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IMMUNE RESPONSES TO TREATMENT WITH NATURAL AND SYNTHETIC ACTH IN BRONCHIAL ASTHMA

By

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Reactions against ACTH were rather commonly noted during the first decade of therapy with pituitary extracts of animal origin. Most of the allergic reactions then reported were probably the result of sensitization against the contaminating foreign proteins in the preparations rather than against the hormone character per se (22). Since the sixties, when highly purified corticotropine came into use, such reactions are less common. The immunological responses may range from fatal allergic shock to the mere occurrence of demonstrable IgE, IgG and IgM antibodies (1, 8, 9, 10, 16, 17, 23, 26, 29, 32).

The naturally occurring ACTH molecule is composed of 39 amino acids. The sequence of the amino acids, 25-33, express the species-specific differences (20). The first synthetic ACTH-preparation produced (tetracosactid) is composed of 24 amino acids linked in the same sequence as the first 24 of the naturally occurring ACTH molecule. This preparation was therefore thought to involve hardly any risk of sensitization when administered to human subjects. However, reactions after treatment with synthetic ACTH-preparations have occasionally appeared both clinically and experimentally. At least two cases of fatal allergic shock induced by tetracosactid have been reported (12, 32) and antibodies against it have

been demonstrated in asthma patients (12, 23) as well as in cases of connective tissue disease (13).

It therefore seems to be of interest to study the incidence of immune response in patients who receive long-term therapy with natural porcine ACTH compared with those receiving synthetic ACTH. The present report is an account of a study on asthmatic patients in this respect during a period of 2 years.

MATERIAL AND METHODS

Patients

Twenty patients, 10 men and 10 women, aged 25-68 years suffering from chronic asthma were systematically studied during 2 years ACTH-therapy. The study included a thorough clinical examination, skin tests, and in skin reactive cases, provocation tests with suspected allergens, laboratory examination of serum samples for antibodies against ACTH and a number of "control" (15) antigens. An allergen of clinical importance could be identified in only two cases (i.e. animal dander and house dust, respectively).

The patients were divided in two groups:

Group 1: Ten patients were treated with Acton 25 IE 7-8 times during 3 weeks. After 1 week's delay they were then given Acton prolongatum 100 IE 7-8 times during 3 weeks and thereafter 100 IE at least every 4th week for 2 years.

Group 2: Ten patients were treated as those in Group 1 but with 0.25 mg doses of Synacthen® instead of Acton and 1 mg doses of Synacthen® Depot instead of Acton prolongatum. In some cases from both groups the prolonged ACTH-doses were given more often when needed because of deterioration of the asthma.

In addition to ACTH and common bronchodilators all the patients were continuously treated with small corticosteroid doses (Table 1).

Intradermal tests were performed with natural and synthetic ACTH-preparations (Acton and Synacthen) in both groups before, and 1, 12 and 24 months after the onset of the study. Serum samples were obtained at the same time. In most of the cases the daily corticosteroid dose was completely dropped during the last 2 days preceding the skin test, and in no case did the daily dose exceed 5 mg of Prednisolon at the time of this test. The ACTH-treatment was also interrupted 3 days before an intradermal test.

Two cases, one of them outside the study proper, being of particular interest, are described in more detail under "Clinical findings".

Normal subjects

Supposedly normal sera were collected from a group of apparently healthy persons subjected to a careful medical health control examination. Data from

the examination will be reported elsewhere (30). The sera from in all 63 persons were chosen to represent both sexes, and ages between 20 and 60 years.

Antigens

Natural ACTH (porcine):

Acton (30 IE/ml, Ferring AB, Malmö) was used for intradermal testing and as antigen in *in vitro* experiments. It was also used for initial treatment in Group 1.

Acton prolongatum (60 IE/ml, Ferring AB, Malmö) was used for long-term treatment in Group 1.

Synthetic ACTH

Synacthen (tetracosactid 0.25 mg = 25 IE/dry ampoule, Ciba-Geigy AG, Basel) was used for intradermal testing and as antigen in *in vitro* experiments. It was also used for initial treatment in Group 2.

Synacthen Depot (tetracosactid 1 mg = 100 IE/ml, Ciba-Geigy AG, Basel) was used for long-term treatment in Group 2.

Homactid (octacosactid 0.5 mg = 50 IE/dry ampoule, Ferring AB, Malmö) was used as antigen in a number of *in vitro* experiments.

Radioiodine ACTH (^{125}I -labelled alpha 1-24 tetracosactid) was obtained from Amersham Radiochemical Centre.

