

Section of Allergy of the Department of Microbiology and Immunology and
Section of Allergy of the "Hospital das Clínicas". Faculdade de Medicina da
Universidade de S. Paulo, Brasil.

EXPERIMENTAL ASTHMA OF THE GUINEA-PIG INDUCED BY BRONCHIAL SPUTUM

By

ERNESTO MENDES

In a previous paper, we demonstrated the possibility of inducing experimental asthma in guinea-pigs by use of human lung tissue (1). Besides asthma, the animals developed antibodies against human lung.

Continuing the studies on the antigenicity of the bronchial sputum of asthmatic patients (2) it has been found that, mainly through the gel-diffusion tests, bronchial sputum not only presents antigens common to lung, liver, kidneys, erythrocytes, intestinal mucus and saliva, but also seems to present its own antigens (3).

For this reason, we decided to investigate if guinea-pigs sensitized by asthmatic patients' bronchial sputum are capable of developing experimental asthma and if they produced antibodies.

MATERIAL AND METHODS

Preparation of the sputum: the bronchial sputum from a patient with the diagnosis of intrinsic asthma with an A Rh-blood group was reduced to fine powder after drying in vacuum. It was then mixed with 25 per cent saline. After stirring, this mixture became homogenous and looked like sputum.

Sensitization

Twenty five per cent sputum in saline mixed with equal parts of Freund's adjuvant was injected intracutaneously in two doses of 1 ml each, with one

With the technical assistance of *A. Germano Silva*.

week's interval, in 10 guinea-pigs having an average weight of 450 g. A control group of 10 guinea-pigs was injected twice, with five days interval, by intraabdominal route with 10 ml of human plasma, A Rh-.

Experimental asthma

The technique described in a previous publication (4) was used. The animals were placed in an appropriate apparatus with a capacity of 18 liters, and they were sprayed with a De Vilbiss No. 40 nebulizer.

The bronchial sputum diluted to 2 per cent was nebulized for 10 to 20' in 12 sessions with intervals ranging from 4 to 11 days. The degree of dyspnea was evaluated in the following way: (+) tachypnea; (++) dyspnea; (+++) dyspnea and convulsions; (++++) death after intense dyspnea.

Schultz-Dale test

Schultz-Dale test, using the ileum, was performed in 3 guinea-pigs, 71, 91 and 93 days after the last sensitizing injection.

Passive Schultz-Dale test

The ileum of a normal guinea-pig was incubated for one hour at 37° C with slices of spleen material taken from a sensitized guinea-pig, mixed with equal parts of Tyrode's liquid. Schultz-Dale test was performed in this passively sensitized ileum.

Gel-Diffusion test

The micromethod on microscopic slides was used (5), each basin measuring about 4 mm and the distance between them being 5 mm.

The gel-diffusion test was performed using: 1) sera of 3 guinea-pigs sensitized with sputum whose blood was collected 3 months after the sensitizing injections; 2) sera of guinea-pigs sensitized with human plasma collected one month after the sensitization. Part of the sera underwent absorption with human ABO red cells.

As antigen we used the same 2 per cent bronchial sputum, 2 per cent human fetal lung tissue in saline triturated in the Waring-blendor and 1 per cent human plasma. In some circumstances the gel-diffusion test was performed using a mixture of plasma and agar or sputum and agar, according to the technique of Björklund (6).

Intracutaneous test

0.10 ml of 10 per cent bronchial sputum was injected intracutaneously in a sensitized and in another normal guinea-pig. The test was performed about 3 months after sensitization.

TABLE I
Results of experimental asthma, Schultz-Dale's, gel-diffusion and intracutaneous tests.

Date	Guinea pigs	Sensitization	Asthma	Schultz-Dale	Gel-diffusion	Cutaneous test
Dec/12/60	10	{ 1 ml of sputum and 1 ml of Freund's adjuvant Same injection	...	...	...	...
Dec/19/60	10		0	...	...	...
Jan/3/61	10		...	...	...	...
Jan/10/61	10		...	...	...	...
Jan/16/61	10		...	...	...	...
Jan/23/61	10	{	...	...	...	...
Jan/27/61	9		2 +	...	}	...
Feb/1/61	9		2 + +	...		...
Feb/8/61	9		0	...		...
Feb/17/61	9		2 + +	...		...
Feb/17/61	9		1 + +	...		...
Feb/24/61	5	...	4 + +	...	...	...
Mar/3/61	8	...	4 + +	...	...	...
Mar/14/61	5	...	3 + +	...	...	...
Mar/21/61	1	...	...	+	6 bands with sputum 2 band with lung	...
Mar/23/61	1	...	...	+	2 bands with sputum 1 band with lung	...
Mar/23/61	1	...	...	+	4 bands with sputum 1 band with lung	...
Mar/23/61	1 (normal)	...	...	+	...	...
Mar/24/61	1	...	...	...	...	...

