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CLINICAL STUDIES ON THE ROLE OF SEROTONIN IN BRONCHIAL ASTHMA

By

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INTRODUCTION

In a previous work we concluded that bronchial testing should be introduced regularly in the routine examination of bronchial asthma, since the most useful human experiments are those using an aerosol of the specific antigen or some other substance believed to influence the antigen-antibody reaction (AAR). Bronchospastic reactions in bronchial asthma are conditioned by the two processes: 1) pulmonary allergy responsible for the release of bronchoconstrictive mediators; 2) increased pulmonary sensitivity of the effectors to these physiological (acetylcholine) and pathological (histamine) substances. According to *Tiffeneau* the cholinergic and histaminic hypersensitivity determines the fundamental pathomechanism of the AAR, it develops with the disease and has a tendency to be prevalent. The pharmacologically active substances released in the AAR are histamine (H), acetylcholine (ACh), heparine, plasma kinines, slow-reacting substances (SRS-A), permeability factors, leukotaxine and serotonin (S).

The role of S in the anaphylactic processes has been considered problematic. The type of anaphylactic response may depend in part upon the site of S release. *Lecomte* found that sensitized guinea-pig lungs liberated only a small amount of S when exposed *in vitro* to the antigen. According to *Brocklehurst*, S has a tendency to relax *in vitro* bronchial muscles. *Cohen* found that endogenous S had been involved in patho-

physiological reactions resulting in hypersensitivity vasculitis of the small pulmonary arteries and arterioles. *Waalkes* stated that S was released *in vitro* from rabbit platelets after the intravenous injection of the antigen into the sensitized rabbit. The action of S on the respiration is transmitted by chemical receptors. *Lévy* found a direct action on the motor receptors and an indirect action through release of parasympathicomimetic and sympathicomimetic substances. S is furthermore thought to be an endogenous substance, or genuine hormone, released *in vivo* during the AAR, yet the diverse clinical symptoms are not inhibited by specific S antagonists. The reason for the divergent findings concerning the role of S in the anaphylactic processes is to be found in the fundamental differences in the mechanism of the anaphylactic reactions of the various animal species examined.

Brocklehurst believes that SRS-A, a slow-reacting substance may be regarded as a major causative agent in asthmatic bronchospasm, as it is produced *in vitro* by the human sensitized lung with prolonged action. The only antagonist so far to support this theory is homochlorcyclizine and the conclusions depend on the exclusion of other agents.

Evidence of the hypothetical role of S in allergic diseases is so far based on *in vitro* experiments. The present study was performed in order to find out whether S acts as a chemical mediator liberated in the course of the AAR and whether bronchial testings with S aerosol is of diagnostic value in bronchial asthma. We approached the problem in three ways:

1. Direct exposure to a S aerosol.
2. Direct inhibition of the S-induced bronchospasm with antiserotonine and antihistamine substances.
3. Examination of the therapeutic effect of inhalations with antiserotonine drugs.

MATERIALS AND METHODS

Selection of patients.—We examined altogether 321 patients: Group I: 246 asthmatics. Group II: 36 cases of various

allergic diseases other than asthma, (vasomotor rhinitis, hay-fever, urticaria). Group III: 39 non-allergic controls (emphysema, chronic bronchitis, pneumonia, silicosis, cardiovascular diseases, gastric ulcer, cirrhosis of the liver).

Bronchial testing.—An aerosol of the solutions was produced by a Dräger nebulizer, inhalations lasted for two minutes or less, stopping at the first feeling of discomfort. We registered the results on a Krogh-type spiograph to which a simple timing device was attached to permit the accurate measurement of the amount of air expelled in one second. We determined the Tiffeneau coefficient (percentage of the total vital capacity (VC) in one second) and the bronchospasm according to Olivier (percentage of the first second VC before and after the inhalation of the testing solution). The same measurements were controlled on the Wyss-Hadorn pneumometer as well. A bronchospasm over 20 % was considered positive after 8 inhalation, the positive reaction to H and ACh had to exceed 30 %. All investigations were undertaken in a symptom-free period, after determination of basic respiratory function tests (VC, maximal breathing capacity, Tiffeneau and pneumometer values, etc.).

Drugs.—1. Serotonin. A freshly prepared 2 % isotonic sodium phosphate buffer solution with pH 7.6.

2. Acetylcholine (Roche). 1 % solution with pH 5.2.

3. Histamine. 1 ‰ solution with pH 5.6.

4. Isolevin (laevo-isopropyl-noradrenaline-bitartaric. cryst. Cilag.).

5. Multergan (phenothiazinyl-10'-1 propyl-2 trimethyl-ammonium-hydrate as methylsulphate. Specia.).

6. Deseril (UML 491 = 1-methyl-lysergic acid-butanolamide bimaleinate. Sandoz). According to Fanchamps Deseril possesses no other pharmacodynamic properties, so that its antiserotonine effect may be considered as highly specific.

