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A COMPARATIVE STUDY OF THE
RESULTS OBTAINED BY TWO METHODS
OF ALLERGEN EXTRACT SPRAYING
IN PATIENTS WITH
CHRONIC NON-SPECIFIC LUNG DISEASE

By

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INTRODUCTION

The study described in this paper was carried out in patients with chronic non-specific lung disease (CNSLD). This category includes those suffering from intermittent or chronic dyspnoea, sometimes of varying intensity, and/or coughing on most days at least three months during at least two consecutive years and/or expectorating, unless another explanation of these phenomena is available.

All patients in our study were suffering from intermittent or chronic dyspnoea.

We have avoided the terms asthma, asthmatic bronchitis and emphysema in order to avoid misunderstandings. If the term asthma does crop up in this paper, then it refers to intermittent attacks of dyspnoea associated with wheezing rhonchi in patients whose lung functions are undisturbed during the asymptomatic intervals.

All patients concerned in this study showed hyperreactivity of the bronchial tree to nebulized histamine. The concentrations used to test this were 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 mg histamine hydrochloride per ml. The dura-

tion of inhalation was 30 seconds per inhalation, and the results were called positive when the vital capacity (VC) and/or the one-second Forced Expiratory Volume (FEV_1) showed a decrease of 20 per cent of the initial value, or more.

Normal individuals tested in the same way show no demonstrable bronchial constriction upon inhalation of one of the above mentioned concentrations of histamine hydrochloride.

The existence of a manifest allergy of the bronchial tree to inhalation allergens can be demonstrated in these patients by inhalation tests; dissolved allergen extracts are used in these tests. (6, 7, 8, 10, 12, 3, 4, 5).

Generally speaking a correlation exists between the strength of the skin reaction and the hypersensitivity of the bronchial tree for inhalation allergens. The same holds true for the number of positive inhalation tests. Nevertheless there are many exceptions on this general rule. Sometimes it is even possible to find a positive inhalation test when the skin reaction is negative. (9).

Therefore we are convinced, that inhalation tests are very usefull to demonstrate the existence or absence of a manifest allergy of the bronchial tree for these allergens. One of the prerequisites for reliable test results is that inhalation of the fluid in which the allergen has been dissolved (*e.g.* Coca's fluid) causes no reaction; another prerequisite is that the patient is free of symptoms and has used no bronchodilating drugs during the 12 preceding hours. When the absolute values for the VC and FEV_1 are too low ($VC < 2000$ ml, $FEV_1 < 1000$ ml) unreliable test results are also obtained.

The dissolved allergen extract is inhaled by the patient as a nebulized spray. The V.C. and FEV_1 are determined both immediately and 4 minutes after completion of the inhalation test, which lasts a maximum of 10 minutes. A decrease by more than 20 per cent of the initial VC. and/or FEV_1 -value (the highest of 2-3 determinations prior to the test) is evaluated as positive; a decrease by 15-20 per cent is evaluated as doubtfully positive, and a decrease by < 15 per cent as negative.

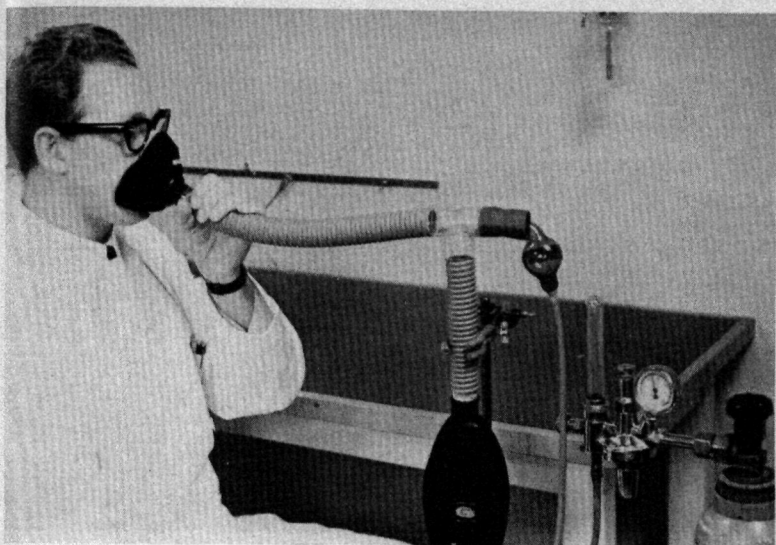


Fig. 1.