RESULTS

The results are condensed in table I.

Experimental asthma.

The guinea-pigs showed signs of dyspnea only 38 days after the sensitization and during the 6th nebulization.

The intensity of the asthma was moderate dyspnea (++) . The control group sensitized with human plasma, was nebulized only once, with 1 % human plasma and the guinea-pigs showed severe dyspnea (+++) after 1 minute.

Schultz-Dale test.

Schultz-Dale test was positive (+) in the animals sacrificed for this purpose (Fig. I).

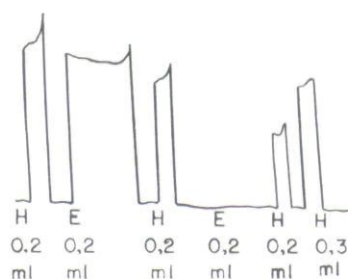


Fig. I.

Schultz-Dale test positive using the ileum of a sensitized guinea-pig.

E = Sputum diluted from 1/100 to 1/400.

H = Histamine phosphate 1/400,000.

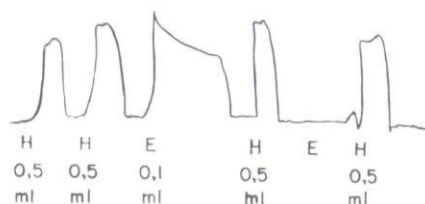


Fig. II.

Passive Schultz-Dale test. Ileum of a normal guinea-pig incubated with spleen material taken from a sensitized guinea-pig.

This test was also positive when the ileum of a normal guinea-pig was sensitized passively with fragments of spleen taken from a previously sensitized animal (Fig. II).

Gel-Diffusion test.

The number of precipitation bands was not identical in the 3 sera. The bronchial sputum showed 6 precipitation bands in one serum and 4 and 2 bands in the other two sera, respectively (Table I).

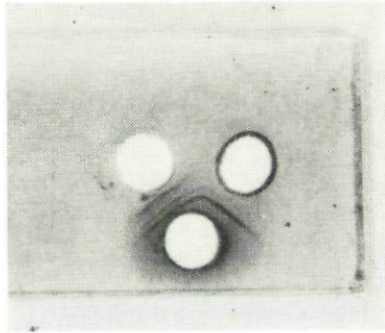


Fig. III.

Gel-diffusion test. Right: serum of guinea-pig and sputum. Left: serum of guinea-pig and lung. A precipitation band with identity reaction is observed.

The serum that showed 6 precipitation bands with sputum produced 2 precipitation bands with lung, while the other two sera presented only 1 precipitation band with lung.

A precipitation band with identity reaction with lung and sputum was observed (Fig. III).

Control group:

The guinea-pig sera anti-human plasma produced 3 lines of precipitation with human plasma but did not show bands of precipitation with 3 different sputum (Fig. IV).

Figure V shows the reactions among the guinea-pig serum anti-sputum against human plasma and 3 different sputum. The sputum showed 4 precipitation lines and only 1 with hu-

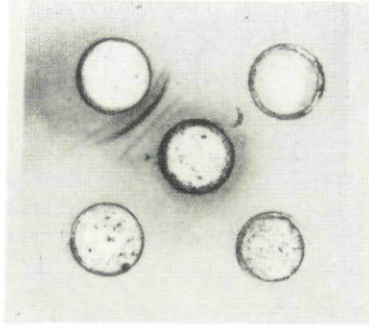


Fig. IV.

Center: guinea-pig serum anti-human serum.
 Left: human serum.
 Right and lower basin: 3 different sputum.

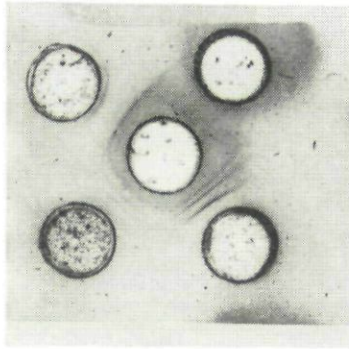
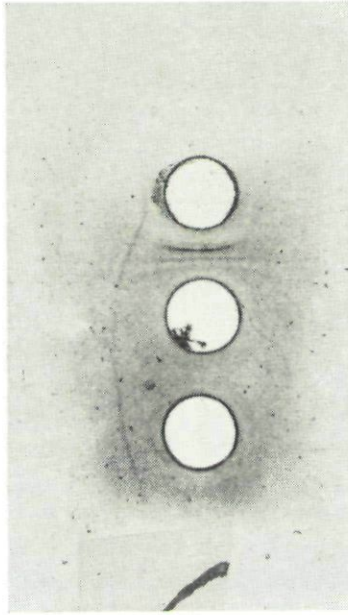


Fig. V.