Synthetic vasopressin:

Postacton (20 IE/ml, Ferring AB, Malmö) was used as antigen in a number of *in vitro* experiments.

Gamma globulin:

Porcine gamma globulin was purchased from the Royal Veterinary College, Stockholm. It was used for detecting sensitization to porcine components by passive haemagglutination and mixed haemadsorption tests.

Intradermal tests

For intradermal tests 0.05-0.1 ml of serial tenfold saline dilutions of Acton and Synacthen were used. A basic solution of 30 IE/ml was called 1:10. The test of immediate wheal and flare type was judged as positive when the sum of the largest wheal diameter and its perpendicular exceeded 20 mm. The testing was started with the concentration 1:100,000 and, if no possible reaction appeared within 10 min, was continued step by step up to 1:100.

Passive haemagglutination tests

Tanned sheep erythrocytes sensitized according to Boydens description (6) were used with Acton and the immunoglobulin. Synacthen, Homactid and

TAB
Clinical and Immunological Data of 20 Asthma Patients

Patient no.	Age (yrs)	Sex	Main daily dose predn. (mg)	ACTH-treatment			
				Acton (I.U.)		Synacthen (I.U.)	
				Total dose before	Added dose	Total dose before	Added dose
Group I							
1	25	F	10	2700	4300	—	—
2	48	M	10	10000	9500	—	—
3	61	M	5	11000	2200	—	—
4	47	M	7.5	3500	9500	—	—
5	65	M	5	2000	2900	—	—
6	60	F	5	700	2400	—	—
7	44	F	5	1500	2400	—	—
8	67	M	2.5	1000	3300	—	—
9	50	M	5	4500	4300	—	—
10	67	M	5	4500	2000	—	—
Group II							
11	63	F	2.5	1500	—	—	4000
12	55	F	5	5000	—	—	4400
13	54	M	5	2100	—	—	2900
14	36	F	5	1000	—	—	4400

Receiving Corticotrophin During a 2-Year Period.

Immediate skin reaction during course of study				IgE anti-ACTH (U/ml)	
A = Acton		S = Synacthen			
Before	1 month	12 months	24 months	Before	12 months
A — S —	A — S 1 +	A — S —	A — S 1 +	23	53
A 1 + S 1 +	A 1 + S —	A 1 + S —	A 1 + S —	46	53
A — S —	A 1 + S —	A — S —	A — S —	42	64
A — S —	A — S —	A — S 1 +	A — S —	ND	ND
A 1 + S —	A — S —	A — S —	A — S —	17	32
A — S —	ND ND	A 1 + S —	A 2 + S —	14	51
A 1 + S —	A — S —	A 1 + S —	A — S —	18	21
A — S —	A — S —	A — S 1 +	A — S 1 +	16	32
A — S —	A — S —	A — S 1 +	A — S 1 +	ND	ND
A — S —	A 1 + S —	A 1 + S 1 +	ND ND	73	33
A 1 + S —	A 1 + S —	A 1 + S —	A 2 + S —	18	21
A 1 + S —	A 1 + S —	A 2 + S —	A 2 + S —	38	29
A — S —	A — S —	A — S 1 +	A — S 1 +	ND	ND
A 1 + S —	A 1 + S —	A 1 + S —	A 2 + S 1 +	ND	ND

TABLE

Patient no.	Age (yrs)	Sex	Main daily dose predn. (mg)	ACTH-treatment			
				Acton (I.U.)		Synacthen (I.U.)	
				Total dose before	Added dose	Total dose before	Added dose
15	56	F	5	600	—	—	2400
16	68	F	2.5	5300	—	—	3400
17	57	M	7.5	7600	—	—	4700
18	38	F	10	1800	—	—	4200
19	61	M	15	1500	—	—	6400
20	47	F	5	6000	—	—	3500

Degree of immediate skin reactions is indicated by signs —, 1 +, 2 +.

—: Negative skin reaction to 1:100.

1 +: Positive skin reaction to 1:100 but negative to 1:1,000

Postacton were attached to sheep erythrocytes by the bisdiazotized benzidine technique described by Stavitsky & Arquilla (27).

The following antigen concentrations were used:

Synacthen (24-tetracosactid)	5 μ g/ml
Homactid (octacosactid)	5 μ g/ml
Postacton (vasopressin)	5 μ g/ml
Porcine γ -globulin	100 μ g/ml
Acton	1.2 IU/ml

The agglutination titrations were performed in serological test tubes and read according to the bottom patterns made by the sedimented erythrocytes.

Further details of the technique are given by the authors in a separate report (15).