Spraying- and inhalationsystem of the Wiesbadener-Doppelinhalator.

For some years, we have used the all-glass "Wiesbadener-Doppelinhalator" for nebulization, adjusting the flow to about 6 litres of air per minute.

The patient inhales the aerosol through a wide, stiff spirometer tube with a diameter of 3 cm and a length of about 50 cm (Fig. 1). The aerosol is introduced at one end of the tube via the inhalator outlet. A perforated rubber stopper or rubber ring is fitted around the neck of the outlet making an airtight seal with the tube.

The other end of the tube is connected with an anaesthesia mask with inflatable rubber edge, which can be fitted snugly over the patient's mouth and nose.

At about 10–12 cm from the inhalator-tube link, a wide T-piece in the tube links it to an anaesthesia balloon of a few litres' volume.

Before the inhalation test, this entire system is filled with the aerosol, whereupon the patient is connected with it. This

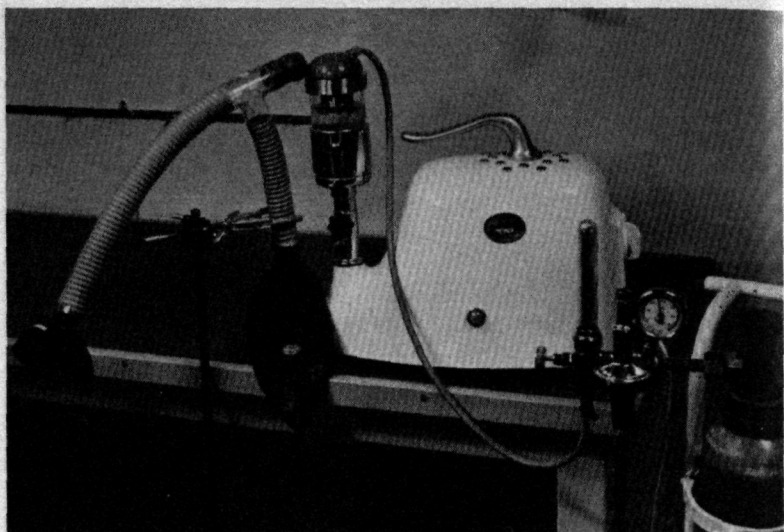


Fig. 2.
The "Ultrason" with inhalation-system.

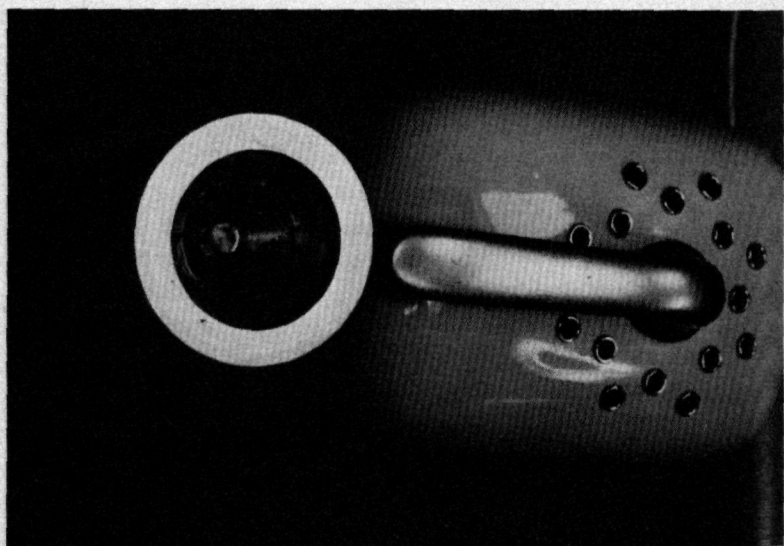


Fig. 3.
The part of the Ultrason, where the aerosol is produced.

method has the advantage that the investigator can be sure the patient is inhaling the aerosol properly. The procedure offers no difficulty in practice.

The method used of course partly determines the results obtained. Therefore, when recently we had the disposal of an apparatus producing an aerosol by means of ultrasonic vibrations (Ultrasol, manufactured by Heijer, Bad Ems - W. Germany), it seemed useful to compare the results of inhalation tests using this apparatus, with those obtained by the method described above. The aerosol produced by the Ultrasol apparatus, too, was guided into an identical tubing system via an air flow at a rate of 6 litres per minute. The methods, therefore, use the same manner of inhalation but differ in the following points (Figs. 2 and 3).

