ACh tests in subjects with allergy connections ($P < 0.001$).

b) Out of the 9 subjects who got severe cough, 7 were smokers; one female subject (UA) got cough only in com-

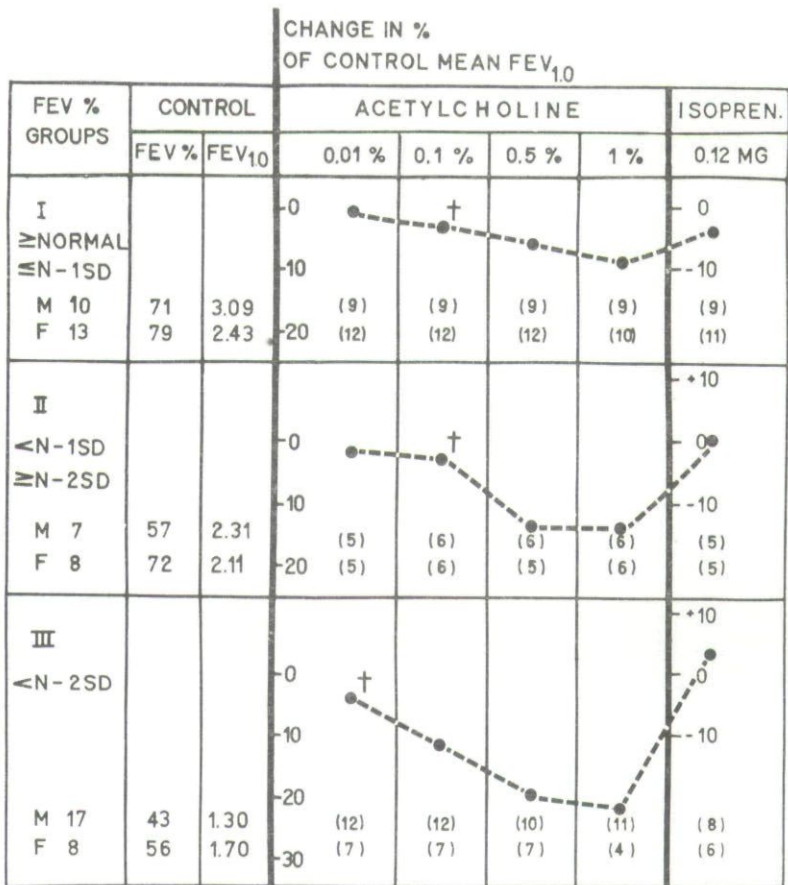
bination with a slight acute cold. Of the 10 smokers in the material, all but one got either cough or ventilatory impairment on ACh inhalation (Table 2). According to χ^2 test there was a significant difference in frequency of cough after ACh in smokers and non-smokers ($P < 0.01$), even when the 4 subjects with familial or with personal allergy were excluded.

The mean control values for the total material were: $FEV_{1.0}$ 3.53 lit., FVC 4.37 lit., $FEV\%$ 81 per cent, PEF 449 lit./min. After inhaling increasing concentrations of ACh, there was an initial small increase, then a successive decrease of the studied parameters. The first significant decrease, recorded after inhalation of 0.5 per cent ACh, concerned PEF ($P < 0.005$). After 1 per cent ACh, PEF, $FEV_{1.0}$ and FVC decreased significantly by 8 per cent ($P < 0.001$), 7 per cent ($P < 0.005$), and 3 per cent ($P < 0.05$), respectively.

B. Patients.

The results of increasing concentrations of ACh in bronchitic patients are given in Tables 4 and 5 and Figs. 1, 2 and 3. After an initial slight increase especially among the males (42), there was a successive decrease in $FEV_{1.0}$, FVC and PEF with inhalation of increasing concentrations of ACh within each group (Figs. 1, 2, 3). The first recorded significant difference between control value and post-drug value was noted in PEF, $FEV_{1.0}$ and FVC after inhalation of 0.01 per cent ACh in Group III ($P < 0.05$). In Groups I and II the first significant change occurred at concentration 0.1 per cent ($P < 0.05$). The P -values for significance of the induced changes grew with further increase of ACh concentrations within the groups (Table 4, Figs. 1, 2, 3).

