Positive “alveolar” responses to antigen inhalation provocation tests: their validity and recognition

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ABSTRACT The validity of inhalation tests in the investigation of extrinsic allergic alveolitis was assessed from the results of 144 antigen and control tests in 31 subjects. A definitive pattern of positive late responses was observed. Reactions to nebulised bird serum and droppings in subjects with bird fancier's lung were identical to reactions after “natural” exposures in aviaries or lofts, and to reactions after “occupational” challenges in subjects with farmer's lung and mushroom worker's lung. In general, positive tests were easily recognised subjectively from symptoms and signs appropriate to an influenza-like illness and undue respiratory effort on exercise. They were associated with significant changes in six readily available objective monitoring measurements—exercise minute ventilation ($\geq +15\%$), body temperature ($>37.2^\circ\text{C}$), circulating neutrophils ($\geq +2500/\text{mm}^3$), exercise respiratory frequency ($\geq +25\%$), circulating lymphocytes ($\geq -500/\text{mm}^3$ with lymphopenia), and forced vital capacity ($\geq -15\%$). These confirmatory monitoring tests had specificities of approximately 95% and sensitivities of 85–48%. Measurement of diffusing capacity, lung volume subdivisions, or resting minute ventilation/respiratory frequency proved to be too insensitive to be useful, as did auscultation and chest radiography. We conclude that responses that do provoke significant changes in these less sensitive tests are unnecessarily distressing and, presumably, unnecessarily hazardous.

The relevance of an environmental antigen to the development of diffuse alveolar disease consistent with extrinsic allergic alveolitis (EAA) is most usefully assessed from a careful clinical history. Confirmatory investigations conventionally depend on serological methods—particularly gel diffusion precipitin tests.^{1–9} While such tests usually demonstrate the presence of precipitating antibody if the antigen in question is indeed responsible for the disease, this is not always the case.^{7–12} Conversely, relatively high proportions of subjects similarly exposed, but apparently unaffected, may also give positive serological results.^{13–17} The antibody response may consequently reflect exposure more than disease.^{18, 19} As yet no laboratory test has proved capable of distinguishing all affected sub-

jects from all who are unaffected, and in an appreciable proportion of cases a definitive diagnosis cannot be made from the clinical and laboratory features alone. This proportion includes, in particular, cases of chronic EAA where experimental periods of antigen avoidance may produce negligible improvement, cases where experimental antigen avoidance is resisted by the subject (or employer) concerned, and cases where a hitherto unrecognised agent is suspected of provoking the disease.

Where diagnostic uncertainty does persist, many specialised centres proceed to inhalation provocation tests. A response is considered to be positive if it reproduces the acute clinical manifestations of EAA. Unfortunately these acute manifestations lack specificity. Influenza-like symptoms of malaise, fever, headache, widespread muscle aching, and anorexia usually mask any evidence of a specifically pulmonary response, although breathlessness and cough may occur.

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Since the provoking agents used in these tests are commonly derived from mouldy vegetation, avian sera or excreta, or contaminated water from cooling systems or humidifiers, the significance of such systemic disturbances may be questioned. This is particularly true of those patients with the chronic form of EAA, which is not characterised by these systemic features.

Regardless of the validity of inhalation provocation tests in the investigation of EAA, there are at present no generally accepted criteria that define positive "alveolar" responses. In some studies the development of fever alone has been considered acceptable confirmatory evidence,^{5,20} while in others fever occurred in control subjects.² Although influenza-like symptoms are the most prominent feature of positive reactions, they are not always reported. Eyckmans and colleagues²¹ and Scadding^{22,23} each described a challenge test with pigeon serum which appeared to provoke fever and leucocytosis. No other features were recorded and yet in the former case pigeon breeder's lung (PBL) was considered to be confirmed, while in the latter Scadding considered PBL improbable.

Objective measurements of pulmonary function have not been reported consistently, probably because significant changes are not always observed. The characteristic findings associated with positive alveolar reactions are restriction of ventilation (occasionally with airways obstruction) and decreased diffusing capacity for carbon monoxide (DLCO),^{2,5,24-28} but both ventilation and DLCO may remain apparently unchanged.^{5,29} Gravity dependent crackles may develop, but are not commonly reported,^{27,29-31} and very few investigators have recorded data regarding the monitoring value of chest radiography.^{29,31}

The purposes of this study were to examine the characteristics and the validity of "alveolar" responses to antigen provocation tests, and to evaluate the potential roles of a number of relatively simple and readily available tests in their recognition.

