

From the Section of Allergy of the Department of Microbiology and Immunology of the Medical School of Sao Paulo and from the "Hospital das Clínicas" (Head: Ernesto Mendes), Sao Paulo, Brazil.

EXPERIMENTAL ASTHMA OF THE GUINEA PIG PROVOKED BY HUMAN LUNG TISSUE

By

ERNESTO MENDES

(With technical assistance of A. Germano da Silva)

Some workers in asthma and allergy believe that, very soon, bronchial asthma may assume a significant role in the picture of the so-called diseases due to autoaggression, to autosensitization or to autoantibodies. Such a prediction stems mainly from the clinical observation of a group of asthmatic patients, classified generally as intrinsic or essential, who, though behaving like those of the so-called allergic group, show no evidences extrinsic allergens causing symptoms. These concepts were rendered more promising when some confused problems of human pathology were explained by mechanisms of auto-immunization.

No experimental work on animals has been published with the sole aim of showing the possibility of autosensitization in bronchial asthma. For this reason, we decided to consider the possibility of inducing experimental asthma in guinea pigs through lung extracts, and to search in these animals for antibodies against pulmonary tissue.

MATERIAL AND METHODS

A) Experimental asthma in guinea pigs

Experimental asthma was elicited by introducing previously sensitized guinea pigs in an adequate glass container of 18

liters capacity, where an atomizer of the De Vilbiss type, no. 40, was installed, according to the technique already described in a previous paper (4).

Lung extracts were prepared as follows:

a) *Pure pulmonary extracts*

Lungs of human fetuses were obtained by surgery, under all aseptic precautions, from stillborn children, whose death was due to obstetric difficulties or to operative causes. Small pieces of pulmonary tissue were mixed with sterile saline solution and put in a Warren Blender grinder until they were rendered into extremely small particles. They were washed and centrifuged several times, according to the technic employed by Cavelti (1) in the preparation of organ extracts.

The extract of human foetal tissue was ground in a glass grail and washed 10 times in saline by centrifugation, always discarding the overflowing fluid. The sediment was diluted to 20 % in saline solution buffered with merthiolate at 1:10.000.

It was kept for 24 hours in the refrigerator at 5° C, and the sediment was washed three times more with saline through centrifugation. When the sediment was still reddish, it was washed by filtration in glass wool, 200 ml of saline solution being passed continuously. After centrifugation, the sediment was diluted to 20 % in saline buffered with merthiolate at 1:10.000.

One part was filtered through fine sterile gauze, and the other through a Seitz filter, being labelled respectively Lung Extract no. 1 and Filtered Lung Extract no. 2.

b) *Cultures of pulmonary extracts with bacteria from the respiratory tract discharges.*

Finely divided fragments of lung tissue, washed several times in saline, were added to other substances in a culture medium, in the following amounts:

Human foetal lung	5,0 g
Difco peptone	5,0 g
Sodium chloride	2,5 g
Glucose	5,0 g
Distilled water	500 ml

Several organisms obtained from respiratory tract discharges of 5 asthmatic patients, and grown in culture broth, were sown in this mixture, which was collected in 2 liter flasks.

After 72 hours at 37°, there was abundant growth of bacteria, which were sown in pure broth and sent to the Dept. of Bacteriology (Dr. Mello), where they were recognized as: non-hemolytic staphylococcus, yeasts and *B. subtilis* 50 ml of merthiolate no. 45 were added, to make sterilization; one portion was filtered through thin gauze, and the other through a Seitz filter; the first was called Cultured Lung Extract no. 3, the other, Cultured and Filtered Lung Extract no. 4.

c) Sensitization with lung extract

Sensitization was effected in 2 groups of guinea pigs with Cultured Lung Extracts no. 3 and 4 as follows:

A first group of 4 guinea pigs with an average weight of 450 g was submitted, on 9/11/56 and 16/11/56, to injections of lung extract by the intraperitoneal and intramuscular route (Table no. 1).

TABLE I

Group of 4 guinea pigs sensitized with lung extract and Freund's adjuvant and sprayed later with lung extract.

Sensitization with adjuvant	Sprays	Dyspnea
9/11/56		
16/11/56		
	30/11/56	-
	18/12/56	+ (2 guinea pigs)
	18/1/57	-
	31/1/57	-
	2/5/57	-

0.5 ml of Cultured Lung Extract no. 3 and 0.5 ml of Cultured and Filtered Lung Extract no. 4 were injected intraperitoneally.

1 ml of a mixture of 8 ml of a lung paste, cultured and filtered through gauze with 24 ml of Freund's adjuvant, was injected intramuscularly. This adjuvant was made to the following formula:

BCG dried and killed by heat	7.5 mg
Falva	4.5 ml
Mineral oil	7.5 ml

The second group of 8 guinea pigs, numbered from 1 to 8 and weighing an average of 400 g, was sensitized in the same way with 2 injections of 1 ml lung extract intraperitoneally and 1.25 ml of the lung-adjuvant mixture in 7/5/57 and 10/5/57 (Table no. 2). Five minutes after the second intraperitoneal injection of lung extract, two guinea pigs presented slight convulsions.

d) *Evocation of asthma spraying with lung extract*

All the 4 guinea pigs of the 1st group were put into an adequate glass container of 18 liters capacity. The lung extract no. 4 filtered through Seitz was sprayed by means of a De Vilbiss type apparatus no. 40, conveniently connected to the container.