A comparison between the three groups showed that there was a clear-cut post-drug decrease obtained in the first group containing subjects with supposedly normal $FEV\%$. In Group II there was a more pronounced decrease, *e.g.* around 14 per cent of basal values for $FEV_{1.0}$ and PEF and somewhat less for FVC. In Group III the average decrease amounted to

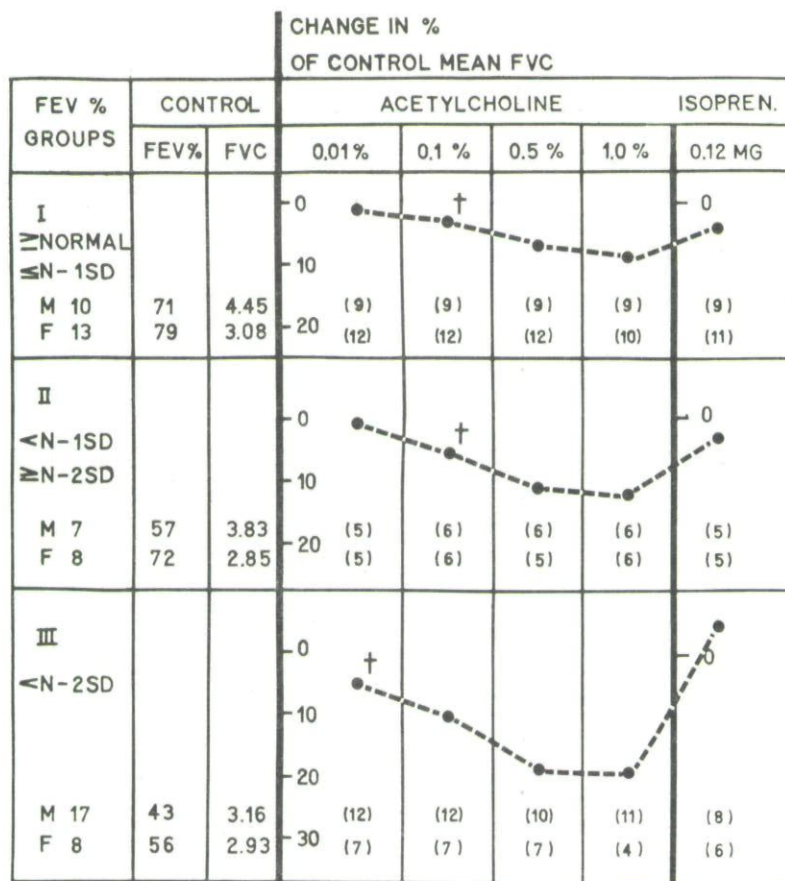


† FIRST SIGNIFICANT DECREASE

Fig. 1.

Effect on mean FEV_{1.0} by acetylcholine inhalation in bronchitic patients with different severity of airways obstruction.

19–22 per cent for FEV_{1.0} and FVC, and 27 per cent for PEF. The number of individual positive tests in the three FEV% groups was compared by χ^2 test in relation to the number of subjects in each group (Table 5). There was a significantly higher frequency of positive tests in Group III