Subjects and methods

Sequential inhalation provocation tests were completed in 29 subjects in whom EAA was suspected, and in two control subjects (two of us) so that a definitive result considered to confirm or exclude EAA was obtained. In six of these subjects there was already convincing evidence of EAA from the histories, physical examinations, chest radiographs, lung function studies, precipitin tests,

and clinical courses after antigen avoidance. With the remaining subjects the evidence was not conclusive. The majority of the subjects studied were exposed domestically to budgerigars (known parakeets in North America), pigeons, canaries or poultry and were possibly affected by bird fancier's lung (BFL). In four subjects farmer's lung or mushroom worker's lung was suspected.

Most subjects were investigated as outpatients with intervals of a week or so between tests. Some were admitted to hospital and had tests on consecutive days, though usually not more than two antigen challenges were carried out during any one admission. Informed consent was obtained from the subjects and the protocol was approved by the hospital ethical committee.

INHALATION PROVOCATION TESTS

Nebulised avian antigens were inhaled through a face mask and delivered via a Wright nebuliser from two sources: avian serum (dilutions of 1/1000, 1/5 in carbol-saline) for 5-30 minutes, and extract of avian droppings (3-40 mg/ml in carbol-saline) for 5-30 minutes.³²

Before each test a modified skin prick test³³ was performed to identify subjects at risk of developing severe immediate hypersensitivity responses. No such subjects were found.

Sequential tests, involving approximately fivefold increments in antigen dose, were carried out until either an unequivocally positive response was obtained or the maximum dose (avian serum 1/5 nebulising 0.5-1.0 ml of neat serum) had produced no response. We believe this dose is roughly equivalent to 24 hours natural exposure in an aviary or loft of approximately 100 birds.³² Failure to respond at this level was deemed to exclude BFL.

The challenge exposures were carried out in a specially designed cabinet. In general, the full exposure of each test was undertaken in three periods of increasing length separated by intervals of 5-10 minutes. This further reduced the risk of any undue immediate reaction. Control tests were performed under identical conditions at some point or points in the series using nebulised carbol-saline alone. The identity of each test extract was not revealed to the subject until the whole series of tests was completed. The colour of each extract, which might have aided its identification, was concealed by the use of a blindfold.

Natural exposure tests with avian antigens were carried out in a budgerigar aviary and in two pigeon lofts of local breeders. Two farm workers and one mushroom worker produced samples of the hay, straw, and mushroom compost respect-

ively involved, and disturbed these in the challenge cabinet environment. One other mushroom worker simply returned to work under our supervision. The duration of natural exposures varied from 10 minutes to five hours.

MONITORING TESTS

Immediately before each challenge, subjects were questioned about current symptoms and underwent routine examination of the chest. Challenges were carried out during the morning, usually between 0800 and 1000 hours. Subjects were seen at frequent intervals during the next 36 hours, or until any positive response subsided, and any unusual symptoms were recorded. Monitoring investigations were carried out immediately before challenge and were repeated at least once later the same day, usually between 1600 and 1930 hours. In the event of a positive response, the timing of repeated investigations was influenced by the time at which symptoms reached their peak.

Oral temperature was measured immediately before each exercise test (see below) and at hourly intervals throughout the day.

Venous blood was taken immediately before each exercise test. Total leucocyte counts were measured in a Coulter counter, evening samples being tested the next morning after storage in a refrigerator. Smears were made from freshly taken blood for differential cell counts.

Pre-challenge pulmonary function studies included measurement of forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) using a dry Vitalograph spirometer and, during the earlier part of the study, measurement of DLCO by the single breath method³⁴ and the subdivisions of lung volume by the helium dilution technique.³⁵ Most patients learned to use the Vitalograph spirometer themselves, and so were able to record tracings at hourly intervals. In general, two or three tracings were made on each occasion, the one recording the greatest FVC being chosen for analysis.

Because early "positive" tests induced increased breathlessness on exertion which was not associated with significant changes in resting lung function nor with appreciable breathlessness at rest, a simple test of lung function during exercise was devised. This consisted of measurement of minute volume and respiratory frequency during three consecutive five-minute periods: sitting at rest; walking on a level treadmill at a comfortable constant rate (one to three miles per hour according to individual ability, and identical in all subsequent tests); sitting during recovery. The minute volume

was measured by breathing from a dry gas meter, and the frequency was recorded by a pneumotachygraph in the breathing circuit.