Center: guinea-pig serum anti-sputum.
 Upper basin right: human serum, with 1 precipitation line.
 Upper basin left: sputum, with 2 precipitation lines.
 Lower basin: two different sputum showing to the left 3 lines
 and to the right 4 lines.

man plasma. Besides, there was not absolute identity between human serum and sputum. Figure VI shows the gel-diffusion technique according to *Björklund*, between human plasma and guinea-pig serum anti-human plasma. The agar contains 20 % of sputum. The sputum did not prevent the reaction. Figure VII shows the reaction between guinea-pig

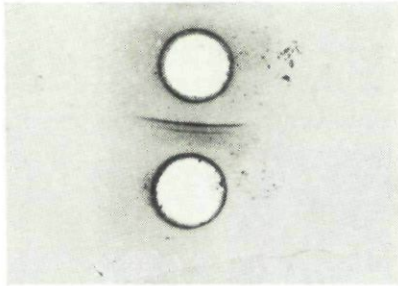
*Fig. VI.*

Center: guinea-pig serum anti-sputum.

Upper basin: sputum with 3 precipitation bands.

Lower basin: human serum, without precipitation bands.

The gel used in this test was mixed with 20% of human serum. Observe the persistence of 3 precipitation lines with sputum and absence of precipitation with human serum.

*Fig. VII.*

Upper basin: human serum.

Lower basin: guinea-pig serum anti-human serum.

The gel used in this test was mixed with 20% of sputum.

Observe 2 precipitation lines.

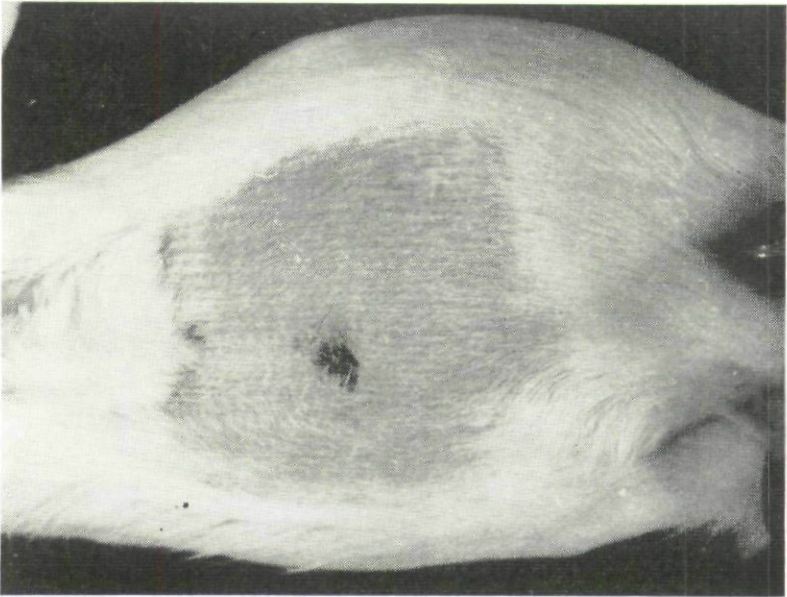


Fig. VIII.

Delayed cutaneous reaction performed with sputum about three months after sensitization.

serum anti-sputum against sputum and human plasma. The agar contains 20 % of human serum, The human plasma did not prevent the reaction.

Intracutaneous test.

One of the sensitized guinea-pigs showed a typical delayed reaction (48 hours) after intracutaneous injection of bronchial sputum. The normal control guinea pig presented only a slight erythema. This test was made about 3 months after the sensitization (Fig. VIII).

DISCUSSION

Bronchial sputum of asthmatic patients is antigenic for guinea-pigs and it is possible to produce experimental asthma in sensitized animals. The immunological mechanism of experimental

asthma was proved by the presence of precipitins, Schultz-Dale's test and delayed cutaneous reaction. The passive transfer of sensitization to the normal animal could be demonstrated in an ileum fragment from a normal guinea-pig, after previous contact with the spleen of a sensitized animal.

Bronchial sputum contains small amounts of albumin and other protein fractions of human serum, which could be revealed by immunological technique (8).

It is obvious to suppose that the bronchial sputum of any infection, possesses the capacity of sensitizing laboratory animals, like rabbits.

However, contrarily to what one might think, serum albumin contained in the bronchial sputum is not fundamental for the sensitization of guinea-pigs, due to the following reasons:

1) Sera from guinea-pigs sensitized with human serum yielded 3 to 4 lines of precipitation with the homologous serum whereas no reaction was observed when the same guinea-pig sera reacted with 3 different sputum (Fig. IV). Thus, a diverse antigenicity between sero-albumin and bronchial sputum was demonstrated.

