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IMMUNOELECTROPHORESIS AS A DIAGNOSTIC MEANS IN ALLERGIC DISORDERS

By

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The object of the present study has been to investigate whether immunoelectrophoresis represents an adequate means by which to localize antibodies or other alien proteins in allergic disorders. Hitherto it has been considered possible by routine electrophoretic technique to ascertain a small rise in the gamma-globulin fraction.

MATERIAL

A total of 18 patients suffering from allergic disorders have been examined; a group of 10 healthy subjects has served as control. The group of allergic patients included: 10 cases of bronchial asthma, 3 of pollinosis, 4 of vasomotor rhinitis, and one case of Chlorpromazine induced icterus (Table 1).

Examinations have included: E.S.R., leucocyte and eosinophilic cell counts, radiography of thorax and sinuses; the findings are recorded in Tables 2 and 3.

Patients in the control group presented no symptoms of previous nor of present allergic disorders.

METHOD

The immunoelectrophoresis apparatus used here is of the type: Agafor 3 (Scheidegger) in Fig. 1 seen as a rectangular box of transparent plexiglass, centrally divided by a trough over which a slab is placed accomodating 8 slides.

TABLE 1

Reg. no.	Sex	Age	Diagnosis	Duration of disorder
3153	female	7	bronchial asthma	5 years
22830	female	41	bronchial asthma	20 "
22230	female	41	status asthmaticus	10 "
22627	male	62	bronchial asthma	6-7 "
22271	male	57	bronchial asthma	31 "
19027	male	53	bronchial asthma	8 months
22144	male	40	bronchial asthma	1 month
914	female	13	bronchial asthma	7 years
748	female	10	bronchial asthma	8-9 "
15322	male	10	bronchial asthma	3 "
22701	male	8	pollinosis	7 "
19013	female	11	pollinosis	6-7 "
22239	female	16	pollinosis	11 "
22580	male	34	vasomotoric rhinitis	20 "
7034	male	12	vasomotoric rhinitis	4 "
22671	female	18	vasomotoric rhinitis	14 "
22085	male	62	vasomotoric rhinitis	5-10 "
8041	female	66	Chlorpromazine induced icterus	2 months

TABLE 2

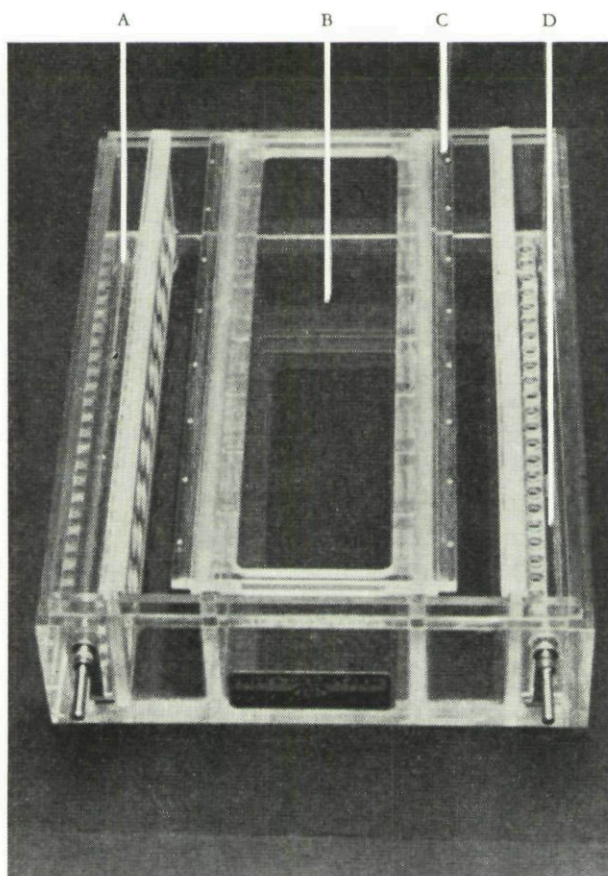
Reg. no.	Hgb/g/l	ESR	Leucocyte count /mm ³	Eosinophilic cell count /mm ³	Intradermal reaction
3153	147	9	19900	910-340	+
22830	142	1	—	180	+
22230	135	5	8500-16600	60-1880	+
22627	108	25	6100	—	0
22271	147	25	4100	70-40	0
19207	134	2	8700	810-580	0
22144	137	34	4800	100-210	—
914	140	8	6200	1760-1120	+
748	142	15	7400	200-510	+
15322	127	10	14000-5000	1660-1180	+
22701	118	23	5300	270-480	+
19013	133	10	5400	300-710	+
22239	132	—	4000-8000	170-460	+
22580	154	3	8600	30-60	+
7034	136	6	5600	80-240	—
22671	141	5	9300-9000	740-1360	+
22085	120	24	5500	360	+
8041	124	13	6800	50	0