The significance of objective changes associated with positive antigen tests was assessed by comparison (a) of positive antigen tests with paired control tests in the same subjects, and (b) of positive antigen tests with similar antigen tests in the subjects shown to be non-reactors. The paired comparisons (a) were tested statistically with a paired *t* test in the case of body temperature and FVC, and Wilcoxon signed ranks tests in the case of neutrophil and lymphocyte counts, exercise minute volume, and exercise respiratory frequency. Comparisons between antigen tests (b) were made with a two-sample *t* test for body temperature and FVC, and with Mann-Whitney U tests for the other measurements.³⁶

Results

One hundred and forty-four inhalation provocation tests involving 31 subjects were completed satisfactorily. The result of each test was judged at the time as positive, equivocal, or negative. Positive tests were defined from two standpoints. Firstly, the subjects concerned developed symptoms of sufficient degree to convince them that they were directly provoked by the earlier challenges despite their not knowing whether the nebulised challenges involved antigen or control exposures. Secondly, we the observers were also convinced of the positive nature of the test, taking into account not only the character and timing of the symptoms described, but also the physical signs, and the responses to any earlier provocation tests. When either party was not fully convinced, though suspicious, the result was defined as equivocal. When neither party was suspicious, the result was deemed negative.

Fifteen antigen challenges provoked equivocal results. Fourteen of these occurred in seven subjects who gave unequivocally positive results after further challenges with the same or higher doses of antigen. In the remaining subject, further antigen challenge produced negative results.

A diagnosis of EAA (with respect to the antigen investigated) was considered to be confirmed in those subjects giving unequivocally positive responses—"reactors"—and to be excluded in those who failed to respond in this manner after completing the full series of tests—"non-reactors". The two control subjects both proved to be non-reactors, and the six subjects who already showed "convincing evidence" of EAA all proved to be

reactors. An analysis of the results is given in table 1. Positive responses after each type of antigenic challenge were indistinguishable.

The result category of each test and the reactor status of each subject were consequently determined at the time of the challenge studies without regard to the objective monitoring parameters. The relative merit of each potential monitoring test was then assessed by comparing the changes associated with positive and obligatorily negative tests (all control tests, and all antigen tests in the non-reactors).

MONITORING TESTS

Symptoms

Tests associated with the development of characteristic symptoms that the subjects regarded as definite are recorded in table 2. Influenza-like symptoms predominated—malaise, fever, widespread aches and pains (particularly headache), anorexia—and occurred with all positive reactions. Respiratory symptoms were less striking. Few subjects were troubled by undue breathlessness at rest, though all noted this on exertion—particularly during the exercise test. Several also devel-

Table 1 Analysis of "alveolar" responses to 144 inhalation provocation tests in 31 subjects

Subjects	Antigen source	Challenge exposure and test result									
		Control	Serum (ml)			Droppings (mg)			Natural (hours)		
			< 0.1	0.1-0.5	≥ 0.5	< 10	10-50	≥ 50	< 1	1-5	≥ 5
Reactors											
Avian											
1†	B	N	P								
2	B	N	N	N	P						
3	B	N		NEE						N	
4†	P	N	NN	P						P	
5	B	NNN	E	N*	E					EP	
	C			P							
6	B	N	N	N	P						
7	B	N	N	PP						NN	
8	B	N				N	E	P		P	
9†	B	N	N	NP		N				P	
10	B	NNNN	E	EE	N*P					E	
	Y		N	N							
11†	B	N	EP								
12	B	N							N	P	
13	B	NN	N	NN	P						
14	B	N	P							E	
15†	P	N	N	N	P				E		
Non-avian											
16	H	N								P	
17†	F	N								P	
18	S	N							P		
Non-reactors											
Avian											
19	P	N	N	N	N						
20	B	N		N	N		NN			NN	
21	B	N	N	N	N						
22	B	N	N	N	N						
23	B	N	N	N	N						
24	B		N	N	N					N	
	P								P		
25	B	N	N	N	N						
26	B	N		N	N						
27	B		N	N	N					N	
28	B	N			N	N	N	N			
29	B			NN	N	N					
30	B	N	N	N	N	N	N	EN			
Non-avian											
31	M								N	NN	

B=budgerigar, C=canary, F=mushroom farm, H=hay, M=mushroom compost, P=pigeon, S=straw, Y=poultry, N=negative, E=equivocal, P=positive.

*These two discordant "negative" results were obtained in two subjects who gave consistently weak responses to antigen challenge irrespective of the challenge dose. One test was carried out on the day immediately after an "equivocal" response, and this may have exhausted the limited responsiveness of the subject concerned. The other subject gave responses that did not reach their peaks until the day after challenge. This was not appreciated at the time of this anomalous test, and no subjective data were recorded for the relevant following day. The test did nonetheless produce an objective monitoring score of 2/6 (see text below).