† FIRST SIGNIFICANT DECREASE

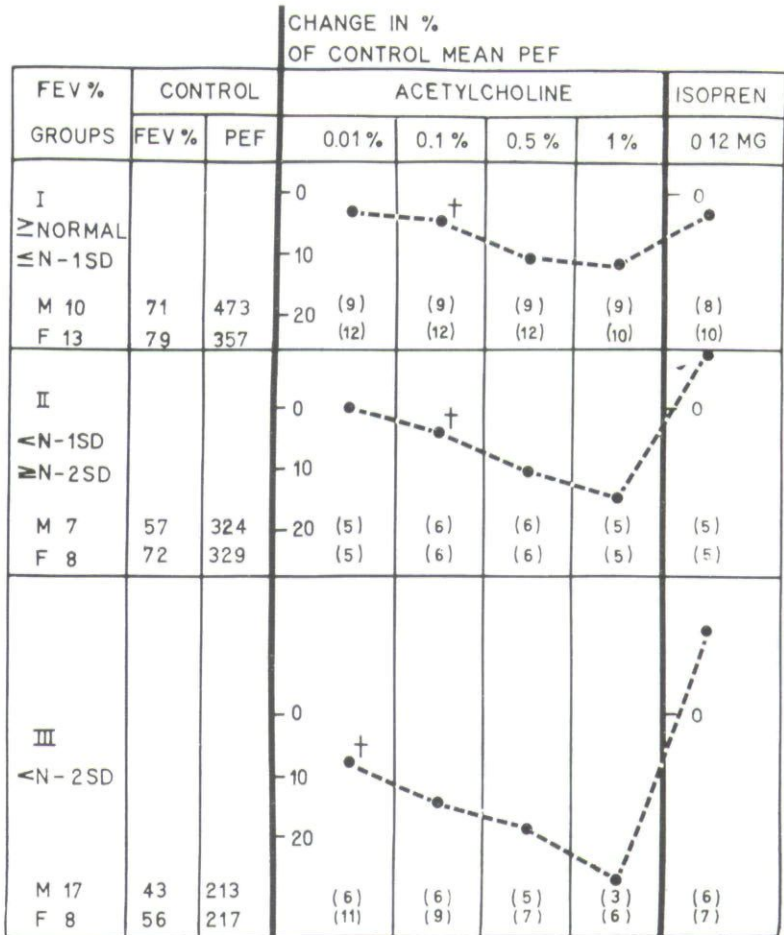
Fig. 2.

Effect of mean FVC by acetylcholine inhalation in bronchitic patients with different severity of airways obstruction.

as compared to Group I ($P < 0.001$), while there was no significant difference shown between Group I and II.

Thus, the ventilatory effect of inhaling acetylcholine increases 1) with increasing concentrations; 2) with the severity of pre-drug airways obstruction (Fig. 4).

Inhalation of 2-3 breaths of isoprenaline after the highest



† FIRST SIGNIFICANT DECREASE

Fig. 3.

Effect on mean PEF by acetylcholine inhalation in bronchitic patients with different severity of airways obstruction.

concentrations of acetylcholine brought the FVC back almost to control values in Groups I and II. In Group III there was an additional bronchodilatation after isoprenaline compared with basal volumes.

Two or more ACh tests were performed in 11 female and

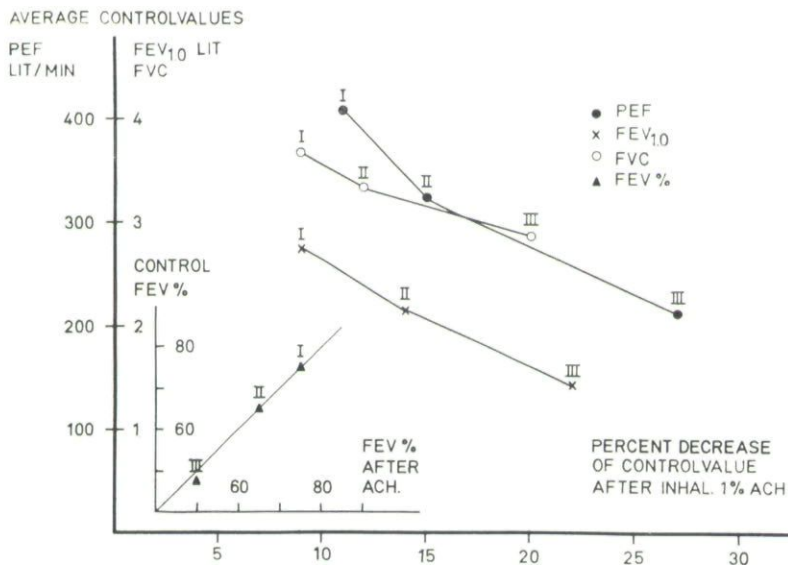


Fig. 4.

Relation between absolute basal values and per cent decrease of ventilatory parameters induced by 1 per cent acetylcholine. (Note that the per cent effect increases with decreasing absolute values, except concerning FEV%).

I, II and III denotes the three FEV% groups.