The diagnostic dose of S was 1 mg; 0.25 mg Deseril and 12.5 mg Multergan had been used for a single test.

The experiments were carried out in three phases:

1. *Diagnosis*.—a) 321 patients inhaled S and H; b) 205 patients received additionally ACh solution.
2. *Examination of the effect of antiserotonine and antihistamine substances in S, H, and ACh-induced bronchospasm* (inhibitory effect of Deseril and Multergan). Deseril and Multergan were administered either 15–30 minutes prior to, or simultaneously with S and H.
3. *Examination of the therapeutic effect of antiserotonine (Deseril) inhalation in bronchial asthma*, in 12 patients with marked bronchial spasm following S, H and ACh inhalations.

RESULTS

We assumed that S acts as a pathological mediator in the bronchospasm of bronchial asthma through the same mechanism as H. In order to confirm this theory we compared the effect of these substances in 321 cases, the results are summarized in Table I. No significant difference could be noted between the two testing methods.

TABLE I
Comparison of inhalations with serotonin and histamine (321 cases).

Diagnosis	Num- of cases	Reaction to serotonin		Reaction to histamine	
		Positive	Nega- tive	Positive	Nega- tive
I. Bronchial asthma	246	146 (59 %)	101	126 (51 %)	121
II. Allergic diseases except asthma	36	8 (22 %)	28	13 (30 %)	23
III. Non-allergic	39	8 (20 %)	31	6 (15 %)	33

Following these preliminary investigations we tested the sensitivity of 205 cases (selected from the 321 patients) with the physiological agent (ACh) as well. Results are listed in Table II.

TABLE II
Correlation between positive bronchial reactions to serotonin, histamine
and acetylcholine (205 cases).

Diagnose	Num- ber of cases	Positive bronchial spasm after inhalation of					
		Serotonin		Histamine		Acetylcholine	
		Total	Alone	Total	Alone	Total	Alone
I. Bronchial asthma	158	96 (60%)	29 (18%)	80 (50%)	9 (5%)	81 (51%)	5 (3%)
II. Allergic diseases	23	11	5	5	Ø	5	2
III. Non-allergic	24	4	Ø	8	2	9	3

The findings were grouped according to whether these substances produced bronchospasm alone, or in correlation with one, or both of the other mediators. It is seen that S had been more often involved in the bronchoconstrictive reaction than H or ACh in the asthmatic group. The high number of positive reactions to S in group II is presumably due to the frequency of rhinitis and hayfever cases, diseases we consider as precursors of bronchial asthma ("preasthmatic state" according to our definition).

In group III the number of positive reactions to S was comparatively low if the high incidence of H and ACh-induced bronchospasm is observed in the controls. It has to be emphasized that this group consisted prevalently of patients with chronic pulmonary diseases (e.g. emphysema, chronic bronchitis), where bronchomotor hyperexcitability is a well-known fact. However, in the absence of bronchial asthma—or its equivalents—S fails to elicit bronchial spasm.

Table III demonstrates in detail the results of S, H and ACh inhalations. It can be noted that 29 asthmatics responded to S alone, a fact that justifies the examination of S sensitivity in accordance with other diagnostic methods in bronchial asthma.

Table IV presents the correlation between age distribution and positive bronchial testing results in 246 asthmatics. Allergen (dust, feathers, bacteria, etc.) sensitivity is the prominent

finding in youth, in elder patients the diagnostic value of different tests is approximately equivalent. In middle age, S sensitivity and response to the bronchodilator Isolevin, seem to correspond, suggesting that S—or some other S-like substance—may account for the moderate, yet persistant bronchial spasm.

TABLE III
Results of serotonin, acetylcholine and histamine inhalation in groups I, II and III (205 cases).

Diagnose	Number of cases	Positive bronchial spasm with							Negative
		ACh	H	S	ACh + H	ACh + S	H + S	ACh + H + S	
I. Bronchial asthma	158	5	9	29 (18%)	20	16	11	40 (25%)	28 (17%)
II. Allergic diseases	23	2	Ø	5 (21%)	Ø	1	3	2	10 (43%)
III. Non-allergic	24	3	2	Ø	3	1	1	2	12 (50%)

The inhibitory effect of antiserotonine (Deseril) and antihistamine (Multergan) substances is summarized in Table V. Reactions to S and H had been determined prior to the investigation. We included cases of bronchial asthma where the bronchial spasm had exceeded 40 % for both agents and the inhibitory effect of the antagonists had been 100 % (no bronchospasm after combined Deseril and S-, respectively H-inhalations). Though Deseril seems to reduce H sensitivity to a certain extent (28 cases, 32 %), the antiserotonine effect had been more pronounced (64 cases, 75 %). It follows that in certain cases antiserotonin drugs may be beneficial in bronchial asthma, as will be discussed later on.

