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THE EFFECT OF DILUTE ANTIGENIC AEROSOL IN THE PROVOCATION OF BRONCHIAL SPASM

By

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In latent allergic asthma acute transient episodes of bronchial asthma can be precipitated by the inhalation of an aerosol of a causative antigen. The technique is of recognized clinical value both for determining the effect of suspected allergens and for hyposensitization treatment (*Herxheimer & Prior 1952*). In hyposensitization, increasing antigen dosage is achieved either by increasing the period of inhalation or by increasing the concentration of the extract. Experience over many years has shown that dilutions of the original extract to as low as 25 per cent of the original concentration can be used as equivalents if the inhalation period is prolonged in the same proportion, (*e. g.* four-fold with a 25 per cent dilution). With higher dilutions, however, this relationship does not seem to apply. Experiments described in this paper will show that in higher dilution the antigen becomes progressively more effective.

MATERIAL AND METHODS

Four asthmatic patients (2 males and 2 females between 25 and 45 years) were informed about the nature of the experiments and agreed to take part. They had previously been proven to be bronchially sensitive to fungal spores (one to *Pullularia-Aureobasidium-pullulans*, one to *Cladosporium herbarum*, two to *Coprinus micaceus*) and their skin was sensitive to extracts of these spores.

The standard extract of the fungal spore concerned (the concentration of which varied between 3 and 20 per cent w/v) was nebulized into a close-circuit recording spirometer as previously described (*Herxheimer 1949*). The nebulizers

used were commercial ones and the same nebulizer and nebulizing pressure was used for the same subject in all experiments. When the subject had shown no reaction to inhalations of physiological saline, three basal values for vital capacity (non-forced, V.C.) and for the peak expiratory flow rate (P.F.R., *Wright & McKerrow* 1959) were established. Following the inhalation of the fungal spore extract, the V.C. and P.F.R. were recorded 1, 3, and, when necessary, 5 and 10 minutes later. A positive reaction was recorded when the V.C. and P.F.R. fell by 10 per cent or more of the mean basal value. Very often the patients noticed a wheeze at the same time, or a short cough developed at the peak of vital capacity inspiration. These symptoms served as corroboration of a mild asthmatic attack. The necessary exposure time for precipitating the attack with the standard solution having been found, the standard solutions were diluted 2 to 10 fold before the next testing some weeks later. The exposure time was increased though to a lesser extent than the degree of dilution indicated, preliminary experiments having shown that a positive reaction might occur earlier than would be expected. Short periods of further exposure were added as found necessary, until a positive reaction occurred.

At further intervals of 6-8 weeks, experiments with further dilutions were added. With each subject 2 or 3 series of exposures with increasing dilutions were carried out. Long experience with this type of experiment usually made it possible to gauge the length of the exposure period necessary to precipitate an

TABLE 1
Exposure Times (E.T.). Subj. B. Antigen: Coprinus.

	Conc. of extr. w/v.	Expected exposure time according to dilution (sec.)	Observed exposure time	
			Seconds	Percentage of expected E. T.
Series 1	1:5*	—	22	—
	1:20	88	48	55
	1:100	440	150	34
	1:500	2200	450	21
	1:2000	8800	900	10
	1:4000	17600	1500	8.5
Series 2	1:5*	—	20	—
	1:20	80	46	58
	1:40	160	78	49
	1:160	640	180	28
	1:800	3200	600	19

* Standard concentration of extract.

TABLE 2
Exposure Times (E. T.). Subj. H. Antigen: Pullularia.

	Conc. of extr. w/v.	Exposure times expected according to dilution (sec.)	Observed exposure time	
			Seconds	Percentage of expected E. T.
Series 1	1:20*	—	100	—
	1:40	200	175	87
	1:100	500	330	60
	1:300	1500	630	42
	1:600	3000	1110	37
	1:1200	6000	2460	41
Series 2	1:10*	—	75	—
	1:100	750	510	68

* Standard concentration of extract.

attack without producing severe dyspnoea. The mild attacks provoked usually cleared spontaneously within 15-30 minutes and it was seldom necessary to introduce isoprenaline aerosol into the circuit in order to terminate them.

RESULTS

The results are shown in Tables 1-4. It will be seen that the exposure time (ET), which is a measure of the amount of inhaled allergen, becomes proportionately shorter every time the dilution of the extract is increased. This is evident from the decreasing percentages of the initial ET in the last column. The smallest percentages have invariably been found with the greatest dilution, with one exception of subject H., in whom in his first series the percentage in the last 3 dilutions remained about the same.