Mixed haemadsorption tests

The procedure followed was a two-dimensional radial agar diffusion technique previously described (13, 14). The antigen solutions were spread over

(continued)

Immediate skin reaction during course of study				IgE anti-ACTH (U/ml)	
A = Acton		S = Synacthen		(U/ml)	
Before	1 month	12 months	24 months	Before	12 months
A 1 + S —	A 1 + S —	A 1 + S 1 +	A 2 + S 1 +	18	34
A — S —	A — S —	A — S —	A — S —	19	20
A 1 + S —	A 1 + S —	A 1 + S —	A 1 + S —	30	64
A — S —	A — S —	A — S 1 +	A — S 1 +	21	22
A 1 + S —	A 1 + S —	A 1 + S —	A — S —	23	27
A 2 + S —	A 1 + S —	A — S 1 +	A — S —	15	61

2 + : Positive skin reaction to 1:1,000 but negative to 1:10,000.

ND : Not done.

glass slides, dried and fixed with acetone. Agar and test sera were applied with the aid of a plastic template. After 40 h diffusion the sera and the agar layer were removed, and antibody attached to the antigen on the bottom glass was traced by indicator cells, i.e. antiglobulin coated erythrocytes prepared as described in a previous report (13). The procedure for determining the antibody titers in this system has also been described previously (14). Further details of this application of the technique have been described by the authors in a separate report (15).

Immunofluorescence tests

Unfixed frozen sections of human pituitary were used as antigen. They were incubated with test serum for 30 min, rinsed for 3 min in three successive cuvettes, incubated for a further 30 min with anti-human globulin conjugate, washed as after the first incubation, and mounted with buffered glycerol. PBS pH 7.4 was used for washings and as a diluent. The procedure was performed at room temperature. The preparations were examined in a Zeiss fluorescence microscope using a dry dark field condenser, exciter filter BG 3 and barrier

filter 44. The light source was a HBO mercury lamp. The fluorescent conjugate (24, 28) was a commercial preparation from the National Bacteriological Laboratory (3, 4). It conformed to proposed standard requirements (5) by containing four precipitating antibody units and having a w/w F/p ratio of 5.9×10^{-3} (7, 19, 21).

Estimations of total IgE serum concentrations

The commercial Phadebas IgE test (Pharmacia Ltd, Uppsala, Sweden) was used for this purpose (31).

Radioimmunoassay of IgE anti-ACTH

A radioimmunoassay differing in some respects from the classical radioallergosorbent test (RAST) was used tentatively for this purpose. 0.1 ml of a ^{125}I -ACTH solution containing 10 mg of antigen/ml was mixed with 0.1 ml of undiluted test serum. Two control tubes were included, one containing positive serum + unlabelled ACTH and the other negative serum + ^{125}I -labelled ACTH. After 1 h at $+20^\circ\text{C}$ and 2 h at $+4^\circ\text{C}$, 1.0 ml of Sephadex-anti-IgE complex containing 1.0 mg of gel/ml was added and the tubes containing the mixture were incubated for 2 h at $+20^\circ\text{C}$ in a rack rotating slowly around its longer horizontal axis. The Sephadex particles were centrifuged for 5 min at 1,000 g and washed 3 times in saline. The pellet after the last washing was resuspended in a drop of saline and its γ -radioactivity was measured in a Nuclear Chicago γ -counter. The amount of ACTH adsorbed to the Sephadex complex was estimated from a standard curve. The IgE concentration in the sample was then estimated from the formula:

$$\text{pg IgE} = \frac{\text{Pg ACTH}}{2 \text{ molar w. ACTH}} \cdot \text{molar w. IgE}$$

The IgE was finally expressed in units. One unit corresponding approximately to 1 mg.

RESULTS

A. Clinical findings

1. *Signs of clinical allergy to ACTH*: Repeated and detailed clinical examinations were performed on the two groups of asthma patients selected for experimental treatment with Acton and Synacthen, respectively (see Table 1). The treatment resulted in the expected improvement of the asthma but caused no sign of clinical allergy to ACTH in any patient in either group during the 2-year observation period.

TABLE 2

Incidence of Intradermal Reactions Against Acton and Synacthen in Two Groups of Asthma Patients During ACTH-Treatment.

ACTH-preparation for treatment	Number of patients reacting in intradermal tests against							
	Acton				Synacthen			
	Months after treatment				Months after treatment			
	0	1	12	24	0	1	12	24
Acton (n = 10)	3	3	4	2	1	1	4	3
Synacthen (n = 10)	7	7	6	5	0	0	4	4

However, a serious anaphylactic attack elicited by Synacthen was noted in a patient not originally included in the study (see case 1 under case reports). In one case (pat. no. 3 in Group 1) high antibody titres against ACTH were found to exist in the absence of clinical ACTH-allergy (case 2 under "Case reports").