†Subjects who already showed "convincing evidence" of EAA before challenge.

Table 2 *Distribution of symptoms and clinical signs developing during the 36 hours after challenge, according to result category*

Result category	Tests	Number of tests* associated with			
		Symptoms		Clinical signs	
		Flu-like	Dyspnoea	Appearance	Breathing
Reactors (n=18)					
Antigen tests					
Positive	23	22/22	23	16/20	18/18
Equivocal	14	11	12	3/13	3/10
Negative	25	0	1	0	0
Control tests	24	0	2	0/23	0/23
Non-reactors (n=13)					
Antigen tests	49	0	3	0	1
Control tests	9	0	0	0	0

*Tests were excluded from tables 2-4 and figures 1 and 4 whenever the records were incomplete. Corrected totals are given where appropriate.

oped a dry cough and difficulty taking a deep satisfying breath, but none felt wheezy. In addition, three reactors developed nausea (one with vomiting) and two others diarrhoea.

In all but three subjects, symptoms began after two to nine hours and reached maximum intensities after between six and 20 hours of the start of exposure. In one subject with farmer's lung, symptoms began after about one hour, while in two avian reactors the onset of symptoms was delayed for 12-18 hours after the start of exposure. With both these avian reactors the maximum intensity of the symptoms experienced was not reached until the next day. All positive reactions were consequently of the "late" type, and all persisted for a minimum of several hours.

Clinical signs

Table 2 gives the distribution of characteristic clinical signs that the observers regarded as definite, according to result category. Facial appearance proved to be the most informative clinical sign and the observers soon learned to recognise the onset of positive responses from the loss of normally cheerful and active expressions. At this stage the subjects themselves rarely admitted to any change in well-being. As the response worsened, an unwell appearance was generally readily apparent. Increased respiratory effort on exercise during the symptomatic phase of positive tests was usually obvious, but this was rarely true at rest.

Auscultatory signs were uncommon. In five patients relatively strong reactions were associated with the development of fine inspiratory crackles in the lung bases. In four patients, basal crackles noted before challenge seemed to become more extensive, and in three others crackles appeared to develop at the extreme lung bases, but these

were not consistently heard. No subject developed wheezes.

Body temperature

Fever characteristically accompanied positive reactions and was usually symptomatic. The mean changes in body temperature according to result category are given in table 3.

Table 3 *Mean maximum body temperatures during the 36 hours after challenge, according to result category*

Result category	Tests	Mean temperature (°C) per subject* before and after challenge		
		Before	After	Change
Reactors (n=18)				
Antigen tests				
Positive	23	36.5	37.9	+1.4
Equivocal	14	36.5	36.8	+0.3
Negative	22	36.5	36.7	+0.2
Control tests	22	36.5	36.7	+0.2
Non-reactors (n=13)				
Antigen tests	47	36.3	36.7	+0.4
Control tests	8	36.3	36.7	+0.4

*Because individual subjects underwent different numbers of tests, mean values for each subject are used in tables 3 and 4. Each subject consequently influenced the results to an equal degree.

The results obtained from positive tests in the reactors cannot be compared in a strict statistical sense with those from the negative antigen tests in the non-reactors because different challenge exposures were used. By considering challenges using budgerigar serum (BS) alone, the significance of any differences was evaluated more satisfactorily. A subgroup of challenge tests was selected comprising all positive tests to BS (10 tests in nine reactors—mean nebulised dose, 0.38 ml neat BS), and all tests to high dose BS in non-reactors (11 tests in 11 non-reactors—mean nebulised dose, 0.75 ml neat BS). These were the tests that had determined whether each budgerigar fancier was a reactor or non-reactor, respectively. Data from these selected tests and the corresponding control tests showed almost identical mean changes to those obtained overall (reactors: positive tests +1.5°C versus control tests +0.3°C, $p<0.01$; antigen tests: reactors +1.5°C versus non-reactors +0.4°C, $p<0.01$). This confirmed that the increase in body temperature associated with positive tests was of a statistically significant degree.

Figure 1 compares the distributions of maximum body temperatures after positive and obligatorily negative tests. Measurements of 37.2°C or less were obtained with 95% (73/77) of the obligatorily negative tests, while measurements exceeding

Fig 1 Distributions of maximal body temperatures during the 36 hours after challenge.

37.2°C were obtained with 78% (18/23) of the positive tests. Body temperature exceeding 37.2°C during the 36 hours after challenge was consequently a valid diagnostic feature of positive responses, having high specificity (the diagnostic end-point having been chosen to identify approximately 95% of negatives) and moderate sensitivity (78% of positives identified).