The antihistamine Multergan proved to be moderately antagonistic (38 %) to S and inhibited the H-induced bronchospasm in a little over half of the cases examined. It has to be emphasized that all drugs had been administered in aerosol in order to study their action in the bronchopulmonary system, the shock organ of bronchial asthma.

TABLE IV
Distribution of positive bronchial tests in different age groups.

Tests	Age in years		
	7-35	36-50	Over 51
Serotonin	54 %	62 %	58 %
Acetylcholine	53 %	46 %	62 %
Histamine	48 %	52 %	70 %
Allergen	86 %	57 %	67 %
Isolevin-test	46 %	69 %	71 %

TABLE V
Inhibitory effect of Deseril and Multergan on experimental bronchial spasm in asthmatics.

Nr. of cases	Inhibitory effect of Deseril on experimental bronchial spasm after inhalation of	
	Serotonin	Histamine
85	64 (75 %)	28 (32 %)

	Inhibitory effect of Multergan on experimental bronchial spasm after inhalation of	
	Serotonin	Histamine
18	7 (38 %)	10 (55 %)

The therapeutic value of Deseril had been tested in 12 selected asthmatics. The selection was based on the satisfactory inhibitory effect of Deseril on experimental S, H and ACh bronchospasm. Prior to treatment routine bronchial tests were undertaken (determination of VC, Tiffeneau and pneumometer values), furthermore, reactions to S, H and ACh and their inhibition with Deseril had been observed. All patients had been under our control for several years prior to the investigations, they had been hospitalized throughout the treatment. All cases had proved to be resistant to current anti-asthmatic therapy.

Deseril was administered in aerosol (1 ml ampoule, containing 0.5 mg antiserotonin substance, was inhaled daily for 10 consecutive days), VC, Tiffeneau and pneumometer values were determined before and after each treatment. Half of the patients received no other medication, nasal polyps had to be removed in one case, but the improvement attributed to Deseril had been already apparent prior to the surgical intervention. The efficacy of the usual antiasthmatics (epinephrine and theophylline derivatives) and of corticosteroids was enhanced by Deseril in two cases, response to these drugs had been less satisfactory prior to aerosol therapy. Age and duration of asthma seemed to have no noticeable influence on the therapeutic results. However, the actual state of the basic disease may be of importance, since the best results were obtained in patients with moderate prolonged breathing discomfort without violent attacks of dyspnea. The effects of Deseril inhalations are summarized in Table VI.

In 4 cases (No. 1, 2, 3, 5) three tablets of Deseril (3×1 mg) were given daily for 6–10 days in order to examine the effect of oral antiserotonin treatment in asthma. In cases 1 and 2 (slight, or no improvement after Deseril aerosol) the oral route was attempted *after* aerosol therapy proved to be ineffective, in cases 3 and 5 the tablets were administered *prior* to Deseril inhalations. *All patients failed to respond to oral medication as well.* No secondary effects were noted apart from transient drowsiness when given orally.

DISCUSSION

Theoretically either excessive production of chemical mediators (ACh and H) or hyperexcitability of bronchomotoric effectors may be responsible for the bronchoconstrictive effect of the AAR in bronchial asthma. In recent years attention has been directed to the possible role of S and S-like substances in the pathomechanism of allergic diseases. Up to the present most authors based their observations on *in vitro* experiments and very little had been produced in the field of clinical ob-

TABLE VI
Therapeutic results with Deseril aerosol (12 cases).

Name	Age	Sex	Deseril + additional therapy	Bronchial tests before and after treatment with Deseril aerosol						Clinical results
				Vital capacity ml		Pneumometer L/sec		Tiffeneau %		
				Before	After	Before	After	Before	After	
1. M.J.	40	F	prednisolone orally	500	1400	0.50	2.50	80	78	Deseril enhanced the effect of prednisolone
2. M.L.	54	F	antiasthmatics	600	600	0.50	05.0	66	80	no effect
3. S.J.	36	F	Ø	900	1900	1.75	3.50	100	100	improved
4. W.E.	50	F	prednisolone orally	1800	900	2.25	1	77	66	no effect
5. D.L.	26	F	Ø	1500	2200	1.75	3.50	42	100	improved
6. H.F.	23	F	Ø	1500	2300	3	3	66	80	improved
7. M.L.	29	F	Ø	1600	2400	1.75	3.50	75	95	improved
8. H.Gy.	28	F	prednisolone (Di- Adreson F aqu.) i.v.	700	2300	0.50	2.50	71	82	improvement due to steroids
9. M.J.	30	F	antiasthmatics	1800	2700	1.75	.375	74	100	improved
10. M.E.	30	F	polypectomy	1800	2300	4	5	90	100	improved
11. T.J.	49	F	Ø	400	1000	1	2	83	100	improved
12. V.L.	40	F	Ø	1500	2600	3	5	84	100	improved

