

## IMMUNOLOGIC STUDY IN DRUG REACTIONS IMMUNOELECTROPHORETIC ANALYSIS OF SERUM AND BULLOUS FLUID IN SOME DRUG ERUPTIONS

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### Summary :

10 cases of bullous drug eruptions due to salicylates and sulphonamides were examined immunoelectrophoretically. Serum total protein was within normal limits. Bullous fluid protein was markedly diminished. Sera of patients of bullous drug eruption due to salicylates showed absence of Ig.G. in one case, Ig.A. in another case and Ig.M. in three cases. On the other hand sera of patients of bullous drug eruption due to sulphonamides showed absence of Ig.M. in two cases and its decrease in three cases. The fluid of salicylates bullous drug eruption showed absence of Ig.G. in one case and Ig.A. in another case. There was also decrease of Ig.A. in one case and decrease of Ig.M. in two cases. The bullous fluid of sulphonamide drug eruption showed absence of Ig.A. in one case and Ig.M. in two cases. Other abnormalities are mentioned.

### Introduction :

Cutaneous drug reactions are manifestations of a hypersensitivity state produced by the drugs or by a chemically related agent. Some drugs are antigenic by themselves, others form haptens as a result of irreversible binding with tissue proteins usually by covalent bonds (1).

The allergic reactions result from the antigenic characters of the drugs and

are independent of their pharmacological activities. Drugs may induce any type of allergic response by the formation of either reagins, precipitating antibodies or may induce delayed type hypersensitivity.

Fixed drug eruption almost certainly has an immunological basis but the evidence is conflicting and difficult to interpret. Histological and histochemical studies suggest that the dermis is primarily involved (2 & 3) and that cell bound antibodies are probably implicated (2).

Although paper electrophoretic studies have been previously done for sera and fluid of bullae in drug reactions (4) yet the sensitivity of this technique is too low to detect even normal amounts of immunoglobulins A and M (5).

The purpose of this work is the study of any possible change in the sera and fluid aspirated from bullae in some cases of bullous drug eruption by the immunoelectrophoretic technique.

### Material :

For the purpose of this study, three groups of patients with past history of bullous drug eruptions, were admitted to hospital.

### GROUP I :

Five patients were included in this group, three males and two females. Their age ranged from 18-36. All five

patients had a past history of bullous drug eruption after the administration of salicylates. Patients were accustomed to take salicylates as an analgesic to relieve headache.

#### GROUP II:

The other five cases constituted group II. Of these five cases, three were females with an average age of 25 years, the other two were males with an average age of 25 years. All had a past history of drug reactions following the intake of sulphonamides.

#### GROUP III:

10 normal persons (from the personal working at the hospital and medically free from any disease) were collected at random. The results of this group were taken as our normal control.

Prior to the administration of the offending drug, blood sera were collected from all the patients. These blood sera were utilized for the estimation of their total proteins and for the study of their immuno-electrophoretic pattern.

Patients were then given the offending agent (aspirin tablets for group I and sulphonamide tablets for group II). Within 24 hours, the eruption developed in all patients, it took the following morphologic appearances:

—Plaques of erythema and oedema located on the dorsal surfaces of both hands and lower lip. Large clear bullae developed on these plaques shortly after. (case 1, 2, 3, 5, 6).

—Erythema Multiforme like picture in the remaining cases. Blood sera were collected from all the patients 24 hours after the appearance of the bullae and kept in deep freeze. Under complete and thorough aseptic conditions the fluid of the bullae was aspirated and sufficient quantity could be obtained.

Blood sera were utilized for total protein estimation and immuno-electrophoretic pattern formation. Fluid bullae were utilized for an immuno-electrophoretic pattern formation.

#### Methods:

- Total serum protein and fluid proteins were estimated by the method of King 1959 (6).
- Immuno-electrophoretic pattern of sera and fluid proteins was carried out according to the micromethod devised by Scheidegger (7) and the double migrate of Blanc (8). The antihuman-serum (Poly valent) Von kaninchen, Biotest-serum Institute Gmb H. was used in this work.

#### Results:

The average range of total serum proteins in all three groups was within normal limit. On the other hand the total fluid proteins of bullae were markedly diminished as compared to their specific sera or to normal controls. No abnormality was detected in the serum immuno-electrophoretic pattern of the patients prior to drug administration. The results of total sera and bullous fluid proteins and the normal controls are shown in table I.