2. *Intradermal tests:* Twenty previously Acton-treated cases were tested intradermally with Acton and Synacthen before, and 1, 12 and 24 months after the onset of a course of either Acton or Synacthen treatment. The results in each case have been recorded in Table 1. A comprehensive compilation of the results (Table 2) shows that the number of patients reacting to Acton was high even before the treatment, and was not further increased either by Acton or by Synacthen treatment. On the other hand, only one patient reacted initially to Synacthen, but the number of patients reacting to that substance increased during the first 12 months of Acton or Synacthen treatment.

3. *Case reports:* Two cases of particular interest to the study are described in further detail immediately below:

Case 1. 110610-900 M.F.

62-year-old female patient with "endogenous" bronchial asthma since 1940. Since 1959 she has been treated continuously with corticosteroids and since 1968

with ACTH. Acton prolongatum 60 was given subcutaneously, usually once a month. In May 1970 she noticed a swelling at the site of the Acton injection. An intradermal test at that time became positive with Acton in the concentration 1:1,000 and with Synacthen 1:100. A subcutaneous test dose of Synacthen was, however, given without reaction. Between 15/7 1970 and 27/5 1972 the patient was then given seven injections in all of Synacthen Depot in 25 mg doses without complications. On the 27th of May 1971 the patient got her Synacthen injection after the usual interval. She was in good condition, without asthma. Within 1 min after the intramuscular injection of 25 mg of Synacthen Depot in 1 ml the patient suddenly complained of tickling in her lips and breathing distress. She rapidly developed a fulminant asthmatic attack with apnea, falling blood pressure and involuntary urination. Artificial oxygen ventilation with a bag and mouthpiece was promptly instituted. Epinephrine 0.5 ml subcutaneously, and 10 ml of theophyllamine and 16 mg of Decadron intravenously were given rapidly. With this treatment the anaphylactic shock was relieved in about 10 min. The further course was uneventful. Blood for antibody examination was taken 4, 13, 19, 30 and 37 days after the anaphylactic attack.

IgE class anti-ACTH determinations on four serum samples from the patient showed considerably elevated values slowly decreasing during the first month after the attack. The total IgE concentration measured in three sera was not elevated. Agglutinating antibodies against Synacthen were present initially and rose to a maximum about 20 days after the attack. They then rapidly decreased (Table 3). Non-agglutinating antibodies, as demonstrated by the mixed hemadsorption test, were present initially against Synacthen and Postacton (Fig. 1). These antibodies decreased or disappeared during the first month after the attack. Antibodies to Acton and porcine globulin were demonstrated in titres 1/640 and 1/160, respectively. Antibodies against human foetal adrenal and pituitary were not demonstrated.

TABLE 3

Antibody Titers in Asthma Patient (Case 1) after Allergic ACTH-Shock.

Days after allergic shock	IgG-IgM-antibody titers against			IgE U/ml	
	Synacthen	Homactid	Postacton	Total (RIST)*	Anti- ACTH†
4	80	10	20	< 10	61
13	320	10	20	< 10	58
19	640	40	20	< 10	45
30	80	40	20	ND	40
37	10	40	20	ND	ND

* Radioimmunosorbent test.

† Radioimmunoassay (cf. text).

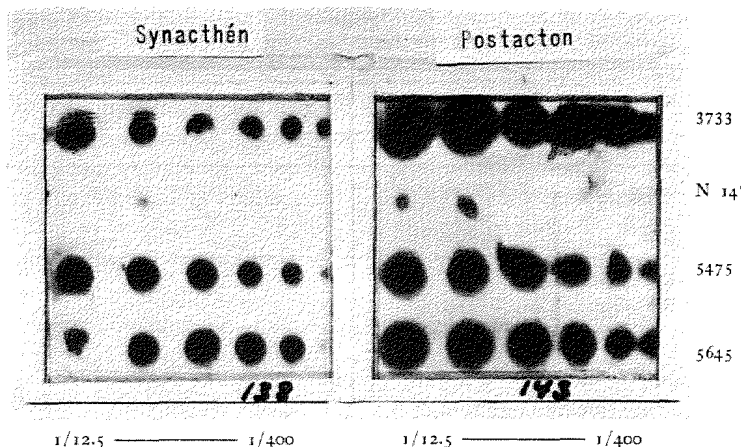


Fig. 1.