Five sprays of 30 minutes duration were performed, the first one on 30/11/56 and, the fifth, nearly 5 months later, on 2/5/57 (Table no. 1).

Only after the spray of 18/12/56 did two guinea pigs show apparent dyspnoea.

A control group of 4 guinea pigs received a 5 % peptone spray for 30', and did not show any signs of bronchial spasms.

The second group of 8 guinea pigs was sprayed 5 times, from 8/5/57 to 3/1/58 (Table no. II). In the first sprays Cultured and Filtered Lung Extract no. 3 was used, at 1:50; in the last spray (3/1/58) pure Lung Extract no. 2 was used,

at 1:50 in saline solution with merthiolate prepared on 2/10/57. Only with the 4th spray, approximately 3 months after sensitization, did one of the 5 animals (guinea pig no. 4) show typical asthma; this occurred also in guinea pig no. 2 with the last spray. The spraying time varied from 30 to 40 minutes.

TABLE II

Group of 8 guinea pigs sensitized with lung extract and Freund's adjuvant and sprayed later with lung extract.

Sensitization		No. of guinea pigs	Spray	Dyspnea	Observations	Precipitins	
Intraperitoneal	Intramuscular with adjuvant					Gel diffusion	Ring
7/5/57	7/5/57						
10/5/57	10/5/57	8					
			31/5/57	—			
		5	1/8/57	—	Bleeding		Negative
			13/8/57	—			
			26/8/57	no. 4 +++			
			16/9/57				
			26/9/57		Bleeding	+ no. 1 & 4 w. lung and liver	
		3	3/1/58	no. 2 +++			
			pure extract				
			13/1/58		Shultz-Dale Guinea-pig no. 1, positive		

B) Assay of precipitins against lung

On 1/8/57, 2 cm blood were taken from the heart of 5 guinea pigs. The serum was withdrawn and put into the freezer at -5°C . On 12/8/57 the precipitins were determined by the ring method, using cultured lung extract and pure lung extract. The result was negative. (Table no. II).

On 26/9/57 another sample of blood was taken by puncture of the heart, and, on 2/10/57 and 17/10/57, precipitins were

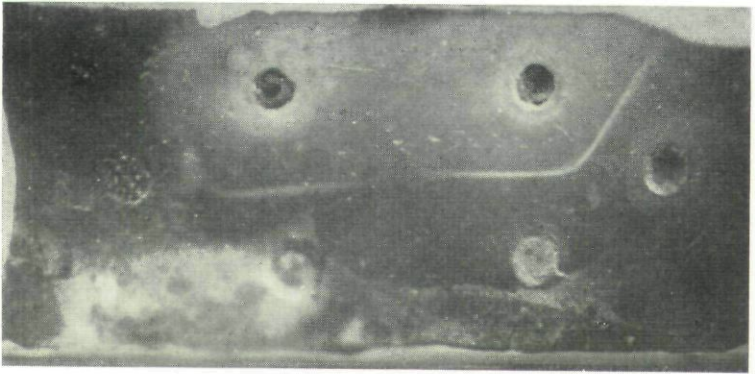


Fig. 3.

Gel-diffusion test on a glass slide.

Right: Band of precipitation, provoked by lung antigen and serum of a guinea pig sensitized against human lung.

Left: Band of precipitation, provoked by antigen of human liver and serum of a guinea pig sensitized against human lung.

assayed by the gel method of Ouchterlony, modified by Hartmann and Toilliez (3). Pure and cultured lung extracts nos. 1 and 4 were employed, and liver extract was used as control.

The extracts were buffered, showing a pH 7.

On 2/10/57, the precipitins were determined, by means of cultured lung extract. The sera of the guinea pigs nos. 1 and 4 showed lines of precipitation with liver and lung extract (fig. no. 3).

On 17/10/57, the sera of the same animals revealed precipitation reactions with pure lung extract (fig. no. 3).

The serum of the guinea pigs nos. 2, 3 and 5 did not present precipitation with any extract.

C) *Shultz-Dale test*

On 13/1/58, guinea pig no. 1 was killed by cranial concussion and the Shultz-Dale test was performed with 3 different portions of the ileum. Pure lung extract at 1:10 was used as antigen.