Leucocyte counts, exercise tests, and spirometry
Data on the other objective monitoring parameters were analysed in the manner described for body temperature. Increases in circulating neutro-

phil counts, exercise minute volume, and exercise respiratory frequency, and decreases in circulating lymphocyte counts and FVC were found to be statistically significant and to have useful diagnostic value (sensitivities of approximately 50% or more were obtained after choosing end-points that produced specificities of about 95%). The details are summarised in table 4. For each of these measurements, the subgroups of tests used for statistical analysis showed almost identical mean changes to those observed overall.

Marked increases in the numbers of circulating leucocytes were observed in association with

Table 4 Mean maximal changes of objective monitoring tests during the 36 hours after challenge, according to result category

	Tests	Body temperature (°C)	Leucocyte counts/mm ³		Exercise 5 minutes %		FVC	
			Neutrophils	Lymphocytes*	Minute volume	Respiratory frequency	% (litres)	
Reactors (n=18)								
Antigen tests								
	23	+1.4	+ 5405	- 351	+ 32	+ 40	- 20	(-0.61)
	14	+0.3	+ 1446	- 46	+ 7	+ 6	- 11	(-0.36)
	25	+0.2	+ 625	+ 346	0	+ 1	- 5	(-0.16)
	24	+0.2	- 412	+ 704	- 3	0	- 7	(-0.22)
Non-reactors (n=13)								
	49	+0.4	+ 404	+ 444	+ 1	+ 2	- 5	(-0.19)
	9	+0.4	- 234	+ 268	- 5	+ 3	- 6	(-0.19)
Statistical comparisons†								
	p <	0.01	0.01	0.01	0.01	0.01	0.05	
	p <	0.01	0.01	0.01	0.01	0.01	0.01	
Diagnostic end-point								
	> 37.2	≥ +2500	≥ -500‡	≥ +15	≥ +25	≥ -15		
	95%	96%	97%	95%	94%	97%		
	78%	68%	52%	85%	64%	48%		

*From morning to evening; †Using selected tests with BS and corresponding control tests; ‡With lymphopenia.

positive tests, and these changes were found to be fully attributable to increases in the neutrophil fraction. With 12 of the positive tests absolute neutrophils (>7500 neutrophils/ mm^3)^{37 38} developed.

The lymphocyte counts were also affected by positive tests, though in the converse manner and to a much less marked degree. It can be seen from table 4 that the mean lymphocyte counts associated with control and negative tests increased mildly after challenge, while there was a small decrease associated with equivocal tests and a more marked decrease with positive tests. The absolute changes in lymphocyte counts did not prove to be of diagnostic value when considered alone, because there was considerable overlap between the distributions associated with positive and obligatorily negative tests. However, a decrease in lymphocyte count of 500 cells/ mm^3 or more did prove to be of diagnostic value when coupled with the development or worsening of absolute lymphopenia (<1500 lymphocytes/ mm^3).^{37 39}

There were no significant changes in the numbers of circulating eosinophils.

The results of the exercise tests are expressed in terms of percentage rather than absolute changes because there were variations between the subjects in treadmill speeds and prechallenge ventilatory patterns. The exercise period, in comparison with the resting period preceding it and the recovery period succeeding it, was associated with the greatest increase after positive tests and the least change after obligatorily negative tests. Measurement of minute volume and respiratory frequency during the resting period consequently proved to be much less sensitive in distinguishing positive from obligatorily negative tests. For both tests an end-point of $\geq +25\%$ produced a specificity of 94%, but sensitivities were only 40% and 30% respectively. Combining data from the 10 minutes of exercise + recovery proved to be less useful than data from the exercise period alone.

With positive responses quite marked individual variations in tidal volume and respiratory rate were noted during the exercise tests, but there was no overall tendency to rapid shallow breathing. The mean tidal volume did not change significantly and calculations with an assumed anatomical dead space of 150 ml showed a mean increase in alveolar ventilation of 30% in association with positive tests. There was a mean fall of 3% with corresponding control tests.

Similar mild mean decrements in FVC were noted with each subgroup of obligatorily negative tests. These are probably attributable to the bias

of choosing the minimum of multiple measurements. The mean maximum fall in FVC of 20% after positive tests was accompanied by a similar mean maximum fall in FEV_1 of 19%, indicating the ventilatory disturbance produced was one of restriction, not obstruction. The mean ratio FEV_1/FVC was 81% both immediately before challenge and at the time of the maximum fall in FVC.