Antibodies against tetracosactid (Synacthen) and vasopressin (Postacton) as demonstrated by the mixed hemadsorption test in asthma patient with anaphylactic shock elicited by Synacthen (case 1 under Case Reports). Reactions produced by sera obtained 4 and 13 days after the shock (27. 5. 71).

3733/70: Positive control serum.

N 148 : Negative control serum.

5475/71: Serum sample 1. 6. 71.

5645/71: Serum sample 9. 6. 71.

Case 2 090519 H.E.

63-year-old man without atopic family history. After a severe infective bronchitis in 1959 he developed a bronchial asthma requiring hospital care and corticosteroid treatment on several occasions. Since 1961 he was continuously treated with corticosteroids and given Acton prolongatum at monthly intervals. He sustained two cardiac infarctions in 1964 and 1972 and ultimately died from the one in 1972.

Rather unexpectedly, in the absence of clinical symptoms, this patient showed high IgG and IgE antibody titres against ACTH and vasopressine, and also an elevated total IgE concentration 12 months after a course of Acton treatment. However, skin tests with Acton caused no reaction. There were no agglutination reactions against porcine serum components and the agglutination reactions against synthetic ACTH-preparations were as high as those against Acton in spite of the fact that he had never been treated with any synthetic preparation (Table 4).

TABLE 4
Antibody Titers in Asthma Patient (Case 2) after Acton Treatment.

Time for serum sample	IgG-IgM antibody titers against				IgE U/ml	
	Synacthen	Acton	Postacton	Porcine γ -globulin	Total (RIST)*	Anti-ACTH†
Before treatment	80	ND	< 10	< 10	205	42
After 1 month	80	ND	< 10	< 10	ND	ND
After 12 months	2560	2560	2560	< 10	370	64

* Radioimmunosorbent test.

† Radioimmunoassay (cf. text).

B. Agglutinating antibodies examined by the passive haemagglutination test

The two comparable groups of asthmatic patients treated with Acton and Synacthen respectively, had already before the treatment course a considerable incidence of low titered reactions against both natural and synthetic ACTH. However, no increase of incidence or mean titres was noted in either of the groups during treatment. In the Acton-treated group the reactivity rates against Synacthen and Acton were nearly the

TABLE 5
Agglutinating Antibody Titers Against Synacthen, Acton, Postacton and Porcine γ -Globulin.

Patients	No. of patients reacting at titre level ≥ 10 and ≥ 40 against							
	Synacthen		Acton		Postacton		Porcine γ -globulin	
	≥ 10	≥ 40	≥ 10	≥ 40	≥ 10	≥ 40	≥ 10	≥ 40
Acton group (n = 10):								
Before study	10	3	10	4	4	1	3	
After 12 months treatment	8	2	10	4	10	4	2	
Synacthen group (n = 10):								
Before study	10	2	9	2	5	0	3	
After 12 months treatment	7	1	7	1	7	1	4	

same before and after treatment. In the Synacthen-treated group the rates were slightly lower after than before the treatment. Individual titre rises were noted in a few cases during the first 12 months of treatment. In contrast to the findings just described, titres and incidence of reactions against Postacton (synthetic vasopressin) were significantly increased during the ACTH treatment (Table 5). This occurred in the Acton as well as in the Synacthen treated group and slightly more so in the former than in the latter.

Time of appearance of immune response against ACTH

The results presented in this report on the 20 systematically studied asthma patients refer mostly to the sera drawn 12 months after the onset of treatment. These sera were chosen because, when comparing sera drawn before and 1 month, 12 months and 24 months after resumption of ACTH-therapy, the mean titres rose slightly during the first month and then somewhat further during the following year. Two years after the onset of therapy they had usually returned to pretreatment figures (Fig. 2 a). The fraction of sera reacting with a titre rise was usually small (5–10 per cent) increased slightly during the first month after onset of therapy and then remained stationary.

It was noted previously that the only statistically significant drug-induced titre rise was that against Postacton. This reaction had its maximal mean titre of 1/40 after 12 months and then subsided.

The recruitment of cases responding in this way was considerable, rising by 7 per cent during the first month and a further 30 per cent during the following 12 months (Fig. 2 b).