14 male bronchitics. In most instances the first run was made only with 1 per cent ACh, which was the sole concentration used at the start of these studies. The difference in pairs of per cent effect with equal ACh concentrations showed no significant change between the two tests. At 1 per cent ACh 10 subjects of 18 showed a larger percentual reduction in the first run. The difference between pairs of absolute pre-drug control values was not significant. In 5/8 the basal value was lowered in those who showed a larger per cent decrease after the drug inhalation.

In Table 5 the collected individual responses to ACh in the three groups are correlated with history of bronchial irritability and allergy. There was a very large and similar percentage of reactivity (80 per cent) in all the three groups concerning cough during the ACh test and history of weather

sensitivity (60–68 per cent). In Group I only 2 subjects, (Nos. 3 and 8) denied a history of bronchial irritability, and 3 (Nos. 26, 30, 131) did not cough during the ACh tests. Corresponding figures in Group II were 1 (No. 7) and 3 (Nos. 8, 106, 135), and in Group III 4 (Nos. 4, 6, 19, 21), and 5 patients (Nos. 5, 23, 27, 104 and 114), respectively. Of these subjects Nos. 5, 8 and 114 appeared in two groups; all 3 coughed in one of the two tests performed on them.

Cough was elicited at lower ACh concentrations than those at which a decrease in dynamic capacities was recorded. There seemed to be no certain difference between the groups in this respect. On the other hand, the ventilatory response was significantly increased with higher group number—the number of patients with negative test was halved from group to group, and the number of positive tests at low ACh concentrations grew with increasing basal airflow obstruction (Table 5).

Patient No. 101 showed positive ventilatory tests in periods with smoking before and after a 2 years' stop, during which she had a negative ACh test.

DISCUSSION

Various stimuli act non-specifically irritating on the mucosa of the airways in normal man and in animals, thereby initiating an increase in air-flow resistance (56). The cough is considered to be due to excitation of afferent airway receptors, and is often strengthened by bronchoconstriction (46, 56). Further studies suggest that the same receptors act in eliciting both cough and reflex bronchoconstriction, maybe with different thresholds for the two events (55).

In the present study on 20 apparently healthy subjects, there was a significant decrease in average ventilatory capacities after inhalation of 0.5 per cent acetylcholine. In 7 of 20 subjects significant ventilatory changes were elicited; cough was even more common. There was a statistically proven relation between smoking and induction of cough during the ACh test in normals. This may reflect an increase in irritability, a sus-

picion further confirmed by the positive tests obtained in subject UA only during a slight cold (Table 2) and in patient No. 101 only during smoking periods.

Among the bronchitic patients there were more reactors than among the healthy subjects, and they reacted to lower concentrations with more severe airways obstruction. There was no obvious difference between the groups with different degrees of pre-drug airflow obstruction, in frequency of bronchial irritability, smoking habits or history or tests of allergy. Cough was elicited in the same proportions in all groups. The ventilatory effect was, however, greater in the groups with severe airways obstruction. Due to the necessary deletion of subjects who reacted strongly on low ACh concentrations, the decrease with the higher ACh concentrations was always calculated from a lower number of patients; with the eliminated subjects included, there would have been a considerably higher percentual effect in the last group.

Tests with different concentrations of ACh have been performed most extensively by *Tiffeneau* (45, 46, 48). He called the ACh concentration that induced in "effet tangible", "dose liminaire" (DL). On 32 normal subjects he reported the lowest DL to be 4000 μg as compared to 50 μg in the present material. *De Vries et al.* consider that toleration of a 30-second inhalation of ACh in amounts exceeding 256 μg rules out bronchial hyperreactivity (50). *Pillot et al.* found that 97 per cent of 164 normal subjects did not decrease their $\text{FEV}_{1.0}$ by as much as 10 per cent after inhalation of 1 per cent ACh for 3 minutes (37). In the present small "normal" material there is a somewhat larger frequency of reacting subjects; this may be due to the composition of the group or to the technique with longer inhalation and the use of mean values. The ventilatory effect was reported by *Tiffeneau* to last for at least 5 minutes, sometimes up to one hour, and to be independent of the degree of response (46). In the present study the average post-drug values were relatively constant for 5-7 minutes although in some subjects a linear postdrug increase in ventilatory capacity with time was observed. There

was not any slowly increasing effect with higher ACh concentrations, indicating marked hypersecretion and oedema, but the small volume deficit seen as compared with control values after isoprenaline in the normal group, may be due to some of these more slowly reversible events. As in other studies, there were no signs of systemic effect from the ACh (17).