1. The way in which the aerosol is produced is fundamentally different.
2. The output per litre of air was 12 mg for the Wiesbadener-Doppelinhalator and 120 mg for the Ultrasol. At a flow of 6 litres per minute, the output for the Wiesbadener-Doppelinhalator is thus 72 mg and that for the Ultrasol is 720 mg. The output values were determined by our biochemist—dr. G. F. Blom—who used a selfdesigned technique to be described in a later publication.
3. Although exact data are not yet available, it can be considered a fact that the particle size of the Ultrasol aerosol is smaller than that of the Wiesbadener-aerosol.

In addition it can be pointed out that inhalation tests with the Ultrasol can be reproduced with an accuracy of about 80 per cent.

PROCEDURE

In each series of patients with CNSLD who showed either intermittent or chronic dyspnoea, both methods of spraying were used, both with Coca's fluid and with the dissolved allergen extracts. We used the above mentioned criteria to

assess results as negative, doubtfully positive, and positive. As regards the inhalation tests with Coca's fluid, however, we used criteria of greater strictness; a decrease in VC and/or FEV₁ by 10 per cent of the initial value or more was recorded as a positive result. This was done deliberately, to ensure complete elimination of a possible aspecific hyperreactivity to this fluid in the inhalation tests with allergen extracts (which of course would impede their interpretation). It must be admitted, however, that non-specific hyperreactivity to the allergen itself can also exist.

RESULTS

Table 1 presents the results of inhalation test with Coca's fluid as obtained with the (calibrated) Wiesbadener-Doppel-inhalator (WD) and the Ultrasol (US).

TABLE 1

Wiesbadener			Ultrasol		
Decrease in VC and/or FEV ₁			Decrease in VC and/or FEV ₁		
20% or more	10-20%	< 10%	20% or more	10-20%	< 10%
0	6	108	8	16	90
(0%)	(5.3%)	(94.7%)	(7%)	(14%)	(73%)

This table shows that nebulization of Coca's fluid with the Ultrasol produces a larger number of non-specific reactions than that with the WD.

For a further study of this property of the US, we carried out inhalation tests with citric acid solutions (0.1, 1.0 and 3.0 per cent) in a series of 100 random patients with CNSLD. Table 2 presents the results obtained and shows that 60 per cent of all patients tested, showed a decrease in VC and/or FEV₁ by 20 per cent or more after inhaling one of the citric acid solutions with the aid of the US.

Patients with a history of unmistakable attacks of asthma and asymptomatic intervals, seemed to show a lower rate of

TABLE 2

Inhalation Tests with Citric Acid Solutions in Patients with CNSLD, Using the Ultrazol. All the Patients in this Table Belong to the Same Group of 100 Patients.

	Inhalation of citric acid solutions				
	0.1% concentration Decrease in VC and/or FEV ₁ > 20 %	1% concentration Decrease in VC and/or FEV ₁ > 20 %	3% concentration Decrease in VC and/or FEV ₁		
			10-20%	>20%	<10%
100 patients with CNSLD	37 (37%)	9 (9%)	24 (24%)	14 (14%)	16 (16%)
42 patients with history of asth- matic attacks	12 (28.6%)	2 (4.8%)	14 (33.3%)	6 (14.3%)	8 (19%)
58 patients with symptoms of chronic dyspnoea	25 (43.1%)	7 (12.1%)	10 (17.2%)	8 (13.8%)	8 (13.8%)
44 patients with FEV ₁ % VC of 60 or more after 25 mg thiazinamium i.m.	10 (22.7%)	4 (9.1%)	15 (34.1%)	5 (11.4%)	10 (22.7%)
56 patients with FEV ₁ % VC < 60 after 25 mg thiazinamium i.m.	27 (48.2%)	5 (8.9%)	9 (16.1%)	9 (16.1%)	6 (10.7%)

non-specific hyperreactivity to citric acid than did patients with chronic dyspnoea or dyspnoea on effort. The same applies when patients with a FEV₁ per cent VC¹ of 60 or more after administration of 25 mg thiazinamium² i.m., are com-

¹ Value of FEV₁ expressed as a percentage of the VC.

² Thiazinamium (Multergan®) is an antihistaminic agent with a strong atropine-like character.

pared with those whose FEV₁ per cent VC under identical conditions is < 60.

