

Inhalation tests in monkeys passively sensitized with human serum

M. RADERMECKER, F. GEUBELLE and J. SALMON

*Institut de Médecine et Clinique Pédiatrique, Hôpital de Bavière,
Université de Liège, Belgium*

Summary

Rhesus monkeys were passively sensitized to grass pollen by intravenous injection of human reaginic serum. Sensitized animals, who consistently exhibited local or general anaphylactic reactions following the intradermal, intrabronchial or systemic injection of the specific allergen, tolerated the allergen perfectly well when given it by inhalation. No significant changes in pulmonary resistance, dynamic lung compliance or tidal volume were observed in unanaesthetized, passively sensitized monkeys following administration of an aerosol containing pollen. An increase in respiratory rate was noted immediately after aerosolization in sensitized and unsensitized monkeys.

Inhalation of histamine in some animals exerted a bronchoconstrictor effect which was promptly relieved by administration of a sympathicomimetic bronchodilator. A positive test with histamine was characterized by symptoms of dyspnoea, auscultatory findings of wheezing rales and by a significant increase in pulmonary resistance. These results suggest that sensitization of the bronchi by reagents *per se* cannot explain the occurrence of bronchospasm upon inhalation of the allergen. Other local or general factors may be required to produce the clinical expression of the bronchial anaphylactic reaction.

Introduction

Monkeys may be passively sensitized to ragweed or grass pollens by intravenous (i.v.) injection of a sufficient amount of human reaginic serum. Sensitized animals consistently exhibit positive skin tests or general anaphylactic reactions following intradermal or intravenous injection of the appropriate pollen (Layton & Greene, 1964; Patterson *et al.*, 1965).

Asthma has also been reported to occur in passively sensitized monkeys after bronchial challenge with an aerosol containing the specific allergen (Patterson *et al.*, 1965, 1967; Patterson, 1969). In these experiments, however, pulmonary resistance to air flow was estimated indirectly from maximal flow values. As the latter provide only

a rough estimate of resistance, the present study was designed to verify the effect of inhalation of the specific allergen and histamine on total pulmonary flow resistance and other ventilatory parameters in monkeys passively sensitized to grass pollen.

Material and methods

Experimental protocol

Eighteen rhesus monkeys (*Macaca mulatta*) of both sexes weighing 5–10 pounds were studied without any anaesthesia or premedication. Dynamic lung compliance (C_1 dyn) and pulmonary flow resistance (R_1), as defined below, were measured the same day, before and after administration of an aerosol containing grass pollen (2 mg), and before and after inhalation of an aerosol containing histamine (2 mg). These experiments were repeated 24 hr later.

Fourteen monkeys were then passively sensitized to grass pollen by i.v. injection of human reaginic serum. As controls, four monkeys received normal human serum (40 ml/kg).

The effect of inhalation of grass pollen and histamine on the mechanics of breathing of the animals was again studied twice, and at 24 hr intervals on the 3 days following passive sensitization. In some animals, the same measurements were done 4 and 6 days after the sensitizing injection.

Bronchoscopy was performed in four anaesthetized sensitized monkeys to allow direct administration of grass pollen into the trachea. During bronchoscopy, R_1 was measured immediately before, during and 15 min after administration of the aerosol.

Passive sensitization

The reaginic serum used for passive sensitization was pooled from four patients who were exquisitely sensitive to grass pollen and who had never been desensitized. Its P-K titre measured in the monkey was 100. Systemic passive sensitization was obtained by i.v. injection of allergic serum (40–60 ml/kg of body weight). The total dose was divided into two equal parts given at a 5 hr interval. Twenty-four hours later and on the following days the passive sensitization of the animals was verified by intradermal, intramucosal and i.v. injection of grass pollen.

Skin tests. Five minutes following an i.v. injection of a 0.5% solution of Evans blue dye (1.5 ml/kg), the monkeys were skin tested by the scratch or intradermal technique (0.05 ml) using a 1×10^{-5} or 1×10^{-6} solution of grass pollen (Pasteur, Paris). A positive reaction was characterized by an extravasation of dye occurring within a few minutes specifically at the site of injection of the allergen. The intensity of the reaction was measured 15 min later by recording two perpendicular diameters (mm) and using the mean of these two values. The intradermal injection of saline or grass pollen (1×10^{-3} solution) was without effect in the monkeys prior to sensitization.

Intrabronchial tests. Saline or grass pollen (0.05 ml, sol. 1×10^{-5}) was injected into the mucosa of the stem bronchi in four monkeys passively sensitized to the allergen. Animals were first subjected to bronchoscopy under general anaesthesia. Five minutes following i.v. administration of Evans blue dye (1.5 ml/kg, sol. 0.5%), the intramucosal injection was made using a long rigid tube (length: 40 cm; diameter: 2 mm) passed through the bronchoscope and terminating in a thin needle (26 G) for intrader-

mal use. The other end of the tube was connected to a 1 ml tuberculin syringe. The site of injection was observed for 15 min.