The reproducibility of "dose liminaire" for ACh has been reported by Tiffeneau to be equal after several months. *De Vries* found small differences in DL histamine at the same time of the day on 5 successive days in 17 subjects (47, 51). Double measurements in 25 subjects in the present study showed no significant change in average ventilatory effect with the same ACh concentrations. Nine tests showed a higher threshold value, 4 a lower, and 11 were not changed, among them 4 negative tests.

In the present study there was a larger average ventilatory effect in initially more obstructive patients. The question arises as to whether a larger degree of excitability is the cause of the more accentuated airways obstruction in these patients, or if their initial increased obstruction gives rise to a more notable effect from hyperreactivity in the form of a still more aggravated airways obstruction. Similarly small changes in the diameter of already-constricted blood vessels give rise to larger reductions in flow than when the same diameter decrease is induced from a larger lumen (24). A clear-cut airway constriction induced from normal predrug values is likely to be caused by a primary hyperreactivity, while a moderate decrease elicited from already-lowered volumes seems less convincing as a sign of primary hyperreactivity. The non-significant difference concerning history of allergy and signs of bronchial irritability between groups with different airway obstruction implies no larger degree of excitability in the more obstructed subjects.

De Vries et al. reported that changes in threshold values for histamine were not always correlated to changes in lung function (52). Ganglion-blocking drugs increased VC and FEV_{1.0} significantly, while the hyperreactivity was reported

to have a "tendency" to decrease. When the χ^2 test is applied to De Vries' material, however, there was a significant decrease of reactivity related to real changes of $FEV_{1.0}$ ($P < 0.01$). Consequently, in supine position, when lung volume and airway diameters are decreased, an increase in reactivity to histamine was shown by *Bouhuys* (10).

In a study somewhat similar to the present one, *Sadoul & Saunier* classified 232 males first clinically and then according to predicted lung capacities and volumes. Larger differences in effect of ACh between the groups were obtained when the classification was based on functional rather than on clinical parameters (38). In 22 per cent of the patients with only cough ("tousseurs"), a marked lowering of $FEV_{1.0}$ occurred after ACh inhalation; these patients showed signs of primary hyperreactivity similar to the 26/38 patients in Groups I and II in the present investigation (Table 5).

The causes of bronchial hyperreactivity seem to be at least twofold. First, mechanical irritation by particles or irritating gases elicits reflexes from different receptors in walls and mucosa in the airways, causing cough and bronchoconstriction *mediated mainly by the vagus* (55, 56). Pulmonary stretch reflexes also react to changes in airway caliber and pulmonary gas volume; their activity increases with a decrease in pulmonary compliance due to bronchoconstriction or vascular congestion (56). In normal man, the bronchial obstruction induced by irritation can be blocked by atropine, which makes a vagal reflex the probable cause (20, 33, 56).

Second, more localized and specific forms of airways constriction have been recognized as being caused by *liberation of histamine* due to, for example, cotton dust, barium sulphate emboli, or serotonin from venous emboli (26, 34). This local mechanism is shown by decrease in lung compliance, increase in pulmonary resistance and expulsion of air from the lungs. The anatomical substrate is thought to be a constriction of smooth muscles mainly in the respiratory bronchioles and alveolar ducts (34). Similar results were found with microaerosols ($< 1 \mu$) on the very sensitive excised lungs of guinea

pigs (21). In allergic reactions the bronchoconstrictive effect seems to depend on the degree of bronchial reactivity to released histamine, acetylcholine, and other substances (49). The effect of these mediators and of cigarette smoke remains local, as has been shown in unilaterally provoked bronchoconstriction in man (3, 13). Reactivity to ACh was considered by *Tiffeneau* to be due to abnormal excitability of bronchial smooth muscles, glands, and vessels, and has been shown to parallel sensitivity to histamine and several physical and chemical agents (32, 48, 50, 52). Consequently, acetylcholine has been used for revealing this general phenomenon.