Lung volumes

Routine measurement of the subdivisions of lung volume after initial positive tests did not demonstrate great changes, and this practice was discontinued after paired positive and control tests were monitored in four reactors. A modest fall in inspiratory capacity (mean -9%) without change in functional residual capacity (mean -1%) was the most prominent finding associated with positive tests, though a mild reduction in expiratory reserve volume (mean -7%) was also noted. The vital capacity and the total lung capacity were reduced appropriately (means of -8% and -5% respectively), and there was a small increase in residual volume (mean $+3\%$) appropriate to the reduced expiratory reserve volume and the unchanged functional residual capacity. Similar changes were observed in a further reactor in whom measurements after control challenge were not obtained.

Single breath diffusing capacity

Diffusing capacity for carbon monoxide was measured in association with 12 positive challenge tests in 10 reactors. Appreciable decreases of 14–45% occurred with three of the more severe reactions only. Figure 2 compares the distributions of changes in DLCO after positive and obligatorily negative tests. Only decrements of 15% or more could be considered diagnostically significant, and with this end-point only two of the positive tests could be identified.

Chest radiographs

No unequivocal changes were noted in 12 inspiratory and five expiratory radiographs of 12 subjects undergoing positive responses. In six films there was some suspicion of increased haziness/nodularity, for which technical differences may have been responsible.

TOTAL MONITORING SCORE

In assessing the diagnostic value of the various monitoring tests studied, the ability of each to identify obligatorily negative tests correctly (the specificity) was related to the converse function of identifying the unequivocally positive tests

Fig 2 Distributions of percentage changes in DLCO during the 36 hours after challenge.

correctly (the sensitivity). The endpoints required to give specificities of about 95% produced useful sensitivity levels (approximately 50% or more) for 10 of the tests studied—symptoms and signs appropriate to an influenza-like illness and undue respiratory effort on exercise (the four subjective observations), and body temperature, circulating neutrophil and lymphocyte counts, exercise minute volume and respiratory frequency, and FVC (the six objective tests).

By allowing a score of one for each of these 10 tests giving diagnostically significant changes, a maximum total monitoring score of 8–10 was possible for the overwhelming majority of tests (not all tests were used in each challenge). The mean scores per challenge test and the mean scores expressed as a percentage of the maximum

score possible are shown according to result category in table 5. Obligatorily negative tests showed, by design, mean scores not exceeding 5% of the maximum possible, while positive tests and equivocal tests (given by the reactors) were associated with mean scores of 77 and 31% respectively. This total monitoring score proved to be of greater diagnostic value than any of the individual tests considered alone. All the positive challenge tests were associated with scores of 4 or more, while all the obligatorily negative tests were associated with scores of 3 or less (fig 3).

If a score of 3 or more is chosen to define a positive result, a specificity of 99% is obtained. This definition would allow eight of the 15 equivocal tests to be reclassified as "positive." All eight had occurred in reactors.

SEQUELAE

In general the systemic response, as measured by the influenza-like symptoms, unwell appearance, temperature increase, and haematological changes, subsided spontaneously within one to two days. The pulmonary response, particularly increased breathlessness on exertion and cough, sometimes persisted longer (in one case for as much as two to three weeks), and for this reason the routine use of prednisone (commencing at 30 mg daily and tailed off over 5–10 days) was introduced. With this modification, symptoms were generally satisfactorily controlled within a day or two.

The possibility of long-term sequelae after these provocation tests was considered in 13 of the reactors who were restudied after intervals ranging from 0.5 to 33 months (mean nine months). Only two considered they had deteriorated. Pulmonary function tests in the remainder showed that lung function had recovered to at least prechallenge levels, and often to within the predicted normal range.

One subject improved initially when she gave up her birds and was treated temporarily with modest doses of corticosteroids (prednisone 20–30 mg daily). Weight gain and increased incapacity from severe osteoarthritis soon led to reduction in corticosteroid dosage, and her respiratory symptoms and lung function thereafter worsened steadily. Continuing budgerigar exposure during frequent "baby sitting" activities at her son's home was eventually discovered.

One other subject admitted to mildly increased breathlessness on exertion when reinvestigated seven months after her positive challenge. She had denied this during the few months immediately after it, and serial lung function studies did not

Table 5 Total monitoring scores, according to result category

Result category	Tests	Monitoring scores			
		Total (T)	Mean per test	Maximum possible (M)	T/M × 100%
Reactors (n=18)					
Antigen tests					
Positive	23	164	7.1	213	77
Equivocal	14	42	3.0	135	31
Negative	25	10	0.4	213	5
Control tests	24	5	0.2	227	2
Non-reactors (n=13)					
Antigen tests					
	49	17	0.3	469	4
Control tests	9	4	0.4	83	5

Fig 3 Distributions of total monitoring scores according to result category.

give confirmatory evidence of deteriorating lung function. It seemed probable that increasing angina of effort was the true cause of her reduced exercise tolerance.