In the former group, it can be maintained, the airways obstruction is for the greatest part or completely reversible, while in the latter group a more or less irreversible airways-obstruction has occurred (normally we find a FEV₁ per cent VC of 70 or more).

We find the same situation in inhalation tests with Coca's fluid (Table 3). This table shows that, of 35 patients with a FEV₁ per cent VC < 60 after i.m. administration of thiazinamium, 4 showed a decrease in the VC and/or FEV₁ exceeding 20 per cent, and 13 showed a decrease by 10-20 per cent after the inhalation. In the group of patients with a FEV₁ per cent VC > 60 after i.m. injection of thiazinamium, the correspondings figures were 1 and 3, respectively, out of 66 patients.

TABLE 3

Inhalation Tests with Coca's Fluid in Patients with CNSLD, Using the Ultrasol.

	Inhalation of Coca's fluid Decrease in VC and/or FEV ₁		
	10-20%	>20%	<10%
35 patients with FEV, <60% of VC after 25 mg thiazinamium i.m.	13	4	18
66 patients with FEV, >60% of VC after 25 mg thiazinamium i.m.	3	1	62

We must therefore conclude that patients with chronic dyspnoea or more or less permanent expiratory disturbances, seem to show a more pronounced tendency towards non-specific hyperreactivity of the bronchial tree.

These findings will have to be borne in mind in interpreting inhalation tests with allergen extracts with the aid of the US.

Consequently Table 4, indicating inhalation tests with allergen extracts using the WD and the US in the same patients,

presents only data on patients whose inhalation tests with Coca's fluid, by both methods of spraying, were definitely negative, *i.e.* in whom the decrease in VC and/or FEV_1 was < 10 per cent of the initial value.

The table shows that a total of 217 inhalation tests with allergen extracts included 36 positive results (16.6 per cent with decrease of VC and/or $FEV_1 > 20$ per cent) using the WD, and 77 positive results (35.5 per cent) using the US. For doubtfully positive results (decrease in VC and/or FEV_1 by 15–20 per cent), the corresponding figures were 18 (8.3 per cent) and 40 (18.4 per cent), respectively.

This means that the use of the US produces twice as many positive and doubtfully positive inhalation tests.

There is an unmistakable correlation between the number of positive inhalation tests and the type of allergen used. Using a fungus extract in a total of 36 inhalation tests, we obtained 3 positive and 6 doubtfully positive results with the WD, and 14 positive and 7 doubtfully positive results with the US.

Using a feather extract in a total of 26 inhalation tests, the WD figures were 0 positive and 2 doubtfully positive results, while the corresponding US figures were 5 and 7, respectively.

With both spraying methods, threshing-dust produces only few positive inhalation tests (but the US again gave more positive results).

Inhalation tests with house dust extract yielded a relatively high rate of positive results with both methods, *viz.*: WD: 32 positive (35.6 per cent) and 6 (6.7 per cent) doubtfully positive, and US: 54 (60 per cent) positive and 12 (14.4 per cent) doubtfully positive; again the number of positive inhalation tests was larger when the US was used.

These experiments show that the use of the Ultrasol ensures a considerably larger number of positive inhalation tests.

However, in view of the remarks on non-specific hyperreactivity, the question arises as to whether the larger number of positive inhalation tests using the US is based on hypersensitivity of the bronchi to the allergens in question, or depends (at least in part) on non-specific hyperreactivity. It is

possible, after all, that non-specific hyperreactivity to the allergen extracts itself exists.

However, the following arguments favour the conclusion that positive inhalation tests with allergen extracts using the US are based on allergy.

1. A distinct correlation exists between the intensity of the skin reactions and the number of positive inhalation tests, as demonstrated in Table 4.

Unfortunately, the number of inhalation tests carried out for each allergen separately and arranged in accordance with the number of positive and negative skin reactions, is not sufficiently large to warrant definite conclusions. It is obvious, however, that in a total of 73 inhalations tests in patients with ++ or +++ skin reactions (wheal diameter 10–15 and > 15 mm, respectively), 48 tests were positive (65.6 per cent), while 11 were doubtfully positive (15.1 per cent).

In 84 patients with + skin reaction (wheal diameter 6–10 mm), these values were 20 (20.8 per cent) and 16 (19 per cent), respectively. In 53 patients with negative skin reactions, 9 positive (17 per cent) and 11 doubtfully positive (20.8 per cent) inhalation tests were seen.