The basic source of hyperreactivity in bronchitis seems to be localized in the bronchial muscle or mucosa as indicated by tests with antihistaminic, anticholinergic, ganglion-blocking and sympathomimetic drugs (20, 28, 46, 50, 52). Injuries to nerve endings, leading to functional denervation, may cause a "hyper-sensitization by denervation", inducing hyperreactivity to various stimuli (12, 23). Further, the β -receptors in "asthmatics" have been claimed to be partially blocked, which might disturb the balance in the autonomous regulation of airway size (15). A lowering of the threshold for neurogenically mediated muscular excitation would result in an exaggerated effect from drugs and mediators.

Irritant gases as well as infections injure the tissue and induce inflammation as reflected in "chronic bronchitis" or "asthma" (27, 35, 36, 57). Injured tissues exhibit an increase in histamine-forming capacity (HFC) and they are highly sensitive to histamine, ACh and antigens (7, 8, 29, 30, 31, 39).

In a tissue with an allergic reaction, histamine is liberated suddenly and disappears fast; the event is followed by a longer period of increase in the histamine-forming capacity. This has been offered as an explanation for delayed response and the failure of antihistamines to effect delayed reactions (30, 53). These findings suggest that patients with chronic inflammatory pulmonary changes may have a larger than normal histamine-forming capacity or even an increased histamine

content in their diseased airways. When additional injury occurs—*i.e.* due to an acute infection or the impact of various particles, cold or SO_2 —histamine or serotonin may be released in quantities large enough to induce bronchoconstriction. Addition of histamine or acetylcholine directly in aerosol or released from an antigen-antibody reaction would act similarly.

The high prevalence of smokers and patients with allergy connections in any material of "bronchitics" may obscure different causes for bronchial hyperreactivity in the material.

The spirometric method used in these studies has been claimed to be as efficient in revealing induced airway obstruction as other more elaborate methods (44). With a body plethysmograph, on the other hand, lung volume and airway resistance can be interrelated and much smaller changes in flow-resistance can be detected (19). Thus *DuBois et al.* have found that maximal expiratory and inspiratory flows decrease only when airway resistance is at least doubled. For clinical evaluation of hyperreactivity, however, dynamic spirometry seems to be useful. Wash-out methods have also proved to discriminate small changes in airflow resistance and resulting induced unevenness in pulmonary ventilation (14, 9). In a coming publication will be shown that the single breath N_2 test is also a very sensitive indicator of the effect caused by broncho-irritating agents. As the size of inhaled particles determines where in the airways they will be deposited, tests with monomorph aerosols should be made to elucidate if various drugs act in different regions, and if various clinical "entities" such as "asthma" and "emphysema" are due to, for example, different location in airflow obstruction (18).

SUMMARY

Nebulized acetylcholine hydrochloride (ACh) was inhaled in increasing concentrations in a standardized way by 20 (10 M, 10 F) apparently healthy subjects and by 58 bronchitics (30 M, 28 F). Dynamic spirometry and peak flow measurements were performed before and after each ACh concentra-

tion. The ventilatory effect was expressed in per cent of the average basal parameters; induction of cough was noted. The reproducibility of test results in healthy and bronchitic subjects was good.

In 7 healthy subjects reacting with ventilatory impairment, there was shown a connection with allergic disease, while cough was connected with smoking to a high degree. The peak expiratory flow first showed the ventilatory impairment in the healthy group.

The bronchitics were classified according to increasing degree of obstruction in three FEV% groups: I) $> \mu-1$ SD, II) $< \mu-1$ SD $\geq \mu-2$ SD, III) $< \mu-2$ SD. There was a similarly high frequency (80 per cent) of cough, weather sensitivity, and allergy in all groups regardless of different FEV%. The initiated ventilatory effect increased within the groups with increasing ACh concentration and between the groups with the severity of pre-drug airways obstruction. As an expression of a similar degree of hyperreactivity, a larger decrease in airflow seems to occur in previously obstructed airways than in normal ones.

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