C. Antibodies against foetal pituitary

Antibodies against foetal human pituitary were demonstrated in three of the 20 asthma patients. The reactions were of low titre (1/10) in all three cases. In one case reactivity was present both before and after onset of therapy. In an-

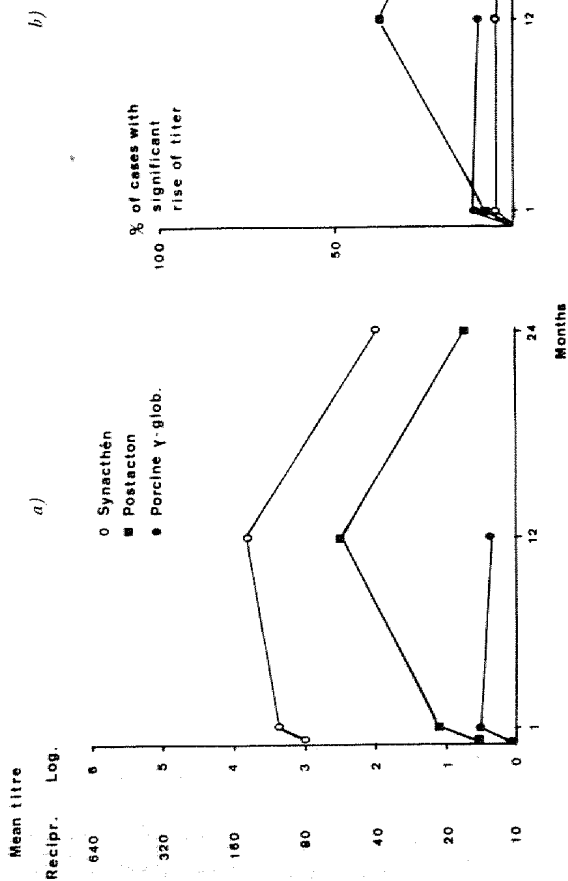


Fig. 2.

Agglutinating antibody response to tetracosactid (Synacthen) vasopressin (Postacton) and porcine γ -globulin during 2 years ACTH treatment in 20 asthmatic patients previously treated with natural ACTH (Acton and Acton prolongatum).

a) Mean titre.

b) Recruitment of cases.

other it appeared after the onset of treatment and in a third patient it was present before but absent 1 year after the onset of treatment.

D. *IgE determinations*

The total IgE contents were determined by the Phadebas method in sera from 17 asthma patients and six supposedly normal persons. Assuming the normal serum concentration of total IgE to be 150 U/ml, only one asthma patient (one of the two with atopic asthma) had an elevated concentration which was present both before and after ACTH "booster" treatment (Table 6).

Specific IgE anti-ACTH antibodies were determined in most of the sera using radioimmunoassay (see Material and Methods). The concentration of such antibodies, measured tentatively in units, ranged between 6.5 and 58 U/ml in 30 apparently healthy persons with a mean of 21 U/ml and a median of 11.6 U/ml (Fig. 3).

Treatment with ACTH caused a change of the IgE anti-ACTH concentration distribution towards higher values in 16 asthma patients with a shift of the median from 17.4 to 26.6 U/ml, and of the mean from 27.1 to 38.5 U/ml (Fig. 3). Before the study, the IgE reactivity rate of the asthma patients against ACTH determined at the concentration level 20 U/ml was not significantly higher than that of normal

TABLE 6
Total IgE in Asthmatic and Normal Subjects.

Group of sera	No. examined	Distribution at different conc. levels			
		< 10	≥ 10	≥ 150	≥ 300 U/ml
Asthma patients:					
Before study	17	8	9	1	0
After 12 months treatment	17	11	6	1	1
Controls	6	6	0	0	0

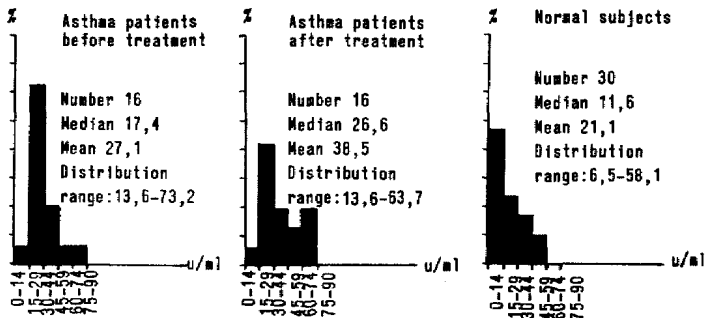


Fig. 3.

Concentration distribution of IgE-anti-ACTH as measured by a modified RIST-technique in sera from asthmatic patients and normal subjects.

individuals in spite of the fact that they all had been treated with ACTH during the last few years. After extra treatment, however, the reactivity rate of the patients was significantly higher than that of the group of normal persons. This was true irrespective of whether the treatment had been with Acton or Synacthen (Table 7). The reactivity rate after treatment was also significantly higher than the rate before treatment.

No significant difference in immunogenic capacity between Synacthen and Acton could be detected in this study. The mean rise of IgE anti-ACTH was 11 U/ml after Synacthen as well as after Acton, and the reactivity rate against ACTH was the same after Synacthen as after Acton (Fig. 3, Table 7).