We found a similar distribution in the case of house dust, although here the number of positive inhalation tests was larger in patients with ++ or +++ skin reactions (79.2 per cent), while that in patients with a + skin reaction was 39.1 per cent and that in those with a negative skin reaction, 23.1 per cent. Thus there was a distinct difference between the

TABLE 4

Inhalation Tests with Allergen Extracts Applied with the WD and the US in the Same Group of Patients with CNSLD. The Inhalation Test with Coca's Fluid was Negative with both Sprays (Decrease in VC and/or FEV₁ 10 per cent. The Table Indicates the Concentrations of the Extracts Used. The Skin Tests were Carried Out with Extracts of Lower Concentrations (Spores of Fungi 1 per cent; Feathers 0.1 per cent; Threshing-Dust 0.5 per cent, and House-Dust 0.5 per cent).

TABLE 4

	Skin reaction	Wiesbadener Decrease of VC and/or FEV ₁			Ultrason Decrease of VC and/or FEV ₁			Total number of inhalation tests
		>20%	15-20%	<15%	>20%	15-20%	<15%	
All inhalation tests together	++/+++	33	5	35	48	11	14	73
	+	2	6	76	20	16	48	84
	±	0	0	7	0	2	5	7
	—	1	7	45	9	11	33	53
		36	18	163	77	40	100	217
Spores of fungi 10%	++/+++	1	1	3	2	2	1	5
	+	2	3	14	9	2	8	19
	±	0	0	4	0	1	3	4
	—	0	2	6	3	2	3	8
		3	6	27	14	7	15	36
Feathers 1%	++/+++	0	1	3	2	2	0	4
	+	0	0	10	1	3	6	10
	±	0	0	0	0	0	0	0
	—	0	1	11	2	2	8	12
		0	2	24	5	7	14	26
Threshing dust (5-10%)	++/+++	1	0	10	2	3	6	11
	+	0	1	31	1	6	25	32
	±	0	0	2	0	1	1	2
	—	0	3	17	1	3	16	20
		1	4	60	4	13	48	65
House-dust (5-10%)	++/+++	31	3	19	42	4	7	53
	+	0	2	21	9	5	9	23
	±	0	0	1	0	0	1	1
	—	1	1	11	3	4	6	13
		32	6	52	54	13	23	90

number of positive inhalation tests in strong positive skin reactions on the one hand, and that in weak positive or negative skin reactions on the other.

For the doubtfully positive inhalation tests, differences between different intensities of skin reactions were not well demonstrable but the same holds true for tests with the WD. We still question, therefore, whether much significance must be attached to the doubtfully positive results of inhalation tests with allergen extracts and whether it would not be advisable to take into account only the clearly positive results. In this detailed review, however, it seemed useful to report these doubtful results.

TABLE 5
*Inhalation Tests with Phosphate Buffer, Using the WD and the US,
in Patients with Asthma.*

Wiesbadener			Ultrason		
Decrease of VC and/or FEV ₁			Decrease of VC and/or FEV ₁		
10-20%	>20%	<10%	10-20%	>20%	<10%
0	0	32	0	0	32

2. As Table 4 shows, a correlation can also be observed between the number of positive inhalation tests and the nature of the allergen. Precisely for allergens with which contact can hardly be avoided, we found many more positive results than for those with which contact hardly ever occurs or can be avoided. This, too, is an argument in favour of the conclusion that positive inhalation tests with US-sprayed allergen extracts are based on allergy. Meanwhile it cannot be denied that the allergen extract itself might give rise to non-specific hyperreactivity. In the context of this study it seemed useful, therefore, to establish whether different results would be obtained with inhalation tests using another preparation for instance purified house dust allergen as developed by *Berrens et al.* (1, 2). The results of these tests are presented in Tables 6

and 7, while in Table 5 the results of inhalation tests with phosphate buffer, used to dissolve the purified house dust allergen have been listed.

TABLE 6

Inhalation Tests with Purified House Dust Allergen, Using the WD (20,000 U) and the US (5,000-20,000 U), in the Same Group of Patients with Asthma.

