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A STUDY ON BACTERIAL SENSITIZATION IN THE INFECTIVE ASTHMA

By

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Infective asthma is regarded as a bronchial asthma syndrome which starts and develops on obvious relation to infections of the respiratory organs. Infective asthma can occur independently or with exogenous allergic bronchial conditions (mixed infective and exogenous allergic asthma). It can also co-exist with chronic bronchitis, in which case there is the possibility of bronchial, peribronchial and alveolar alterations which constitute the anatomical substrate of the pulmonary emphysema (infective asthma complicated by bronchitis, bronchiolitis and pulmonary emphysema) (*Ricci et al.* 1963).

As *Zanussi & Mazzei* say (1961), it is sometimes very difficult "to distinguish on the clinical plane between infective asthma and the other infectious respiratory symptoms capable of sustaining a similar symptomatology, usually presenting all the symptoms of asthmatic bronchitis". *Herxheimer* (1961) and *Orie* (1961) too stress the difficulty of discriminating, both on the clinical and etiopathogenical plane, between the various asthmatic forms of bronchitis.

And yet differences of considerable significance from the pathological point of view have been shown between bronchitis and asthma. Above all, a complex of inflammatory, degenerative processes affecting the terminal and respiratory bronchioles has been ascertained in cases of bronchitis, whereas in asthma no analogous histological changes have been ob-

served in segments so far from the bronchial tree (*Gough* 1961, *Gough & Wentworth* 1960). Moreover, whilst the development of bronchial and peribronchial phlogosis in chronic bronchitis often takes the form of emphysema initially centrolobular and subsequently diffuse, studies of asthmatics who died from status asthmaticus have merely shown the alveoli are distended, destructive changes being absent, and the bronchial tubes are obstructed by an abundance of mucus clinging stubbornly to the walls (*Williams* 1961, *Williams & Leopold* 1959).

For some time now, there has been discussion on the hypothesis that sensitization of the bronchial tissue to bacterial allergens may constitute the most important etiopathogenic element in the infective asthma syndrome. Attempts have been made to reproduce in man asthmatic crises by making the subject inhale suspensions of standard organisms (*Hajos* 1960), or organisms isolated from bronchial secretions (*Ricci et al.* 1961, *Ricci* 1963), or bacterial extracts prepared from cultures of *Neisseria catharralis* isolated from sputum (*Hampton et al.* 1963). The concept of sensitization to substances derived from bacteria disregards the pathogenic powers of the organisms themselves; indeed, it has been proved possible to sensitize with saprophytes (*Dutton* 1956, *Morrow et al.* 1956).

Present interest in this problem was recently emphasized anew by *Hampton* (1964), from both the clinical and the etiopathogenic points of view.

The object and scope of our study was to examine cases of infective asthma with a view to establishing possible sensitization to substances of microbial derivation, using the flora isolated from the bronchial secretion of the patients themselves.

MATERIAL AND METHODS

The study was conducted with 113 subjects of both sexes, 69 of them being suffering from infective asthma and 44 with combined infective and exogenous asthma.

These subjects, who were examined when free from their asthma, were made

to inhale from an intermittent positive pressure inhaler, a mixture consisting of a suspension of organisms (10 million to the millilitre) and the respective filtrates of broth culture in equal parts. The organisms were isolated from the sputum and bronchoscopic aspirates of each patient, using common and individual culture plates. The isolated organisms were subsequently cultivated in a soya trypticase broth for 5 days, then filtered.

The aerosol was given for 15 minutes. The results were considered expressive of "immediate bronchostenotic reaction" whenever the spirographic examination conducted after 15-30 and 45 minutes revealed a reduction in quotient $\frac{F. E. V.}{v. c.}$ equal to or more than 15 per cent in relation to the basic values; the crises which occurred between the 1st and 16th hour of inhalation were considered "delayed". (This is not to be taken in its immunological sense, but merely as a chronological indication). In those cases which produced positive results, a check was carried out by having the patient inhale a mixture consisting of a 0.5 per cent formulated physiological solution and the culture medium. Checks were also carried out by means of bronchial tests conducted with heterogeneous proteins (bovine serum albumin), which always produced negative results.

TABLE I
Table of Case Material
Number of Cases: 113

Type of asthma	Number of cases	Positive to bronchial tests		Chronology of asthmatic reactions		
		No of cases	Per-cent age	Imme-diate only	Imme-diate & de-layed	De-layed only
Infective	69	24	39 %	3	13	8
Infective and exogenous allergic	44	13	29 %	3	4	6

Those tests which produced positive results spirographically were always attended by subjective and objective symptoms of bronchostenosis.

To confirm the specific validity of each test, the inhalation was repeated with the same bacterial strain at least 24 hours after the initial session. The result was considered definitely positive when both tests produced a bronchostenotic reaction with the same characteristics. The inhalation sessions with the various bacterial strains isolated from bronchial secretion were conducted at intervals of at least 24 hours.

TABLE 2
Bacteriological Results of an Examination of the Sputum and Bronchoscopic Aspirates of 113 Patients Suffering from Infective Asthma.