DISCUSSION

The occurrence of immunization against ACTH has been well documented by several authors (1, 2, 9, 16, 17, 23, 32) and in recent years has been described with the hormone part of the ACTH molecule as well as the part carrying the species characteristics, and with synthetic hormone as well as with natural pituitary extracts. Species and organ specific anti-

TABLE 7

Reactivity Rate of IgE-anti-ACTH at the Concentration Level 20 U/ml in Two Groups of Asthma Patients during ACTH-Treatment.

Diagnostic group	Reactivity rate at 20 U/ml	P for difference from normal *	P for increase of reactivity rate during treatment *
Acton group:			
Before study	4/8	0.2	
After 12 months treatment	8/8	< 0.001	< 0.001
Synacthen group:			
Before study	4/8	0.2	
After 12 months treatment	8/8	< 0.001	< 0.001
Both groups combined:			
Before study	8/16	0.7	
After 12 months study	16/16	< 0.001	< 0.001
Normal subjects	10/30		

* P estimated by the χ^2 -test.

bodies against pituitary cell cytoplasm have also been described previously (13), and in this report. Subclinical immunization against ACTH, synthetic as well as natural, appears to be fairly common as can be concluded from the incidence of antibodies to it in ACTH-treated persons (15, 16, 23, 25). Clinical symptoms, on the other hand, seem to be rare but all the more important when they do occur since they are often serious and unexpected.

Clinical symptoms of immunological sensitization against ACTH usually seem to be either an anaphylactic state in persons treated with ACTH because of allergic diseases (12, 32) or a state of refractoriness to ACTH treatment in cases of rheumatism (13, 15, 29).

The two cases separately described in this report have been chosen as they represent what may be regarded as the two principal polar extremes of reactivity in asthma patients.

Case 1 illustrates some characteristic features of the im-

munologic response to ACTH in the allergic patient, i.e. the relative absence of premonitory symptoms, unexpectedness and rapidity of onset of the serious anaphylactic shock, and the relief by adequate treatment. This is in agreement with earlier reports on similar cases (12, 32). Several serological findings in this case are worth particular consideration. It is to be noted that IgE antibodies to synthetic ACTH were present in the serum in relatively high concentrations at the time of the anaphylactic attack and then decreased slowly. The concentration of agglutinating and presumably blocking antibodies with the same specificity, on the other hand, was low at the onset of the attack but rose to a maximum 20 days later, much in the way expected from a "booster" response. It is therefore possible that a predisposition to anaphylaxis had existed in this patient for some time but been balanced by blocking antibodies, and the anaphylaxis may have been elicited by a decrease in the concentration of these antibodies. A contributory cause could be that precipitins played a role by activating the complement system and thereby inducing the formation of anaphylatoxins (C_3a , C_5a) resulting in histamine liberation. It is also possible that aggregated IgE could have activated complement (C_3) via the "alternate pathway" and likewise induced formation of anaphylatoxins (11, 18). However, the complement levels were not examined in this case.

Case 2 also suggests that an anaphylactic sensitization to ACTH may not be altogether uncommon in predisposed persons and that the absence of symptoms may depend on the presence of blocking antibodies. This patient had a high IgE concentration against ACTH but showed no sign of clinical reaction against the hormone. However, he had also the highest agglutinating titre against ACTH noted in this study. The problem of the specificity of this reaction will be discussed in a separate report (15). However, there was no clinical evidence of refractoriness to ACTH-therapy. The patient died from a coronary thrombosis during the period of observation. There was no sign of arteritis or nephropathy that could have suggested the presence of an immune complex disease.

The skin tests for immediate reaction performed with this series of patients gave results that further illustrate the points made above. Obviously, many of the patients were already sensitized to the natural hormone at the onset of the therapeutic trial as judged from the skin test. A sensitization to tetracosactid occurring in some cases, however, was not apparent until 12 months later.

The initial incidence of skin sensitivity to Acton was therefore probably due to the species characteristics which is fully plausible since all these patients had been treated with the natural hormone at some time earlier in their lives. Agglutinating antibodies to both natural and synthetic ACTH were present in high incidence already at the onset of treatment. It may further be noted that a positive skin test to tetracosactid 12 months after the onset of treatment was less common than the presence of IgE antibodies to tetracosactid at that time. This discrepancy is possibly explained by the demonstrated simultaneous presence of agglutinating, i.e. blocking, antibodies. The positive skin reactions demonstrated were weak or moderate which is in agreement with the fact that none of these patients had clinical signs of ACTH allergy. It is, of course, highly probable that much more vigorous reactions would be met with in allergic cases. The points made above, therefore, do not imply that skin tests are not of value for revealing a latent ACTH allergy.