In all tests, a histamine solution of 1:1,000,000 was em-

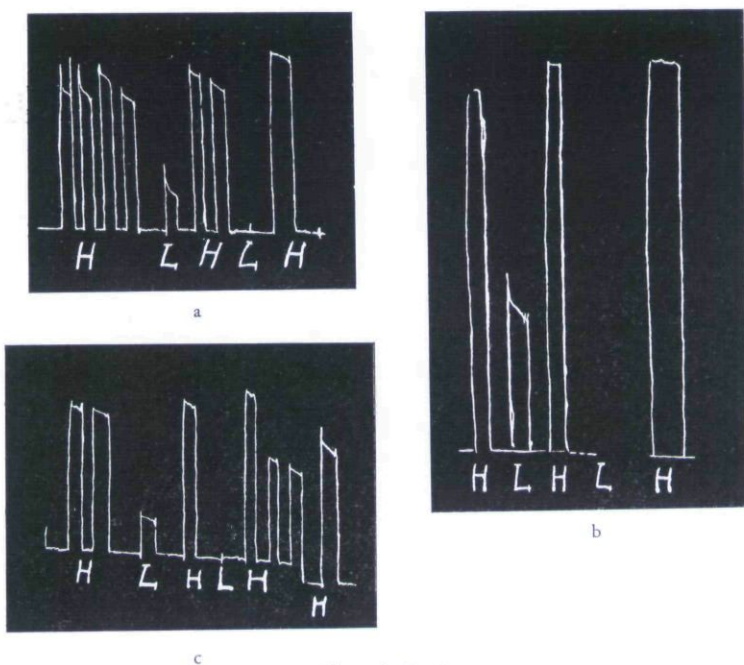


Fig. 4 (a, b, c).

Shultz-Dale test in three preparations of ileum of a guinea pig sensitized with human lung tissue. L = lung; H = histamine.

ployed, in order to verify the reactivity and the contractility of the intestine. In the three intestinal segments, contractions were obtained only with the first addition of the pulmonary antigen. Subsequent additions did not provoke contractions, evidencing immunological exhaustion, confirming the pharmacological reactivity of the intestine (fig. no. 4).

Conclusion: Positive Shultz-Dale Test.

DISCUSSION

In view of the results of these studies, one can admit the possibility of producing experimental asthma in guinea pigs with human pulmonary antigen. Sensitization, however, which may exteriorize itself by asthmatic crisis, was not very strong, as it is when potent antigens are used, like egg white. Indeed, in the

first group of 4 guinea pigs, we only provoked dyspnoea in 2 animals and only with the second spray (Table I).

It is true that these animals had been sensitized with small amounts of lung tissue, whose structure might besides have been changed by bacteria of bronchial discharges, since it served as a culture medium.

Pulmonary tissue, which works as a culture medium for common bacteria in the discharges of asthmatic patients, was used in order to approach most closely the theoretical conditions under which human lung tissue could sensitize the patient himself.

Peptone present in the culture medium could be responsible for the symptoms of bronchial spasms in this group of guinea pigs; this possibility was discounted for no symptoms at all were noticed in another control group where only peptone was sprayed.

The experiments with the second group of guinea pigs (Table no. II) seem to have proved that pulmonary tissue is actually able to sensitize guinea pigs, as demonstrated by the presence of precipitins, by the Shultz-Dale test and by the production of experimental asthma.

The symptoms of bronchial spasms, though obvious, were seen only in two guinea pigs. The presence of precipitins was realized also only in 2 of 5 guinea pigs when gel methods and pure lung extract were employed. The result was negative with the use of the ring method and of cultured lung extracts.

It is interesting to note that the serum of two animals with precipitins against human lung also showed lines of precipitation against human liver. Though the lines were not identical, for liver extract showed 3 lines in one preparation against 1 of lung, work in the future, should show which antigens are common to both organs.

The Shultz-Dale test, performed on one of the animals with a positive result (fig. 4), is another proof that guinea pigs may acquire sensitivity to human lung tissue.

Although there are several facts which favour sensitization

by pulmonary tissue, further work should decide the degree of specificity to lung extracts.

SUMMARY

Guinea pigs sensitized with human lung tissue can show symptoms of bronchospasm when subjected to sprays with the same antigen.

Extracts of human lung tissue, working as culture media for bacteria of the bronchial discharges of asthmatic patients, can also provoke bronchial spasm in sensitized guinea pigs.

Guinea pigs sensitized with human lung tissue can show serum precipitins against human lung evidenced by the gel diffusion tests.

A guinea pig sensitized with human lung tissue gave positive Shultz-Dale tests with pieces of ileum.

The possibility of group reactions with other organs may occur. The future trends in this field of research are discussed.

LITERATURE

1. *Cavelli, P. A. & Cavelli, E. S.*: Studies on the pathogenesis of glomerulonephritis; Production of auto-antibodies to kidney in experimental animals. *Arch. Path.*, 39, 148-152, 1945.
2. *Freund, J.*: Sensitization with organ specific antigens and the mechanism of enhancement of the immune responses. *J. Allergy*, 28, 18-28, 1957.
3. *Hartmann, L. & Toilliez, M.*: Micro-méthode d'étude en gélose de la réaction antigène-anticorps (variant de procédé d'Ouchterlony). *Revue Française d'Études Cliniques et Biologiques*, 2, 197-199, 1957.
4. *Mendes, E.*: Action of prednisone on experimental asthma of the guinea pig. *Acta Allergologica*, 11, 181-187, 1957.
5. *Ouchterlony, O.*: Antigen-antibody reactions in gels. *Ark. Kemi, Min. Geol. B* 26, 1, 1949.

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.