Intravenous injection of grass pollen. In some unanaesthetized animals, previously passively sensitized to grass pollen, the blood pressure, intrathoracic pressure changes, air flow and tidal volume were recorded before and during the first 20 min following i.v. injection of grass pollen (3 ml, sol. 0.1%).

Blood pressure was measured directly in the femoral artery with a catheter inserted under local anaesthesia and connected to an EMT 34 differential electromanometer (Elema-Schönander, Stockholm) (sensitivity: 0–30 cmHg) by polyethylene tubing filled with heparinized saline (10 u/ml). The system was provided with a three-way valve permitting regular flushing with 1 ml of heparinized saline to maintain patency of the catheter.

Mechanics of breathing

The total pulmonary flow resistance (R_1) in $\text{cmH}_2\text{O/l/sec}$ is the quotient of the endo-oesophageal pressure change measured between 'isovolume' points, in the middle of inspiration and expiration, divided by the corresponding flow rate change. It corresponds to the sum of the airway resistance (R_{aw}) and the viscous resistance of the lung tissue (R_{lt}), which results from the friction of the tissues. During bronchoscopy, the pneumotachograph was connected directly to the bronchoscope. The R_1 measured under these conditions thus included the resistances of the lower respiratory tract and of the tube (internal diameter: 5 mm; resistance: 0.5 $\text{cmH}_2\text{O/l/sec}$ in the range of the observed flows).

The dynamic lung compliance (C_1 dyn) in $\text{ml/cmH}_2\text{O}$ is the ratio between the tidal volume and the endo-oesophageal pressure change calculated from the start to the end of inspiration. The values of R_1 and C_1 were determined from ten respiratory cycles and the mean was calculated.

Intrathoracic pressure changes were determined by means of an endo-oesophageal balloon (Buytendijck, 1949) according to the technique used in neonates (Senterre & Geubelle, 1970).

Since the flow resistance of the mask and the pneumotachograph (see below) was low and the endo-oesophageal pressure changes were relatively high, the transpulmonary pressure gradient was not measured and only the endo-oesophageal pressure changes were used.

Flow rate was measured by means of a pneumotachograph (Fleisch, 1956) of low resistance (0.2 $\text{cmH}_2\text{O/l/sec}$) connected to an EMT 32 differential electromanometer (Elema-Schönander) of high sensitivity (0–15 mmH_2O). The pneumotachograph was applied to the animal's face with a fitted plexiglass mask. The dead space of the system (mask plus pneumotachograph) was around 15 ml. The translucency of the mask made it possible to ensure that the system was air-tight and that the mouth and nostrils remained patent.

The *tidal volume* signal was obtained by integration of the air flow signal.

Electric equivalents of the intrathoracic barogram, the pneumotachogram and the spirogram were recorded with a four-channel Jet Recorder (Mingograph, 81, Elema). The time phase shift between the records was under 0.01 sec.

Respiratory frequency (f) and ventilation/min ($\dot{V}$) were calculated. It was thought that the latter parameter would easily reveal any leak in the mask-pneumotachograph system. These ventilatory parameters were measured in unanaesthetized monkeys,

restrained in dorsal recumbency. They were recorded continuously before, during and 15 min after inhalation of grass pollen or histamine. Some technically unsatisfactory records were discarded.

The mean variation of a given parameter (R_I , C_1 dyn, V_T , f or $\dot{V}$) from day to day or following inhalation of pollen or histamine corresponded to the algebraic mean of the individual differences expressed as a percentage of their initial value. The significance of the results was analysed by the Student-Fisher t test at the 5% level.

Aerosols

A 2 ml solution containing 2 mg of a commercial extract of grass pollen (Pasteur, Paris) or histamine (bichlorhydrate) in saline was aerosolized from a 'Vaponefrin' nebulizer. An air pressure of 0.4 kg/cm² was applied to the nebulizer. It has been demonstrated that under these conditions particle size varies from 0.6 to 4 μ and has a mean value of approximately 3 μ (Bryson, 1947). Fifteen litres of air were required to aerosolize the 2 ml solution. The aerosol was collected into a 40 litre capacity rubber bag and administered to the animals through a one-way valve connected to the pneumotachograph and the mask. The resistance of this valve was about 0.3 cmH₂O/l/sec and its dead space 15 ml, which must be added to that of the mask-pneumotachograph system (15 ml).

After inhalation of the aerosol, the one-way valve was disconnected when recording the ventilatory parameters.