Discussion

Although inhalation provocation tests are widely used in the investigation of allergic asthma, their role in the investigation of EAA is not well established. Extrinsic allergic alveolitis is much less common than asthma, and many of the antigens responsible have been recognised in recent years only. Experience is consequently limited. Furthermore, allergic "alveolar" reactions are of the "late" type only, and so the tests provoking them are particularly time-consuming.

A more serious problem concerns the validity of the positive "alveolar" reaction. It could be argued that a systemic response occurring without evidence of pulmonary dysfunction is caused by the presence of contaminating microbial toxins or, after challenge with bird serum, by serum sickness; that systemic hypersensitivity to the test antigen could have been induced by sequential test exposures themselves; and that, irrespective of its cause, systemic hypersensitivity could be unrelated to coexisting lung disease.

That no significant responses occurred in the two control subjects or in 11 other non-reactors, is strong evidence against toxic mechanisms being directly responsible. Positive tests were reproducible and were readily distinguished from control tests despite the identity of the nebulised challenges being unknown to the subjects concerned. Furthermore, positive responses were obtained from all six of the subjects who had already shown convincing evidence of EAA before undergoing inhalation challenge. Five of the reactors gave equivocal or positive responses to their first challenge with the relevant bird serum, confirming that in them, at least, hypersensitivity to bird serum was not a consequence of the test exposures themselves. The three subjects reacting to hay, straw, and mushroom compost respectively also gave positive responses to their first test exposure. All the positive reactions were essentially identical in nature, irrespective of the particular provoking antigen or its means of delivery, and irrespective of whether the subject concerned manifested the acute or chronic form of EAA. The responses were shown to involve significant changes in lung function, despite the dominance of systemic disturbances, and they closely resembled the acute illnesses with which the pigeon breeders and farm workers had presented. We conclude that the evidence provided by inhalation provocation tests of the type described is both valid and definitive in the investigation of EAA. No other currently available test is of equal diagnostic value.

Subjective observations found to be useful in monitoring the alveolar response to challenge were the symptoms and corresponding clinical signs of an influenza-like illness and undue shortness of breath on exercise. All proved to be highly discriminatory, especially when they were considered together. Their significance was limited, however, because they were largely responsible for establishing the result category initially. In this respect the objective parameters were more valuable, since the objective data were not analysed until the study had been completed and the result of each test had been defined. Six objective measurements showed statistically significant changes in association with positive tests. Overall, they were less sensitive than the subjective observations but they confirmed that significant systemic and pulmonary changes occurred only in the reactors, and only after antigen challenges. This validates the use of the subjective parameters in establishing the result category.

Measurement of minute volume during the exercise period of the exercise test proved to be the most useful objective parameter, having a sensitivity of 85% (fig 4). This depended to some extent on performing sequential exercise tests after challenge so that maximal changes could be

identified. The latter were found to coincide with the symptomatic peaks of positive reactions.

Measurement of exercise respiratory frequency proved to be less sensitive than minute volume but was, nevertheless, more useful than any of the other tests of pulmonary function. Increased exercise respiratory frequency may have been partly dependent on the commonly accompanying pyrexia, though pyrexia appeared to have negligible effect on exercise minute volume during heat challenge experiments involving the two control subjects and a well-insulated electrically heated suit. These divergent effects of fever on respiration have been described by Petersen and Vejby-Christensen.⁴⁰ In fact, at the times of the relevant exercise tests, the body temperature of five of the reactors did not exceed 37.0°C.

Measurements made during the initial resting and subsequent recovery periods of the exercise tests did not in any combination improve the diagnostic value of the exercise period alone. The exercise test as described could consequently be simplified by omitting these two unhelpful periods, and by performing a single follow-up study when any symptoms appear to be maximal.