Skin reaction	Wiesbadener			Ultrazol		
	Purified house dust allergen 20,000 U			Purified house dust allergen 5,000-20,000 U		
	Decrease of VC and/or FEV ₁			Decrease of VC and/or FEV ₁		
	>20%	15-20%	<15%	>20%	15-20%	<15%
++/++++	6	2	13	14	0	7
+			7			7
±			1			1
—			3			3

TABLE 7

Comparative Inhalation Tests with 5-10 per cent House Dust Extracts and Purified House Dust Allergen (5,000-20,000 U), Using the US in the Same Group of Patients with Asthma.

Skin reaction	House dust extract 5-10%			Purified house dust allergen 5,000-20,000 U		
	Decrease of VC and/or FEV ₁			Decrease of VC and/or FEV ₁		
	>20%	15-20%	<15%	>20%	15-20%	<15%
++/++++	17	1	3	14		7
+	1	1	5			7
±			1			1
—		1	2			3

Table 5 shows that the phosphate buffer used to dissolve the allergen never caused a non-specific hyperreactivity, no matter whether the WD or the US was used. Comparing the inhalation tests with purified house dust allergen by both spraying methods in the same group of patients (with a history of unmistakable attacks of asthma and undisturbed lung functions

during the asymptomatic intervals), we find that again the US produced a distinctly larger number of positive results (Table 6). On the other hand, Table 7, indicates that with purified house dust allergen slightly fewer positive inhalation tests were seen in the same patients than with 5-10 per cent house dust extract, both US-sprayed. It is therefore possible—but not easily established with certainty—that these differences are based on non-specific hyperreactivity to the house dust extract.

TABLE 8

Inhalation Tests with 5-10 per cent House Dust Extract (US-Sprayed) in Patients with CNSLD with and without an Expiratory Disturbance after i.m. Injection of Thiazinamium. The Inhalation Tests with Coca's Fluid were Negative.

Skin reaction	FEV ₁ % VC < 60 after 25 mg thiazinamium i.m.			FEV ₁ % VC < 60 after 25 mg thiazinamium i.m.		
	Decrease of VC and/or FEV ₁			Decrease of VC and/or FEV ₁		
	>20%	15-20%	<15%	>20%	15-20%	<15%
++/+/+++	6	0	0	35	2	9
+	4	3	3	9	1	19
—	0	1	2	3	1	8

Although we believe we have offered arguments justifying the assumption that manifest hypersensitivity of the bronchial tree to inhalation allergens is better demonstrated with the aid of the Ultrasol than with the conventional Wiesbadener-Doppelinhalator, a critical attitude in the interpretation of inhalation tests with allergen extracts sprayed with the aid of ultrasonic vibrations, remains a necessity.

This can probably be elucidated on the basis of Table 8, which concerns the patients in whom inhalation of Coca's fluid remained negative, but who were divided into a group with a FEV₁ per cent VC < 60 following intramuscular injection of thiazinamium, and another group with a FEV₁ per cent VC > 60 following thiazinamium injection.

In both groups, we regard the positive inhalation tests in

patients with ++ or +++ skin reactions as an expression of allergy to house dust, but in the group of disturbed expiration (considered in view of our findings on non-specific hyper-reactivity in these patients), positive inhalation tests associated with weak positive and negative skin reactions must be assessed with caution.

The use of an apparatus producing an aerosol by means of ultrasonic vibrations would be of importance especially in:

1. investigations into the existence and extent of non-specific hyperreactivity of the bronchi;
2. detection of manifest allergy of the bronchi when the usual inhalation methods give a negative result and either the history or examination raises a suspicion of manifest hypersensitivity to a given allergen.

SUMMARY

In a series of patients with chronic non-specific lung disease (CNSLD), suffering from intermittent or chronic dyspnoea, inhalation tests with allergen extracts were carried out, using two methods of spraying. The one method called for the use of a simple glass inhalator, while the other sprayed extracts with the aid of ultrasonic vibrations.

With the latter method, the number of positive inhalation tests (decrease in VC and/or $FEV_1 > 20$ per cent of the initial value) was about twice as large as that obtained with the former method.

It is concluded from the findings that, spraying allergen extracts with the aid of ultrasonic vibrations, allergy of the bronchial tree to these extracts can be demonstrated in more patients than has so far been possible.

The apparatus in question is also very suitable for a study of non-specific hyperreactivity of the bronchi to, say, citric acid solutions. This property, however, makes it necessary to be critical in interpreting inhalation tests with allergen extracts, particularly in patients with chronic dyspnoeic symptoms and weak positive skin reactions.

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