In some anaesthetized animals subjected to bronchoscopy, the aerosol was administered directly into the bronchi by means of a rubber tube directly connected to the nebulizer and passed through the bronchoscope.

Bronchoscopy

Animals were anaesthetized by i.v. injection of 60–120 mg of sodium pentobarbital (Veterinary Nembutal Abbott). Bronchoscopic equipment (Storz, Tuttlingen, Germany) was composed of a cold-light supply (Universal) of 150 watts and fibre glass bundles transmitting the light to a 'Universal' bronchoscope (5 mm $\times$ 30 cm) with proximal lighting. Endoscopic examination was performed directly through the bronchoscope or by means of a wide-field (60°) fixed focus optic.

Results

Passive sensitization

Skin tests (scratch or intradermal) made with serial dilutions (sol. 1×10^{-2} to 1×10^{-6}) of grass pollen in saline were negative in normal unsensitized monkeys but strongly positive as early as 24 hr following passive systemic sensitization with the corresponding reaginic serum. The mean diameter (mm) of the extravasation reaction observed 15 min after intradermal injection of the allergen (0.1 ml, sol. 1×10^{-5}) ranged from 15 to 20. The injection of saline (0.1 ml) failed to produce any reaction.

Sensitized monkey serum when injected (0.1 ml) into normal homologous skin is capable, even after 1/30 dilution, of locally sensitizing monkey skin against grass pollen. Following systemic passive sensitization, the skin reactivity for the pollen as well as the P-K activity of the monkey serum gradually disappear within 10 days.

Aliquots (0.1 ml) of grass pollen solutions (1×10^{-3} and 1×10^{-4}) were injected into the bronchial mucosa of six normal anaesthetized bronchoscoped monkeys

previously injected with Evans blue dye. No reaction occurred in the 20 min post-injection period. However, the injection of 0.05 ml of grass pollen (1×10^{-5}) into five animals passively sensitized the previous day consistently produced a blue spot at the site of injection. This bronchial extravasation reaction progressed in the same way and was of the same order of magnitude as the cutaneous reaction observed in the same animals following intradermal injection of the same allergenic solution. Injection of saline into the bronchial mucosa of sensitized monkeys had no effect.

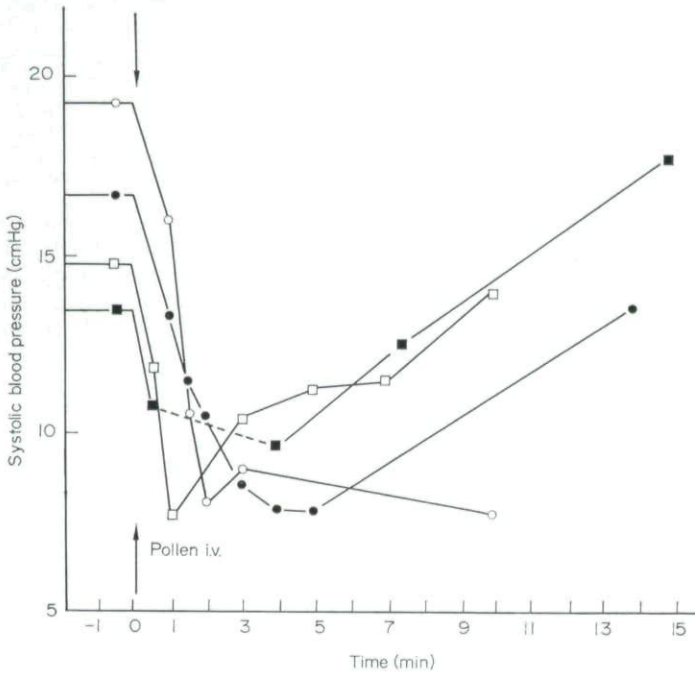


Fig. 1. Effect of intravenous (i.v.) injection of grass pollen (3 ml, sol. 0.1%) on the blood pressure of monkeys passively sensitized to this allergen by i.v. injection of human reaginic serum. Dashed line: during this period it was impossible to record the blood pressure for technical reasons.

I.v. injection of grass pollen (3 ml, sol. 0.1%) was perfectly tolerated by normal monkeys. The same injection in monkeys passively sensitized 24 or 72 hr before invariably led within 30 sec to severe disturbances: excitation, piloerection and prostration. The cardiac and respiratory rates increased. Ventilatory intrathoracic pressure changes could no longer be recorded, the oesophageal peristaltic waves being too frequent. At the same time, there was a drop in blood pressure (Fig. 1). The blood pressure decrease, amounting to 60% of its initial value, was maximum around the fourth minute following injection of the allergen. Afterwards, it tended gradually to recover to normal values. In 5 min the animals developed oedema of the face and penis. One of them had diarrhoea. No rales were audible in the chest and no urticarial lesions could be detected on the trunk or limbs. During this period, R_1 was not significantly modified. All symptoms disappeared within 30 min.