The anode vessel is on one side of the central trough, the cathode vessel on the other, the electrodes being soldered onto the bottom of vessels. The anode vessel as well as the cathode vessel are filled by 10–15 ml of veronal buffer at pH 8.6. Difco Noble Agar, stored in a refrigerator at 4° C has been used. Immediately before use the agar is liquefied in water-bath, 2–3 ml being applied to each slide which prior to use is degreased by alcohol and ether. When the agar on the slides has solidified, troughs and holes are punched and stamped out (cf. Fig. 2) for antibody and antigen, respectively, using special Agafor equipment constructed for this specific purpose. Conduction between anode/cathode vessels and agar on the slides is provided by strips of electrophoresis paper the breadths of which equal that of slides.

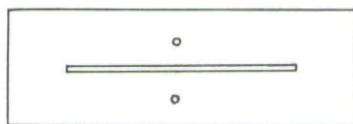
The process of electrophoresis covers 90 minutes at a voltage of about 40, the intensity of current being 400 milli amperes.

TABLE 3

Reg. no.	Radiography of thorax	Radiography of sinuses
3153	chronic bronchitis	bilateral maxillary sinusitis
22830	"	"
22230	localized, mild bronchiectases	"
22627	"	obs.: bilateral maxillary sinusitis
22271	"	leftsided maxillary sinusitis
19207	"	pan-sinusitis
22144	"	"
914	pulmonary emphysema, right intermediate lobe is the site of infiltration	rightsided maxillary sinusitis
748	"	"
15322	peri-bronchitis	"
22701	"	"
19013	pulmonary emphysema	"
22239	"	"
22580	"	"
7034	"	"
22671	"	polyp of the right maxilliary sinus
22085	bilateral basal pleural changes	polyp in the nasal cavity
		bilateral maxillary sinusitis
8041	—	—

*Fig. 1.*

A = anode vessel. B = central trough. C = frame. D = cathode vessel.

*Fig. 2.*

0.005 ml of antigen and 0.10 ml of antibody material is used for the immunoelectrophoresis which upon termination of electrophoresis is filled into the vessels. The plates with agar, antigen, and antibody are exposed to diffusion for 12 hours followed by rinsing in physiological saline for 12 hours and final brief rinsing in distilled water. Plates are stained for 15 minutes using amidoblack, followed by decolorization by water, ethanol, and acetic acid in a ratio of 50:40:10 for 12 hours and final rinsing in a 4 per cent acetic acid solution. For the inspection of plates a photographic projector is used at alternate magnifications.

- (1) Immunoelectrophoresis is carried out on sera from all of the patients.
- (2) The precipitation reaction between antigen and antibody is found to be dependent mainly upon the reciprocal concentrations, environment, pH, and sundry factors. Hence, in cases of pollinosis diffusion of serum from patients has been attempted against concentrations of grass pollen in ratios of $1:10^{-1}$ up to $1:10^{-7}$. Here the procedure has included the alternate use as antigen and antibody of sera from patients with pollinosis on the consideration that concentrations of pollen antibodies in serum are exceedingly small and that electrophoresis probably might operate to provide higher concentrations.¹
- (3) In one case of vasomotor rhinitis presenting positive cutaneous tests (cat's hair + + +) immunoelectrophoresis was performed using as antibody extract of tissue derived from a nasal polyp in the patient concerned. The organ extraction was prepared as follows: 5 to 10 g of nasal polypous tissue was rinsed in 4-5 changes of physiological saline and subsequently homogenized for 5 minutes in a Potter-Elvehjem's homogenizer. Prior to homogenization 0.5 and 2 ml, respectively, of saline was added in order to provide different antibody concentra-

¹ The pollen extract used in the present study has been prepared by the Allergologisk Laboratorium, Copenhagen.

tions in the product. Here extracts of cat's hair (Bencard) served as antigen.