As has already been mentioned, low titres of agglutinating antibodies were present already at the onset of treatment in most cases, and no further increase in mean titre or incidence against Acton, Synacthen or porcine γ -globulin in response to a restricted treatment course could be demonstrated. A rise of titre to a peak after 12 months, however, was noted in a few individual cases. There was also an increased incidence of IgE antibodies to the different ACTH preparations at the onset of treatment. In contrast to the agglutinating antibodies the IgE antibodies were shown to increase during treatment with either Acton or Synacthen. This selected type of reaction by the predominantly IgE antibody class may be peculiar to allergic

patients, while patients with, for example, rheumatic conditions might react by the IgG or IgM class. The significantly increased incidence of agglutinating antibodies may cause the absence of allergy in the latter patients (15, 29). An explanation which clearly remains to be investigated. The agglutination titres against Synacthen were usually somewhat higher after Acton treatment than after Synacthen. This may be due to an adjuvant effect of the species part of the Acton molecule. An increased incidence of antibodies against porcine γ -globulin was noted in the present patients as a result of treatment with natural porcine ACTH (23). However, no increase in titre or incidence of reactions against species antigen was noted during the further treatment course.

Altogether, the presented results support the view that the immunogenicity of ACTH preparations, natural as well as synthetic, is very low. Only after repeated courses of intensive treatment does a significant immunization take place, and even then, clinical anaphylactic symptoms are often probably prevented or reduced by the formation of blocking antibodies. In contrast, the genetically predisposed person may have a vigorous reaction within a short period after the onset of the first treatment, or suddenly and unexpectedly after several years of uneventful therapy. Premonitory symptoms may not be present or may be faint and easily overlooked. If suspicion of allergy against natural ACTH arises a changeover to a synthetic preparation should be made with caution, fully realizing that the hypersensitivity may concern the hormone specific part of the molecule. Whether the species part of the natural hormone molecule has to act as a tolerance breaking carrier to induce primary immunization while synthetic preparations produce only a booster effect, or whether the synthetic substance can act as full immunogens, unfortunately can not be decided from the present study since all patients had been given Acton before participation.

A remarkable finding is that a significant increase of antibody titre after the course of ACTH treatment could be demonstrated only against vasopressin (Postacton). This

effect was produced by both the natural and the synthetic preparation although it was most conspicuous with the natural one. In some cases, the titre rises were considerable. No adverse clinical effects could be ascribed to this condition. However, symptoms such as refractoriness to vasopressin might have presented themselves if conditions for their appearance had been present as they are, for example, in parturient women. The mechanism for this cross immunogenity remains to be clarified and will be further considered in a separate report (15). A migration inhibition experiment was performed with the peripheral blood leucocytes of one of the reported asthma patients (Case 1). It is of interest that this experiment showed a migration inhibition by Postacton but not by Synacthen or Homactid.

SUMMARY

Two comparable groups of asthmatics each with 10 patients were treated during 2 years at scheduled intervals with either natural or synthetic ACTH up to a total dose of 2000-9500 IU. All patients had previously been given the natural but never the synthetic hormone. Intradermal tests with natural and synthetic ACTH were performed before treatment and after 1, 12 and 24 months. Serum samples were also taken on these occasions and analysed for antibodies against ACTH, vasopressin and porcine γ -globulin. No sign of clinical allergy to ACTH was noted in any of the patients during the 2-year period.

The incidence of intradermal reactions against natural ACTH was high at the onset of treatment but was not increased by treatment with either synthetic or natural ACTH, while the reactivity rate against synthetic ACTH was increased after both types of treatment.

The incidence of IgE reactions against synthetic ACTH at the 20 U/ml level was significantly increased after 12 months' treatment with either natural or synthetic hormone. A high incidence of low-titered agglutinating antibodies against

natural or synthetic ACTH was demonstrated before treatment in both the groups, but no significant change in incidence or mean titre against natural or synthetic ACTH or porcine γ -globulin was noted during treatment with the natural or the synthetic preparation. A few patients, however, did display an increased agglutinating titre against ACTH after 12 months' treatment. Rather unexpectedly, most sera reacting with ACTH were found to react also with vasopressin and a significant increase of the incidence of these reactions and of the titres occurred during the treatment with synthetic as well as with natural ACTH.

Two cases have been examined in detail, one because of a fulminant shock after synthetic ACTH and the other because of very high antibody titres without clinical symptoms of ACTH allergy.

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