Mechanics of breathing

Ventilatory parameters of the normal monkey. Fig. 2 is a typical record of the changes in endo-oesophageal pressure, air flow rate and tidal volume obtained in a

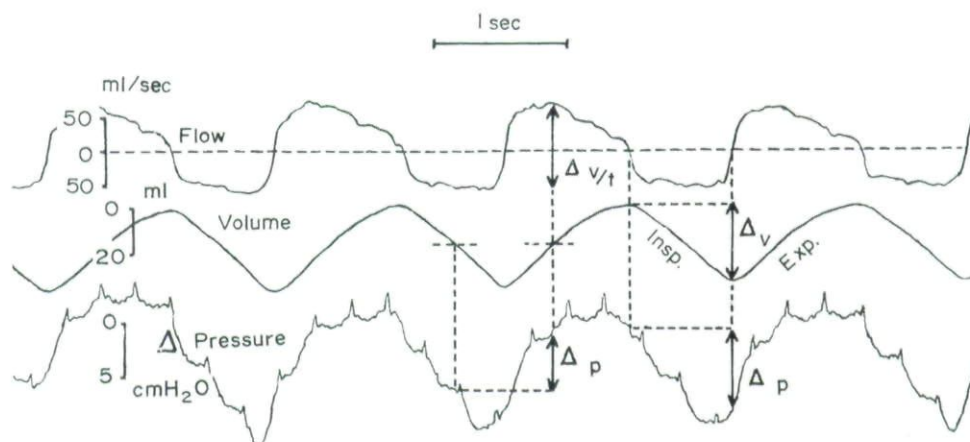


Fig. 2. Simultaneous record of the changes in endo-oesophageal pressure (cmH₂O), air flow rate (ml/sec) and tidal volume in a normal monkey. Pulmonary flow resistance (cmH₂O/l/sec) is the quotient of the endo-oesophageal pressure change measured between 'isovolume' points (in the middle of inspiration and expiration) divided by the corresponding flow rate change. Dynamic lung compliance (ml/cmH₂O) is the ratio between the tidal volume and the endo-oesophageal pressure change calculated from the start to the end of one inspiration.

normal unanaesthetized monkey. It also illustrates our method of calculating pulmonary flow resistance (R_1) and dynamic lung compliance (C_1 dyn). R_1 values, measured at 24 hr intervals in eleven normal monkeys (weighing from 5 to 10 pounds), tended to vary from day to day. The algebraic mean of the individual day-to-day variations, expressed as a per cent of the R_1 value obtained the first day, is 4.34% (standard deviation $\pm 44.4\%$; range: -40.6 and $+116\%$).

The mean values of R_1 , C_1 dyn, f and V_T in thirteen normal monkeys (5–10 pounds) are shown in Table 1. The day-to-day variations of these parameters are neither systematic nor significant. They were not significantly modified following i.v. injection of human serum (40–60 ml/kg). We defined a positive response to a provocation test as a 100% increase in R_1 occurring during or within a few minutes after inhalation of aerosolized histamine or specific allergen.

Table 1. Mean ventilatory parameters determined in thirteen normal rhesus monkeys (5–10 pounds)

	R_1 (cmH ₂ O/l/sec)	C_1 dyn (ml/cmH ₂ O)	V_T (ml)	f (min)	$\dot{V}$ (ml/min)
Mean	36	9.82	40.17	51.3	2.105
S	17.28	5.14	13.87	11.52	963.5
n	24	24	24	24	24
E_m	3.53	1.05	2.83	2.35	196.6

R_1 : pulmonary resistance; C_1 dyn: dynamic lung compliance; V_T : tidal volume; f : respiratory frequency/min; $\dot{V}$: ventilation in ml/min; S: standard deviation; n : number of determinations; E_m : standard error of the mean.

Following inhalation of pollen. Table 2 shows the R_1 of normal and sensitized monkeys before and 15 min after administration of pollen as well as their differences as a per cent of the initial values.

In unsensitized animals R_1 did not change significantly after pollen inhalation, except in monkey 3 where a twofold increase was noted. This positive provocation test could not, however, be reproduced the next day. In sensitized animals also there was no significant change in R_1 following pollen inhalation. The mean variation of R_1 in response to allergen was not different before and after passive sensitization. Moreover, as shown in Table 3, there was no significant variation of C_1 dyn and V_T whereas f increased significantly in both animal groups following inhalation of pollen.