- (4) On the consideration upon which also the above experiments have been based, viz. that antibodies in allergic disorders are in linkage with the reacting organ, we have in one case of Chlorpromazine induced icterus endeavoured to demonstrate a presence of antibody to Chlorpromazine in liver cells. When the Chlorpromazine induced icterus had subsided and the liver function tests had normalized two liver biopsies were taken a.m. Menghini from a 66-year-old female patient, the interval between biopsies covering 14 days. The biopsies were routine homogenized. Immunoelectrophoresis was carried out on serum from a healthy subject who 1 hour before blood sampling had received 50 mg of Chlorpromazine by the intramuscular route; on serum from a healthy subject whose serum had been incubated together with Chlorpromazine in vitro for 1 hour; and finally on serum from a healthy subject whose serum had not been in contact with Chlorpromazine, neither before nor after blood sampling.

Subsequently the patient was exposed to a provocative test using 10 mg of Chlorpromazine administered by the intramuscular route.

The values obtained by: thymol test, alkaline phosphatase, GPT-transaminase, and icterus index were markedly influenced thus showing that the liver cells had reacted to Chlorpromazine at the time of the test.

RESULTS

- (1) A comparison of a control material with results obtained by immunoelectrophoresis experiments in 3 groups of: bronchial asthma, vasomotor rhinitis, and pollinosis failed to show any significant difference between findings.
- (2) In the three cases of pollinosis no precipitation lines de-

veloped, not even after modification of antigen concentrations.

- (3) No pathological precipitation lines developed after tentative immunoelectrophoresis on homogenized tissue from a nasal polyp in a patient presenting vasomotor rhinitis and hence the result obtained must be considered negative.
- (4) Likewise immunoelectrophoresis on liver tissue from a patient with a past history of Chlorpromazine induced icterus failed to demonstrate a presence of antibodies for which reason the hypothesis of an allergic genesis could not be substantiated.

DISCUSSION

The negative results obtained in the present study may be attributable to various causes.

It has been mentioned that the pH level, the reciprocal concentration of antigens and antibodies, the salt concentration and general environment may be of importance in the development of precipitation lines. The patients were never exposed to provocative tests, cutaneous allergies admittedly not always being identical to allergies of the respiratory tract. Besides it had not been shown beyond doubt whether the antigens employed were of a haptenic nature or they were valid antigens.

But, the rather rudimental methods of examination and the minute amounts of protein involved may have combined to provide these results. Reference is made to "International Archives on Allergy and Applied Immunology", 1961 and 1962, in which a series of experiments have been reported by *Perelmutter, Lea, Freedman & Schon* who give an account of the detection of antibodies in patients suffering from Ragweed allergies, their material including treated as well as non-treated patients together with healthy subjects immunized by extracts of ragweed. In these experiments it was found necessary by chromatography of sera from the patients concerned to con-

centrate the antibodies in a well-defined chromatographic group. Sub-pressure dialysis later provided a concentration almost at the gel-limit level. Diffusion in gel a.m. *Ouchterlony* provided precipitation lines as an expression of the presence in the Ragweed extract of several allergens.

On the basis of these studies it must be reasonable to consider with a certain scepticism the establishment of a readily available technique of examination by which to determine allergic conditions, ragweed admittedly being on the most potent allergen.

SUMMARY

The purpose of the present study is to demonstrate by means of immunoelectrophoresis a presence of antibody in patients suffering from allergic disorders with a view to determining the allergens involved. The experimental results were negative.

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