Bacterial strain	Incidence resulting from cultural examination of the bronchial secretion of (113 cases)	Positive results of bronchial tests induced by the various strains (37 cases)
Alpha-haemolytic streptococcus	77	17
Neisseria	69	9
Haemolytic Staphylococcus		
aureus (coagulase +)	26	5
Candida albicans	12	—
Non-haemolytic Staphylococcus		
albus (coagulase —)	11	—
Escherichia coli	11	3
Non-haemolytic Streptococcus	7	—
Klebsiella	8	3
Enterococcus	4	—
Haemolytic Staphylococcus		
aureus (coagulase +)	3	1
Proteus mirabilis	2	1
Non-haemolytic Staphylococcus		
albus (coagulase +)	1	—
Alkaligenes	1	1
Pseudomonas aeruginosa	1	—

NB. In three cases, the tests yielded positive results with two different strains.

RESULTS

The bronchial tests produced positive results in 39 per cent of the infective asthma cases and in 29 per cent of the combined infective and exogenous allergic cases. (See Table 1). In 50 cases of chronic bronchitis studied by the same methods, we failed to find a single case of positive reaction to the inhalation test with bacterial allergens. Most of the

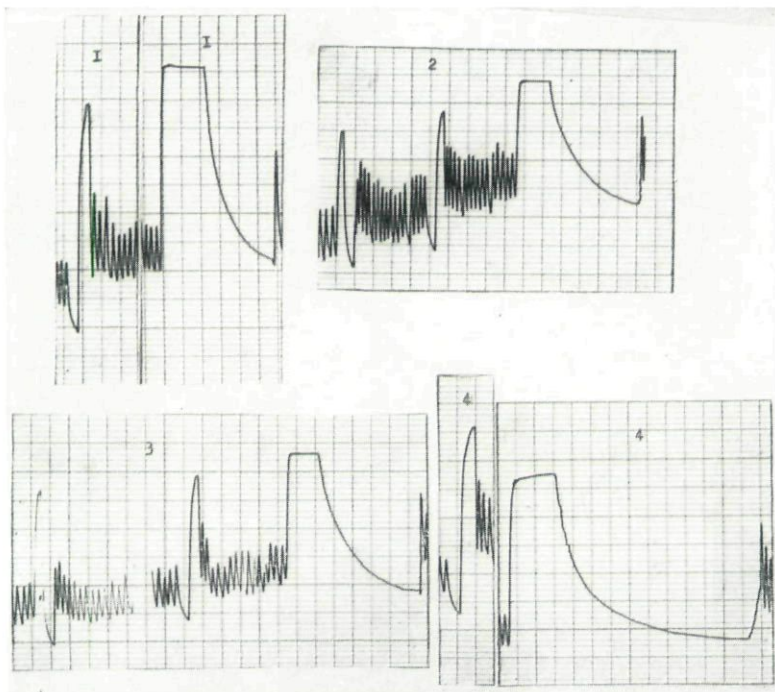


Fig. 1.

B. A., male 33; infective asthma.

1. Initial examination: VC = 4400 ml; FEV₁ = 2640 ml; P. E. = 60 per cent.
2. 5 min. after inhalation of alpha-hemolytic streptococcus isolated from bronchial secretions: VC = 2640 ml; FEV₁ = 1320 ml; PE = 50 per cent.
3. 15 min. later: VC = 2860 ml; FEV₁ = 1430 ml; PE = 50 per cent.
4. 24 hours later: VC = 3630 ml; FEV₁ = 1706 ml; PE = 48.5 per cent.

VC = vital capacity.

FEV₁ = first second forced expiratory volume.

PE = percentage expired ($= \frac{\text{FEV}_1}{\text{VC}}$).

asthmatic reactions were immediate or delayed; in a few of these cases, the asthmatic symptoms took a continuous/ sub-continuous course; in others they were diphasic, with abatement and consequent recurrence of the crises.

Table 2 gives the frequency with which the various bacterial strains were met with in sputum and in bronchoscopic aspir-



Fig. 2.

C. S., male 52, infective asthma.

1. Initial examination: VC = 3960 ml; FEV₁ = 2750 ml; PE = 69.4 per cent.
2. 15 min. after inhalation of *Neisseria* isolated from bronchial secretions: VC = 3520 ml; FEV₁ = 1980 ml; PE = 56.2 per cent.
3. 30 min. later: VC = 3960 ml; FEV₁ = 2310 ml; PE = 58.3 per cent.

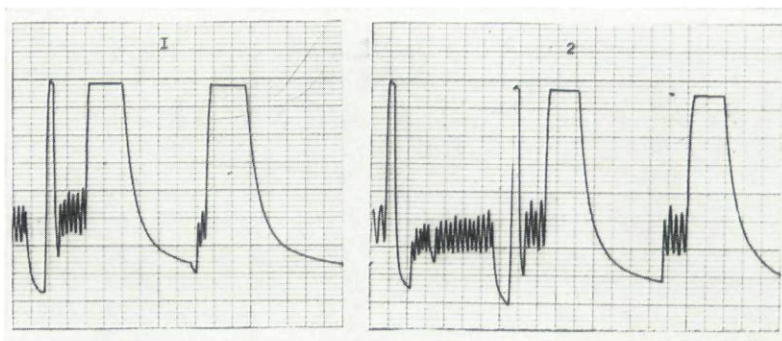


Fig. 3.