Table 2. Effect of inhalation of grass-pollen (2 mg) on monkey R_1 (cmH₂O/l/sec) before and after systemic passive sensitization with serum from pollen-sensitive patient

Monkey	Before sensitization			After sensitization		
	Inhalation of pollen			Inhalation of pollen		
	R_1 before	R_1 after	$\Delta\%$	R_1 before	R_1 after	$\Delta\%$
1	—	—	—	27	23	-14.8
	34	29	-14.7	39	31	-20.5
2	15	28	+86.7	—	—	—
	19	24	+26.3	29	30	+3.4
3	22	66	+200	40	38	-5
	33	31	-6	29	37	+27.6
9	85	74	-12.9	60	102	+70
	60	79	+31.7	40	34	-15
10	46	38	-17.4	30	38	+26.7
	31	29	-6.5	74.8	26	-65.2
11	—	—	—	46	42	-8.7
	—	—	—	42	32	-23.8
12	40.5	30	-25.9	—	—	—
	—	—	—	—	—	—
13	37	27	-27	21	17.5	-16.7
	34	25	-26.5	36	28	-22.2
14	70	62	-11.4	54.6	46	-15.8
	41.6	63	+51.4	—	—	—
15	42.6	30	-29.6	28.5	36	+26.3
	39	40	+2.6	22	15.2	-30.9
16	12.5	20	+60	21.5	26.7	+24.2
	27	26	-3.7	30.5	25	-18
Mean	38.29	40.1	+15.4	37.27	34.86	-4.36
S	18.54	19.22	56.92	14.36	18.59	30.1
E_m	4.37	4.53	13.41	3.38	4.38	7
t	—	—	1.14	—	—	0.62
P	—	—	0.286	—	—	0.556

$\Delta\%$: individual variations as a per cent of initial R_1 value.

Table 3. Mean pulmonary compliance (C_1 dyn), mean tidal volume (V_T), and mean respiratory frequency per min (f) before and after inhalation of pollen in normal monkeys and in monkeys passively sensitized with serum from grass-pollen sensitive patients

		Before sensitization			After sensitization		
		Inhalation of pollen			Inhalation of pollen		
		Before	After	$\Delta\%$	Before	After	$\Delta\%$
C_1 dyn (ml/cmH ₂ O)	<i>M</i>	7.86	8.91	+19.1	10.95	12.23	+12.3
	<i>S</i>	2.89	2.76	30.48	4.15	5.29	19.7
	<i>n</i>	11	11	11	10	10	10
	<i>P</i>	—	—	0.07	—	—	0.08
V_T (ml)	<i>M</i>	36.3	40	+12.6	40.8	40.8	+0.79
	<i>S</i>	12	13.1	27.2	18	19.2	22.65
	<i>n</i>	10	10	10	10	10	10
	<i>P</i>	—	—	0.196	—	—	0.92
f	<i>M</i>	47.3	52.3	+12.7*	50.1	64.6	28.6*
	<i>S</i>	11.7	14.2	11.8	11	18.1	22.9
	<i>n</i>	10	10	10	10	10	10
	<i>P</i>	—	—	0.008	—	—	0.004

$\Delta\%$: mean of individual variations as a % of the initial values; *n*: number of determinations.

* Significant variation ($P < 0.05$).

No clinical or auscultatory symptoms of dyspnoea were noted within the first hour after aerosol administration. In eight animals R_1 was measured 60 min after pollen administration. Individual and mean values of R_1 were not significantly different from the values observed before the bronchial challenge. Similarly, there was no significant change in the mean (%) variation of R_1 measured before and 60 min after allergen inhalation (Table 4). An aerosol containing pollen was insufflated directly under bronchoscopic control into the bronchi of four passively sensitized monkeys. R_1 was measured before and 15 min afterwards. Mean R_1 did not change (9.4 cm H₂O/l/sec) (Table 5). In one animal (21), however, an almost 100% increase in R_1 was noted after bronchial insufflation of pollen. Monkey 22 also showed a brief but definite increase (200%). However, this bronchial obstruction, which was accompanied by audible rhonchi, disappeared ($R_1 = 6.4$ cmH₂O/l/sec) immediately after bronchial aspiration.

Following inhalation of histamine. The effect of the inhalation of histamine on R_1 was studied in twelve monkeys before and after passive sensitization with human allergic serum. Fig. 3 is a typical example of a positive respiratory challenge observed in two normal and three sensitized animals. Such a positive test was characterized by the development of clinical symptoms of dyspnoea and sometimes of high-pitched rales. These symptoms tended to regress quickly following the administration of an aerosol containing a sympathicomimetic bronchodilator.

The inhalation of histamine but not of pollen was poorly tolerated by the monkeys, whether sensitized or not. As a rule, the animals struggled and fought to remove the mask. These reactions made it more difficult to record the various ventilatory para-

meters. Positive provocation tests were obtained more frequently in sensitized monkeys. This might have been due to the presence of reagins in the allergic serum used for passive sensitization, since the phenomenon was not observed after the administration of normal human serum.