The antigen concentrations at the beginning of a series (standard concentrations) vary in 3 of the 4 subjects. The reason for this is that these experiments could, for external reasons, not be repeated at short intervals; they were done at 6-8 weeks' intervals and required 3 years for completion. The allergic sensitivity of the subject naturally varied some-

TABLE 3

Exposure Times (E. T.). Subj. M. Antigen: Coprinus.

	Conc. of extr. w/v.	Expected E. T. according to dilution (sec.)	Observed exposure time	
			Seconds	Percentage of expected E. T.
Series 1	1:5*	—	35	—
	1:50	350	210	60
	1:200	1200	560	47
	1:500	3500	840	25
	1:1000	7000	1620	23
Series 2	1:20*	—	40	—
	1:80	160	130	81
	1:160	320	210	66
	1:300	600	330	55
Series 3	1:600*	—	120	—
	1:3000	600	360	60

* Standard concentration of extract.

what during this long period, and the basic exposure time had to be determined anew for each series. Also, during late summer, when the fungal aerospora increased, the subjects M. and R. became more sensitive, requiring prednisone maintenance doses for 2 or 3 months. During this period a stable basal (standard) ET could not be established, and the series had to be interrupted. Later on, the experiments could be resumed, but had to start from a different standard antigen concentration and ET.

There are certain sources of error in the observation of exposure times. It is almost impossible in very experiment to give the exact amount of aerosol which produces the same degree of bronchial spasm. It may happen, for instance, that with an ET of 300 sec. no spasm is produced, but with the addition of a further 60 sec. spasm becomes apparent. It must be regarded as probable, that elements of spasm of a greater or a lesser degree would have been present if not 60 sec. but

TABLE 4
Exposure Times (E. T.). Subj. R. Antigen: Cladosporium.

	Conc. of extr. w/v.	E. T. expected according to dilution (sec.)	Observed exposure time	
			Seconds	Percentage of expected E. T.
Series 1	1:30*	—	8	—
	1:150	40	24	60
	1:1500	400	120	30
	1:6000	1600	240	15
	1:48000	12800	960	8
Series 2	1:100*	—	20	—
	1:1000	200	160	80
	1:5000	1000	400	40
Series 3	1:6000*	—	180	—
	1:18000	540	450	83
	1:50000	1500	900	60
	1:200000	6000	2400	40

* Standard concentration of extract.

30 or 90 sec. had been added to the exposure time. All the observed exposure times can be regarded as exact only within a range of ± 10 per cent or ± 15 per cent.

DISCUSSION

This investigation was begun many years ago (but not continued), when bronchial testing by inhalation of antigenic aerosols was in its earliest days. It was then realized that allergic asthma was, in nature, provoked not by liquid aerosol particles inhaled in great density but by the spores and pollen which occur in much lesser density (*Cladosporium*, for instance with maxima between 12000 and 19000 spores per cubic metre) in the air (*Hyde & Adams* 1960). From the present results it seems that with the greatest dilutions the strongest reactions can be achieved relative to the dose used. This is the more remarkable, as with the usual concentrated extracts, if

their short inhalation is repeated at suitable intervals, complete hyposensitization can be achieved. In the present experiments, the sensitivity of the bronchial mucosa has certainly not decreased, as it has reacted to a smaller dose of the antigen. It may be that in high dilution the amount of antigen does not matter as much as the time of contact which the antigen has with the mucosa. If this time is considerably prolonged, the antigen particles may penetrate further and make more intimate contact with the antibody present in the bronchial mucosa. It may well be that in nature the duration of contact between pollen or spore and the sensitive respiratory membranes is more important than the actual pollen or spore count in the air. It should be possible to test this theory experimentally by clinical as well as by immunological methods.

SUMMARY

1. Four subjects with latent allergic bronchial asthma received inhalations of their specific antigen aerosol until mild bronchial spasm could be recorded. In subsequent experiments increasing dilutions of the same antigen aerosol were inhaled until bronchial spasm occurred.
2. In all series the inhalation period increased with increasing dilution, but much less than would have been expected according to the proportion of the dilution. In some series the inhalation period increased to only 10 per cent or less of the expected time. This means that the bronchial spasm occurred with the lesser amounts of antigen the more diluted the antigen had been given.
3. No explanation for this phenomenon can be given. It appears possible that apart from the antigen dosage the period of its contact with the bronchial membrane may play a part in its effect.

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