P. E., male 42, infective asthma.

Control with physiological formolated (0.5 per cent) solution combined with culture medium.

1. Basic examination: VC = 4235 ml; FEV₁ = 2640 ml.
2. 30 min. after inhalation of physiological formolated (0.5 per cent) solution combined with culture medium: VC = 4290 ml; FEV₁ = 2640 ml.

ates; also records the percentage of positive asthmatic reactions to the various strains.

In the great majority of cases, cultural examination of the bronchial secretion revealed the presence of flora made up of

several bacterial strains (2, 3, and, in a few rare cases, 4). In the same patients, however, with the exception of three cases, the inhalation test showed a positive reaction to only one of the strains. The three exceptions were found to show positive reaction to 2 different strains. Figs. 1 and 2 show typical spirographic modifications following bronchial testing. Fig. 3 shows the results in a control test.

DISCUSSION

It does not seem possible that the asthmatic reactions induced with the methods outlined above can depend upon random or casual factors considering the following results:

1) In no case was any significant bronchostenotic reaction recorded when the patient inhaled the mixed physiological solution and germ culture medium or the heterogeneous protein solution.

2) In those patients suffering from infective asthma, the test produced a positive result in the case of only one of the bacterial strains.

3) Where positive reaction was recorded, the result was confirmed in a second test.

4) We never obtained positive results in any case of chronic bronchitis, although the test was carried out along the same lines.

5) We think we can exclude the possibility of bronchoactive substances present in the filtrates being pathogenically responsible, since the pharmacodynamic tests carried out with the filtrates themselves failed to reveal histamine or acetylcholine in any appreciable quantity. These tests were carried out biologically, by applying increasing doses of filtrate to the isolated intestine of guineapig, *i.e.* the same doses which, during our tests, caused a bronchostenotic reaction¹.

¹ The biological tests to determine the presence of pharmacodynamically active substances in the broth culture filtrates were conducted at Prof. Gordonoff's laboratory, Pharmacological Institute of the University of Berne.

Parallel to our bronchial tests, we studied, in 30 of the 113 cases examined, the possible presence of precipitating antibodies, employing Ouchterlony's method and using the broth culture filtrates as antigens. The results obtained were consistently negative.

In the same way that bronchial tests with exogenous allergens cause asthma, the asthmatic reactions with bacterial strains isolated from bronchial secretion may be taken to indicate sensitization to microbial allergens present in the respiratory tract. With this method, we believe that we can obtain a diagnostic pointer as indicated above.

SUMMARY

The authors carried out bronchial tests on 113 asthmatic patients, 69 with infective asthma and 44 with combined infective and exogenous asthma, using fractions prepared from organisms isolated from the sputum and bronchoscopic aspirates of the patients themselves. The tests were positive to one of the organisms isolated from the bronchial secretion in 39 per cent of the infective asthma cases and in 29 per cent of the combined infective and exogenous allergic asthma cases. That means that the aerosol, made up of material of bacterial origin, provoked an immediate and delayed bronchostenotic reaction.

The biological tests to determine the presence of pharmacodynamically active substances in the bacterial filtrates showed no evidence of any sufficient quantities of histamine or acetylcholine, at least not in such quantity as to provoke by themselves a bronchostenotic reaction.

The investigation in some of these cases of asthma to determine the presence of precipitating antibodies according to the Ouchterlony method, using the filtrates of broth culture as antigens, has yielded negative results. The bronchial tests carried out on collateral casuistics of chronic bronchitis have constantly given negative results.

ZUSAMMENFASSUNG

Bei 113 Asthmatikern, wovon 69 an infektiösem Asthma und 44 an exogenem Asthma litten, wurden Bronchialteste vorgenommen. Die Tests wurden mit Bakterienfiltraten durchgeführt, die aus Keimen gewonnen wurden, die aus dem Sputum und dem Bronchialsekret dieser Patienten stammen. Bei 39 Prozent der Patienten mit infektiösem Asthma und bei 29 Prozent der Fälle mit kombiniertem – infektiösem und exogen-allergischem Asthma – waren die Tests auf einen der isolierten Keime positiv. Das Aerosol, hergestellt aus diesem bakteriellen Material, hat also eine bronchostenotische Sofort- und Spät-Reaktion hervorgerufen.

Die Anwesenheit von Histamin oder Acetylcholin in den Bakterienfiltraten – wenigstens in solchen Mengen, dass sie selbst bronchostenotische Reaktionen auslösen könnten – konnte durch pharmakologische Untersuchungen ausgeschlossen werden.

Seroprazitierende Antikörper konnten mittels der Ouchterlony-Technik nicht nachgewiesen werden.

Bronchialteste bei einer parallelen Serie von Patienten mit chronischer Bronchitis ergaben durchwegs negative Resultate.

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