Table 4. Pulmonary resistance (R_1) (cmH₂O/l/sec) in normal and passively sensitized monkeys before and 60 min after inhalation of grass pollen (2 mg)

Monkey	Before sensitization			After sensitization		
	Inhalation of pollen			Inhalation of pollen		
	R_1 before	R_1 60 min after	$\Delta\%$	R_1 before	R_1 60 min after	$\Delta\%$
9	85	81	-4.7	60	74	+23.3
	60	56	-6.7		—	—
10	—	—	—	30	39	+30
	—	—	—	74	57	-23
11	—	—	—	—	—	—
	36	27	-25	46	42	-8.7
12	22	29.4	+33.6	40	43.2	+8
	—	—	—	—	—	—
13	37	38.7	+4.6	21	26	+23.8
	34	26	-23.5	36	38.4	+6.7
14	70	75	+7.1	54.6	58.2	+6.6
	41.6	62	+49	—	—	—
15	42.6	42	-1.4	28.4	39.7	+39.8
	39.3	47.7	+21.4	22	18.8	-14.5
16	12.5	16	+28	21.6	25.4	+17.6
	—	—	—	14.6	20.3	+39
Mean	43.64	45.53	+7.5	37.35	40.17	+12.4
<i>S</i>	20.81	21	23.4	18.2	16.6	20.34
<i>E_m</i>	6.27	6.34	7	5.25	4.8	5.87
<i>t</i>	—	—	1.07	—	—	2.11
<i>P</i>	—	—	0.34	—	—	0.06

$\Delta\%$: individual variations as a % of initial R_1 value.

Discussion

The mean R_1 of normal rhesus monkeys, determined in thirteen animals weighing from 5 to 10 pounds, is 36 cmH₂O/l/sec (± 17.28). This value is similar to that obtained in the human newborn (Geubelle *et al.*, 1959). Our data, obtained primarily from young monkeys, tend to suggest that R_1 correlates negatively with body weight. Such a relationship is not unexpected since, in the growing monkey, weight is proportional to height; moreover, it is known that in children R_1 is a reciprocal function of height.

The R_1 in monkeys is subject to day-to-day variations which are not systematic but similar to those observed in healthy children ($10.24\% \pm 42.9$) (Geubelle & Vavrova, 1969).

Table 5. Effect of endo-bronchial insufflation of grass-pollen (2 mg) on pulmonary resistance (R_1) in anaesthetized, intubated and passively sensitized monkeys

Monkey	R_1 (cmH ₂ O/l/sec)		
	Before pollen	After pollen (10–15 min)	$\Delta\%$
11	7.5	7.5	0
12	14	15	+7.1
21	7	13.5	+93
22	9	6.4	-29
Mean	9.4	10.6	+17.78
<i>S</i>	3.2	4.3	52.52
<i>E_m</i>	1.6	2.14	26.26
<i>t</i>	—	—	0.68
<i>P</i>	—	—	0.534

$\Delta\%$: individual variations as a % of initial R_1 value.