The exercise test changes were found to be the result of increased alveolar ventilation. It is of

Fig 4 Distributions of maximal percentage changes in exercise minute volume during the 36 hours after challenge.

some interest that this usually occurred in the absence of a significant reduction in DLCO. Hyperventilation and decreased arterial PCO_2 are characteristic of more advanced stages of both cryptogenic fibrosing alveolitis and EAA, and it is probable that hyperventilation rather than large increases in physiological dead space was responsible. It may be that relatively modest diffuse alveolar insults provoke increased sensitivity of afferent nerve endings and hence hyperventilation, when the degree of any alveolar ventilation/perfusion imbalance is insufficient to impair carbon monoxide transfer. This may also occur before there is a significant decrease in compliance.⁴¹⁻⁴³

The usefulness of the exercise test in demonstrating pulmonary involvement in the reactions observed may be seen in perspective by considering the other tests monitoring lung function which were all measured at rest. Only the repeated measurement of FVC proved to be worthwhile but its sensitivity was less than 50%. Warren and colleagues used flow volume loops and single breath nitrogen washout curves to monitor changes in lung function during positive "alveolar" responses of six patients with bird fancier's lung.²⁸ They considered these to be as sensitive as the measurement of static and dynamic compliance, but in general did not demonstrate any significant changes until the vital capacity became impaired.

The three remaining objective measurements found to be useful diagnostically reflected the systemic response. Fever and neutrophilia are both well-recognised features of positive "alveolar" responses to provocation tests,^{5 24 26 28 44} and both proved to have useful discriminatory value.

The mild but significant decrease in the number of circulating lymphocytes is an intriguing finding which has not been described previously, though Hargreave and Pepys observed that the proportion of lymphocytes decreased with positive tests, while that of the neutrophils increased.⁵ The discriminatory value of the lymphocyte changes was limited (sensitivity 52%) and in only one instance did the lymphocyte count change significantly without the neutrophil count doing likewise. Its chief interest concerns its cause. Could the missing cells have migrated to the lung as a consequence, either of the antigenic challenge, or of the resulting immune/inflammatory response? In its acute phase, EAA is known to be characterised by an excess of pulmonary lymphocytes.^{45 46} Geller and colleagues have recently reported low circulating lymphocyte counts in patients presenting with pulmonary hypersensitivity reactions to nitrofurantoin,⁴⁷ as have Flaherty and colleagues in

describing patients recovering from farmer's lung.⁴⁸ In both reports it was the T lymphocyte count which was reduced, thereby implying involvement of cellular immunological mechanisms. Alternatively, lymphopenia may have been a non-specific consequence of the stress of the positive response, possibly as a result of increased corticosteroid secretion.

Auscultation, chest radiography, and the measurement of DLCO or the subdivisions of lung volume may be expected to demonstrate significant changes only with relatively severe reactions. Such reactions could be readily identified anyway by the more sensitive monitoring observations described here. Only six of the 23 positive reactions (in five reactors) were associated with the development of clear-cut basal crackles and with only two of these did the DLCO decrease significantly. The lowest total monitoring score of these six reactions was 5/8, and the mean was 85% of the total possible—scores that comfortably identify unequivocally positive responses. Looked at another way, inhalation provocation tests that do produce crackles, significant falls in DLCO, or radiographic changes are unnecessarily severe. This implies unnecessary distress to the patient concerned and, presumably, greater hazard.

It is interesting that the two strongest reactions occurred in the two patients with farmer's lung tested by the natural method of exposure. They disturbed samples of their own dry mouldy hay and straw in a challenge cabinet environment for 90 and 40 minutes respectively. This created considerably more dust than they were accustomed to occupationally and both reacted briskly to the first active test. In retrospect, shorter exposures would have been more appropriate. The avian challenges involved sequential increasing doses, the first of which was known to be safe.⁵ The bird fanciers were consequently better protected from unduly strong reactions, and so were less likely to demonstrate changes with respect to the less sensitive tests. Stronger reactions may not always be produced by greater challenge exposures, however, because the responsiveness of some subjects is limited. In these circumstances confirmation of positive reactivity may require that weak (though, for the individual concerned, maximal) responses are appropriately reproducible after further antigen and control challenges.

The device of assessing each provocation test by means of a total monitoring score enabled eight of 15 equivocal tests to be reclassified retrospectively as positive. The subjects concerned might consequently have been spared the dis-

comfort and risk of further challenge exposures. The use of all six objective measurements found to be diagnostically useful was of course time-consuming, and the question arises whether the routine use of all six would be cost-effective. In a sense, none is essential in evaluating the result of most tests because the subjective observations alone were shown to be adequate, being equally valid and rather more discriminatory. If, nevertheless, objective evidence is required to confirm the subjective findings, measurement of exercise minute volume is the most effective of the comparatively simple and easily available monitoring tests considered in this study. It is however the least convenient and the most time-consuming. More practical is the measurement of both body temperature and neutrophil count, one or the other of which is likely to change significantly with more than 90% of positive tests. The cost-effectiveness of using any of the other objective tests in addition is marginal.

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