The immuno-electrophoretic analysis of sera of patients complaining of drug eruption due to administration of salicylates as compared with normal controls showed the following data:

- Case I: There was absence of Ig.G, lipoprotein and antitrypsin (Fig. I).
- Case II: There was absence of Ig.M., lipoprotein and antitrypsin (Fig. II).
- Case III: There were decrease of both Ig.A. and Ig.M.
- Case IV: There was absence of Ig.M. and lipoprotein.
- Case V: There was absence of Ig.M., and antitrypsin.

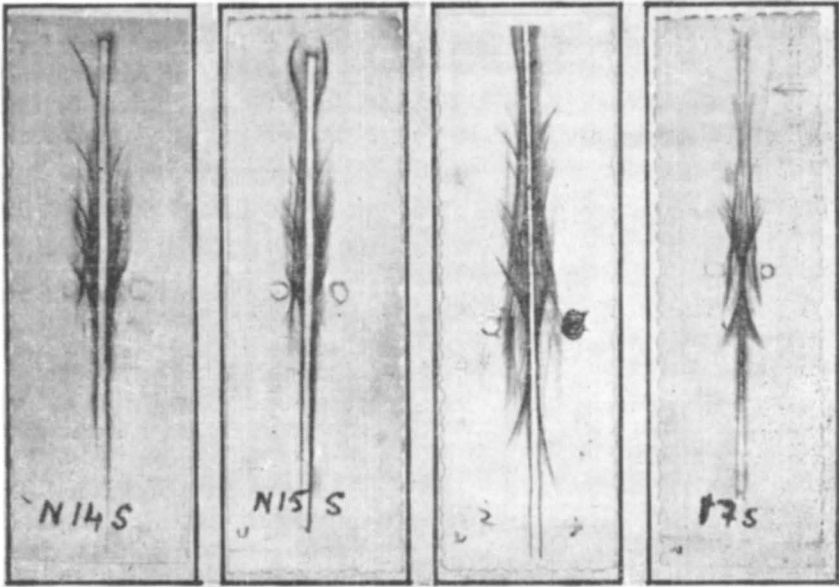


Fig. 1. 2. 3. 4

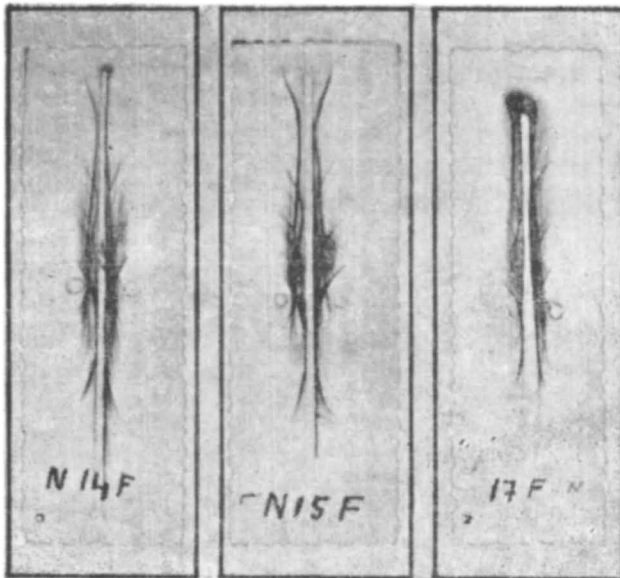


Fig. 5, 6, 7

Drug administered	No.	Sex	Age	Normal serum protein in G/100 ml.	Patient serum protein in G/100 ml.	Fluid protein in G/100 ml.
Salicylates	1	Male	18	6.9	6.0	4.4
	2	Male	30	6.8	5.8	4.2
	3	Male	24	6.9	6.2	4.0
	4	Female	36	6.8	6.4	4.4
	5	Female	28	6.8	6.6	4.2
Sulphonamides	6	Female	21	6.2	6.0	4.2
	7	Female	27	5.8	6.2	4.0
	8	Female	30	6.4	6.2	4.4
	9	Male	20	6.6	6.4	4.0
	10	Male	30	6.8	6.2	4.2

The immunoelectrophoretic analysis of sera of patients complaining of bullous drug eruption due to sulphonamides as compared with normal controls showed the following changes :

Case I : There was absence of Ig.M and decrease in Ig.A. and Ig.G.