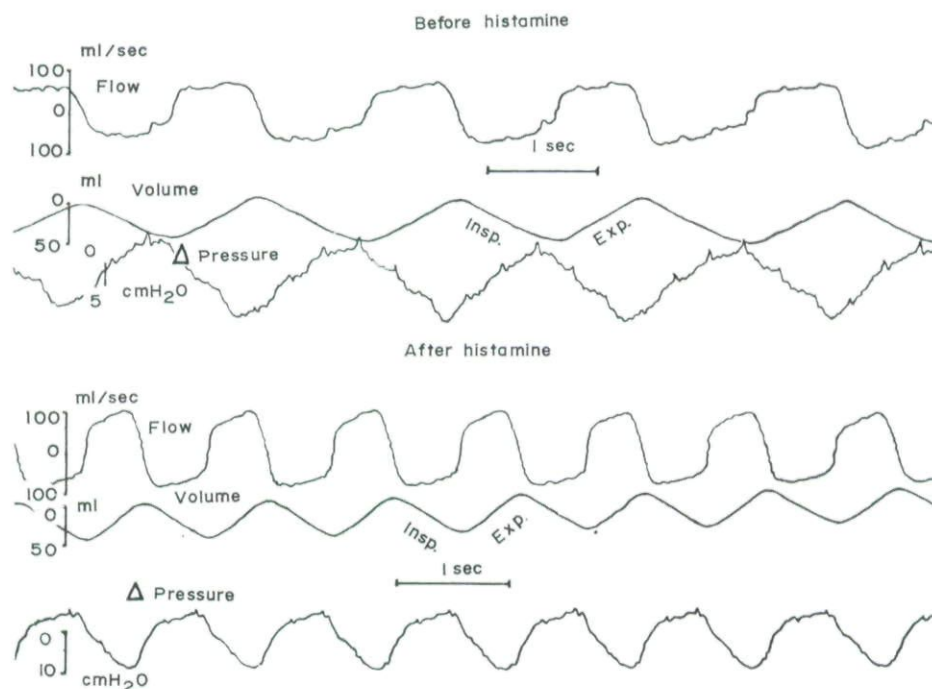


Fig. 3. Positive respiratory challenge with histamine in a normal monkey. The changes in intrathoracic pressure (cmH₂O), air flow (ml/sec) and tidal volume (ml) are simultaneously recorded before and during inhalation of an aerosol containing histamine (1 mg). The aerosolized drug induces tachypnoea and a twofold increase in the intrathoracic pressure changes which required a decrease in the sensitivity of the recording system. Since the air flow rate is not significantly modified, it is calculated that pulmonary resistances are doubled.

During inhalation of pollen or histamine, the additional dead space is about 30 ml. This is 2 or 3 times larger than the monkey's anatomical dead space if the latter is assumed to be similar to that of the human newborn (Strang, 1961). This situation is perfectly tolerated by the monkey breathing pollen. The increased respiratory rate consistently noted immediately after inhalation of pollen or histamine in normal or sensitized animals is most likely due to slight CO_2 retention. On the records obtained 1 hr later, polypnoea is no longer present. The hypercapnoea must be slight since no significant increase in tidal volume is observed.

The inhalation of pollen is perfectly tolerated by the monkeys passively sensitized to this allergen. Their R_1 , as in the case of normal unsensitized animals, does not change significantly 15 and 60 min following pollen administration. Several possibilities should be considered in view of these negative results:

(a) The amount of aerosolized pollen (2 mg) seems to be sufficient. This dose is much greater than that routinely used for bronchial challenges in asthmatic children. Under the same experimental conditions, less than 100 μg and sometimes even less than 10 μg of the same pollen extract is sufficient to provoke a 100% increase in R_1 in pollen-sensitive children (F. Geubelle and S. Leclercq, unpublished observations). Consequently, the amount of allergen given to the monkeys is at least 20 times greater than the usual threshold dose in asthmatic children.

(b) It is very unlikely that a bronchospastic reaction might have been overlooked in our experiments. Endo-oesophageal pressure, tidal volume and ventilatory flow rate were continuously recorded throughout inhalation and the records carefully watched for any increase in R_1 . Moreover, R_1 was measured at the end of aerosolization, i.e. 15 min after the start of the experiment. In the monkey this is more than sufficient time for a cutaneous or bronchial anaphylactic reaction to develop when the antigen is injected locally. Finally, animals were inspected and examined regularly during the first hour after inhalation: no sign of dyspnoea was observed and R_1 was not modified.

(c) The level of grass pollen hypersensitivity passively induced in our monkeys by i.v. injection of human reaginic serum is very close to that seen in allergic patients suffering from hay fever, as shown by the following findings:

(i) Positive skin tests were obtained in all sensitized monkeys with 1×10^{-5} and 1×10^{-6} pollen solutions. The amount of allergen injected under these conditions is similar to the threshold dose for a positive skin test in human beings strongly sensitive to pollen.

(ii) In six animals, the same pollen solutions were injected locally into the bronchial mucosa. In each case, a positive reaction of the same intensity as the cutaneous one was observed. These findings suggest that the level of sensitization of the bronchi is analogous to that induced in the skin.

(iii) Finally, severe anaphylactic reactions were provoked in the sensitized animals by i.v. injection of pollen.

From these findings, it may be concluded that the level of sensitization passively induced in monkeys by i.v. injection of reaginic serum seems to be of the same order of magnitude as that observed in pollen-allergic patients.

Aerosolized histamine (2 mg) administered to normal and sensitized monkeys may produce a definite increase in R_1 which is rapidly reversible after inhalation of a sympathicomimetic bronchodilator. The bronchospasm is usually accompanied by clinical symptoms of breathlessness and sometimes by high-pitched rales. Inhalation

of histamine, in contrast to pollen, is poorly tolerated by monkeys, whether sensitized or not, as suggested by their agitation. This intolerance may result from the vascular effects of the amine or its irritant action on the nasal mucosa. The restlessness of the animals during the inhalation of histamine made it more difficult to record the respiratory parameters and might account for the lack of reproducibility of some results. However, the bronchoconstrictor effect of histamine seems to have been more obvious in monkeys passively sensitized with reaginic serum. Fink & Schlueter (1969) have also observed that monkeys passively sensitized with serum from ragweed-sensitive patients are more sensitive to inhaled metacholine than a control group treated with normal human serum. These findings and our results suggest that sensitization of the respiratory tract by reagins increases its reactivity to certain chemical irritants.