2) A similar fact was observed with the anti-sputum guinea-pig serum, which, by reacting in the same slide against human serum and various sputum samples showed 4 lines of precipitation with the sputum and only one with the human serum (Fig. V).

3) By gel-diffusion, using *Björklund's* technique, after mixing 20 % of human serum to the gel, the anti-sputum guinea-pig serum did not lose its 3 lines of precipitation on reacting with the sputum (Fig. VII).

This fact means that in the guinea-pig serum, certain anti-sputum antibodies are formed which are not neutralized by human serum, therefore, they do not present an immunological relationship to it.

4) The opposite is also true; the guinea-pig serum against human-serum did not lose its precipitation lines with the homologous antigen when sputum was mixed with the gel. This

shows that antibodies against the human serum were not precipitated and neutralized by the sputum (Fig. VI).

5) We observed (3), in a previous paper,, that anti-sputum serum (produced in rabbits) causes, with the homologous antigen, at least 7 or 8 bands of precipitation and only 3 bands with some human sera, demonstrating that in the bronchial sputum there are from 4 to 5 antigens capable of sensitizing which are not common to sero-proteins.

6) The anti-sputum serum produced in rabbits reacted only with 1/3 of various human sera. If the antigen responsible for the sputum antigenicity were exclusively ascribed to albumin-serum, it is obvious to suppose that all human sera should produce a precipitation reaction with the antibody serum, and not only 1/3.

7) Finally, by immunoelectrophoresis we succeeded in demonstrating that in bronchial sputum there are various antigens which do not present any similarity with sero-proteins (8).

All these facts suggest that there are antigens in the sputum which are not common to sero-albumin; the same occurs with saliva as *Ellison et colab.* (7) demonstrated by means of immunoelectrophoresis the existence of specific antigens.

Reaction of identity between lung and bronchial sputum were seen only in the serum that showed 6 precipitation bands.

This fact can be explained because it is well known, according to *Kabat & Mayer* (9) that "the quantity of cross reacting antibody may vary from various individuals of the species immunized, as well as in serum samples obtained from a single animal after varying times of immunization". The antigenic relations between the bronchial sputum and other organ had already been discussed by *Mendes et al.* (3) using sera of rabbits immunized by bronchial sputum.

Various investigations are being made in order to find out about the real nature of the antigens of sputum and whether they present a different immunological behaviour in various respiratory infections.

SUMMARY

1. Experimental asthma was produced in guinea-pigs sensitized by bronchial sputum from an asthmatic patient.
2. The ileum of these guinea-pigs contracted in contact with the bronchial sputum, as seen in the Schultz-Dale test. The same result was obtained with the ileum of normal guinea-pig passively sensitized by spleen of a sensitized guinea-pig.
3. In the sera of these animals, anti-bronchial sputum precipitins were demonstrated by the gel-diffusion technique. These antibodies were not common to antibodies against human serum.
4. Delayed cutaneous reaction was also observed with an intracutaneous injection of bronchial sputum.

REFERENCES

1. *Mendes, E.*: Experimental asthma of guinea-pigs provoked by human lung tissue. *Acta Allergologica* 12, 231-239, 1968.
2. *Bukantz, S. C. & Berns, A. W.*: Studies with sputum. I. Initial observations on the chemical nature and blood group substance content of asthmatic sputum. *J. Allergy* 29, 29-43, 1958.
3. *Mendes, E., Ottensooser, F., Silva, A. G., Lacaz, C. S. & Cintra, A. B. U.*: Antigenicity of asthmatic sputum. *Allergie und Asthma* 7, 197-208, 1961.
4. *Mendes, E.*: Action of prednisone on experimental asthma of guinea-pig. *Acta Allergologica* 11, 181-187, 1957.
5. *Mendes, E. & Silva, A. G.*: Micrometodo de difusion en gel sobre portaobjetos. *Rev. Argentina Alergia* 4, 51-54, 1959.
6. *Björklund, B.*: Immunologic studies of rabbit bone-marrow with the aid of gel-diffusion and serologic techniques. *Int. Arch. Allergy* 4, 340-359, 1953.
7. *Ellison, S. A., Mashimo, P. A. & Maudel, I. S.*: Immunochemical studies of human saliva. I. The demonstration of serum protein in whole and partial saliva. *J. D. Res.* 39, 892-898, 1960.
8. *Mendes, E., Strauss, A., Ferri, R. G. & Cintra, A. B. U.*: Immunochemical studies of asthmatic sputum (in press).
9. *Kabat, E. A. & Mayer, M. M.*: Experimental immunochemistry. Springfield, Charles C. Thomas, 1961. p. 342 and 647.

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.