The antitrypsin band differed in shape and was less in concentration than the normal control. The albumin was present but not in boat shape as the normal control. The prealbumin band was definite, clear and longer (Fig. III).

Case II : Showed the presence of an abnormal band above the Ig.G. zone, with decrease of Ig.M. (Fig. IV).

Case III : Showed absence of Ig.M.

Case IV : Showed decreased Ig.M.

Case V : Showed decreased Ig.M. and lipoprotein.

The immunoelectrophoretic analysis of fluid aspirated from bullae in salicylate hypersensitive patients showed the following abnormalities :

Case I : Showed absence of Ig.G. and antitrypsin. An abnormal faint band above the Ig.G. zone was detected (Fig. V).

Case II : Showed absence of Ig. M., hemopexin and lipoprotein. Ig.A. was decreased (Fig.VI).

Case III : Showed decreased Ig. A. and antitrypsin.

Case IV : Showed decreased Ig. M. and hemopexin.

Case V : Showed decreased Ig. M. only.

The immunoelectrophoretic analysis of fluid aspirated from bullae due to sulphonamides showed the following abnormalities :

Case I : The Ig.G., Ig.A. and Ig.M. were stuck together at the beginning, Ig. M. had a different shape and was nearer to the trough. Ig. A. was less in concentration. Lipoprotein was thicker and more concentrated. There was a band nearer the prealbumin which is not present in the normal.

Case II : Showed absence of Ig. M. and Ig. A. (Fig. VII).

Case III : There was decreased Ig. M.

Case IV : Showed decreased Ig. M. and absence of lipoprotein.

Case V : Showed the same picture as case II.

### Discussion :

The mechanisms of various drug reactions up till now are unknown. Although in some cases most of the circumstantial evidences are in favour of the reaction being of an immunological background, the mechanism is often obscure since most of the reactions cannot yet be reproduced in experimental animals.

In view of the fact that drug reactions may take the form of any of the well known groups of hypersensitivity states, i. e. anaphylactic, cytotoxic, Arthus type and a delayed type of reaction. The present bullous drug eruption may be considered somehow or other to belong to either the cytotoxic or the Arthus type of reaction. On the other hand the drugs used in this work are known to act as simple haptens which without its presence no antigen antibody reaction will take place.

The results obtained concerning the different reactions of immunoglobulins encountered in the investigated cases in either sera or fluid aspirated from the bullae suggest the following remarks :

1. A very interesting and curious abnormal band was encountered above the Ig. G. fraction in both sulphur and salicylates drug eruption in hypersensitive patients. The presence of this band may be of great importance to sustain the hypersensitivity state of the individual.

2. There is a deficiency in Ig. G. in sera and bullous fluid in some cases of both sulphur and salicylate hypersensitivity. A direct explanation is unfortunately not possible but we suggest that deficiency may be either :

A - Due to neutralization effect it exerts on the drug protein complex or,

B - It is still possible that this deficiency by itself predisposes the individual for his drug hypersensitivity state.

We are in fact inclined to believe in the first suggestion since our results showed that this deficiency in Ig. G. is not uniform in all cases, a matter that could be explained easily if we attain the theory of neutralization effect.

In fact the absence of Ig. G. in some cases and the presence of new bands in others may give a good ground to assume that the bullous drug reaction is not the genuine delayed type of hypersensitivity state, since the latter is characterized by the absence of active humoral antibodies.

3. Generally speaking there was discrepancy between the sera proteins in normal individuals and hypersensitive patients. Meanwhile the total fluid protein aspirated from bullae was found by us as well as by other investigators (6) to be lower than the serum protein of the same individuals. This, however, can be explained by the presence of certain intactness of the barrier between the serum and tissue fluids. Also we can assume that the bullous fluid proteins are formed as a result of local destruction of cells. This assumption could not be easily attained in view of the fact that similar and even parallel changes take place in bullous immunoglobulins hand in hand with changes encountered in the serum.

4. The absence of antitrypsin in serum and bullous fluid in some cases of both salicylate and sulphonamide hypersensitivity state may be explained on the assumption that it is utilized in process of bulla formation by interacting with intercellular trypsin. If this hypothesis is true we can deduce that bullae formation in fixed drug eruption may in part result from a biochemical process, the antitrypsin—trypsin interaction is of prime importance. However, this deficiency was not manifested in all the cases examined.

This preliminary report however, it is early to assess, it will be naturally throws some light on the formation of the long path for the future. bullae in fixed drug reactions although

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