servations. In the literature the only available human exposure tests had been undertaken by *Herxheimer*, who ascribed the bronchospastic effect of the S aerosol to the acidity of the solution, which might have an irritant effect on the bronchial mucosa. In preliminary experiments we tested with an unbuffered solution with pH 4.6. Since the bronchospastic reaction that followed the inhalations did not correspond with the clinical findings, we employed on *Brocklehurst's* suggestion the buffered solution to avoid false positive results deriving from striking differences in some cases between the trachea and the intrapulmonary bronchial muscle. It seems that data referring to any one type of tissue may give a misleading indication of the overall condition of the bronchial tree. The S solution elicited reactions similar to the bronchoconstrictive effect of H and ACh, which could be registered both spirographically and clinically. First second VC diminished, and this is not observed when the cough derives from the upper respiratory tract, including the trachea, nor in vasomotor rhinitis and hayfever without asthmatic complications. Clinically wheezing could be noticed all over the lower part of the bronchial tree, and not only over the trachea.

Various methods were attempted to investigate the possible role of S in bronchial asthma, the experiments can follow three routes: 1) direct exposure to S aerosol; 2) determination of the S level in sputum or serum; 3) examination of the effect of S antagonists in bronchial asthma.

1) *Direct exposure to S* and its derivatives gave until lately no conclusive evidence regarding the spasmogenic effect of S. Our experiments proved that the action of S was specific and diagnostically as important as exposure to H and ACh.

2) *Determination of the amount of S* and its derivatives had been attempted by various authors. *Christensson & Ekwall* found the 5-hydroxyindol acetic acid increased in the urine in provoked allergic rhinitis. *Levy* observed the same in the bronchial mucus during the asthmatic attack and according to *Cruchaud* the proportion of serabinding S is elevated in allergic patients. *West* suggested that S is possibly stored in the lungs

of asthmatics during a paroxysm. On the other hand, *Beraldo* found no change of the S content of the sputum of asthmatics. Since our problem had been the investigation of the role of S in clinically symptom-free asthma, in order to differentiate asthma from other non-allergic bronchopulmonary diseases, we did not find it necessary to examine the possible increase of S during the asthmatic attack.

3) Although *substances antagonizing the bronchoconstrictive effect of S* and the therapeutic value in allergic disorders may be enhanced by the synergistic or potentiating effect of other antihistaminic, bronchodilator or sympathicomimetic agents, the results are disappointing in asthma. *Bruun & Letman* treated allergic patients with cyproheptadine and found no response in asthma. *Pautrizel* stated that the S antagonist UML 491 (Deseril) was only effective in migraine. The only data in favour of antiserotonine therapy come from *Cruchaud* who found clinical improvement after treatment with UML 491.

In previous works we have already emphasized that neither H nor H-like substances play a decisive role in the pathomechanism of bronchial asthma. Antihistamines do not inhibit the basic reaction, they can only afford relief from symptoms associated with asthma (itching, hypersensitivity of the upper respiratory tract, nervousness, etc.). In studying the antiserotonine drug Deseril we did not expect to find a new point anti-asthmatic substance, but we wished to investigate how far S could be made responsible for bronchial spasm and whether the liberation of S, or sensitivity to S influenced the course of the disease.

SUMMARY AND CONCLUSIONS

Experiments on the possible role of serotonin in human bronchial asthma had been attempted in three ways: 1) 321 patients received serotonin and histamine aerosol, 205 inhaled additionally acetylcholine. The bronchospastic reaction of serotonin was found positive in 60 % of the asthmatics, thus direct exposure to a buffered 2‰ solution of serotonin seems to be

of value in the differential diagnosis of bronchial asthma. Furthermore, serotonin seems to play an active part in eliciting moderate prolonged dyspnea in elder asthmatics. It is concluded that serotonin is liberated during the antigen-antibody reaction simultaneously with other chemical mediators.

2) The bronchoconstrictive effect of serotonin is specific, since it could be inhibited by serotonin antagonists (Deseril) in 75 % of the cases investigated, whilst antihistamines (Multergan) had the same effect only in one third of the cases.

3) Antiserotonin drugs are ineffective in bronchial asthma when given orally, but may be beneficial in aerosol treatment. The inhibitory effect of antagonists came into evidence only when the drug was administered by inhalation. 6 patients out of 12 selected asthmatics responded well to Deseril inhalations, but it has to be borne in mind that the action is not prolonged and consists merely in diminishing bronchial spasm without apparently affecting the antigen-antibody reaction. Thus antiserotonin drugs may be added to the symptomatic treatment of bronchial asthma in certain cases of pronounced serotonin sensitivity.

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