Patterson and co-workers (Patterson *et al.*, 1965, 1967; Patterson, 1969; Miyamoto *et al.*, 1968) have observed that monkeys passively sensitized to ragweed or grass pollens develop dyspnoea and rhonchi immediately after bronchial challenge with the specific allergen. The level of passive sensitization attained in their animals seems to be analogous to that obtained in the present study, to judge from the P-K titre and the volume of serum used for passive sensitization and from the cutaneous and systemic reactivity to the allergen. Patterson's study does, however, differ from ours on the following points:

(1) Airway resistances were not measured. Patterson *et al.* relied on symptoms of dyspnoea, the occurrence of rales and decreased peak expiratory flow to indicate the presence of bronchospasm. In our experience, these criteria are neither as reliable nor as sensitive as the direct measurement of total pulmonary flow resistance.

(2) Animals were submitted to general anaesthesia and intubated. Both these conditions greatly promote the accumulation of secretions in the bronchial tree and trachea; we have regularly observed signs of dyspnoea in anaesthetized monkeys which were promptly relieved by the aspiration of bronchial secretions.

Our results show that, in the monkey passively sensitized to grass pollen, the inhalation of histamine may have a bronchoconstrictor effect whereas administration of an aerosol containing pollen has no effect on airway resistances in the first hour after the bronchial challenge. Indirect evidence merely suggests that an anaphylactic reaction between allergen and reagins, present in the bronchial mucosa, may be the cause of atopic asthma (Rose & Radermecker, 1971). Our results indicate that in the monkey reaginic sensitization of the bronchi *per se* cannot explain the occurrence of bronchospasm upon inhalation of the allergen. Other local or general factors may be required to produce the clinical expression of the bronchial anaphylactic reaction. Experiments are now in progress to study the effect of β -adrenergic blockade on the bronchial reactivity of monkeys.

Acknowledgment

This work was supported by grant No. 20118 from the Fonds de la Recherche Scientifique Médicale, Brussels, Belgium.

References

- BRYSON, V. (1947) Performance criteria for nebulizers in aerosol therapy. Meeting, American College of Allergists, Atlantic City, New Jersey.
- BUYTENDIJK, H.J. (1949) Oesophagus Druk en Long Elasticiteit. Thesis, Groningen, Oppenheim (ed.).

- FINK, J.N. & SCHLUETER, D.P. (1969) Passive transfer of metacholine sensitivity. Abstr. 28, Am. Ac. Allergy 25th Meeting; Bal Harbour.
- FLEISCH, A. (1956) Le pneumotachographe. *Helvetia Physiologica Pharmacologica Acta*, **14**, 363.
- GEUBELLE, F., KARLBERG, P., KOCH, G., LIND, J., WALLGREEN, G. & WEGELIUS, C. (1959) L'aération du poumon chez le nouveau-né. *Biologica Neonatorum*, **1**, 169.
- GEUBELLE, F. & VAVROVA, V. (1969) Compliance et résistance pulmonaires au débit aérien chez l'enfant sain. *Le Poumon et le Coeur*, **25**, 1085.
- LAYTON, L.L. & GREENE, F.C. (1964) Systemic allergic shock induced in monkeys passively sensitized by intravenous injection of human allergic sera. *International Archives of Allergy*, **25**, 193.
- MIYAMOTO, T., REYNOLDS, L.B., PATTERSON, R., CUGELL, D.W. & KETTEL, L.J. (1968) Respiratory changes in passively sensitized dogs and monkeys as models of allergic asthma. *American Review of Respiratory Diseases*, **97**, 76.
- PATTERSON, R. (1969) Laboratory models of reaginic allergy. *Progress in Allergy*, **13**, 332.
- PATTERSON, R., FINK, J.N., NISHIMURA, E.T. & PRUZANSKY, J.J. (1965) The passive transfer of immediate type hypersensitivity from man to other primates. *Journal of Clinical Investigation* **44**, 140.
- PATTERSON, R., MIYAMOTO, T., REYNOLDS, L. & PRUZANSKY, J. (1967) Comparative studies of two models of allergic respiratory disease. *International Archives of Allergy*, **32**, 31.
- RADERMECKER, M. (1971) Contribution à l'étude de la nature et du rôle pathogène des réagines. Thèse, Arscia éd., Bruxelles.
- ROSE, B. & RADERMECKER, M. (1971) The pathogenesis of bronchial asthma. *Immunological Diseases*, p. 859. Little Brown, Boston.
- SETERRE, J. & GEUBELLE, F. (1970) Measurement of endo-oesophageal pressure in the newborns. *Biologia Neonatorum*, **16**, 47.
- STRANG, L.B. (1961) Alveolar gas and anatomical dead-space measurements in normal newborn infants. *Clinical Science*, **21**, 107.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.