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The Medico-Legal Importance of Immunological Cross Reactions

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Summary. Serum proteins from humans and various animal species were tested for immunological relationship (immunological cross reactions) by immunoelectrophoresis and electric precipitation, using various anti-human immune sera. The presence of similar constituents in the polypeptide chains of protein molecules may give rise to cross reactions, which interfere with the identification of the species, from which the blood stain is originating.

It is proposed that sets of immune sera be tested before use in order to exclude cross reactions. The introduction of anti-human haemoglobin (red cell stroma) immune sera into routine criminological blood stain analysis is recommended.

Zusammenfassung. Es wurde die immunologische Verwandtschaft (immunologische Kreuzreaktivität) von Menschenserum und verschiedenen Tierseren mittels gewissen Anti-human-Immunseren, Immunelektrophorese sowie Elektrosynerese getestet. Die partielle Ähnlichkeit von Polypeptidketten der Protein-Moleküle kann Kreuzreaktionen herbeiführen, die die Bestimmung der Herkunft von Blutspuren erschweren können.

Es wird empfohlen, daß Sätze von Immunseren vor der Anwendung auf Kreuzreaktivität untersucht werden. Die Einführung von Anti-Hämoglobin(Rotblutzellen-Stroma)-Immunseren in die gerichtsmedizinische Praxis wird vorgeschlagen.

Key words: Cross-reacting antibodies — Blood stains.

Introduction

The serum proteins, above all the immunoglobulins, of man and various animal species are in certain respects similar to one another. These structural and functional similarities, which may give rise to immunological cross reactions, are of an evolutionary origin [1—3] and may interfere with the results of criminological analysis for the origin (species specificity) of blood stains [4, 8].

Materials and Methods

Whole sera from man, swine, cattle, goat, rabbit and chicken were compared by immunoelectrophoretic method [9], using various anti-human immune sera (anti-human horse immune sera[1] designated 249, 375, L-111 and L-172, anti-human goat immune serum[2] designated G-26, rabbit immune serum[3] R-129 to human red cell stroma and haemoglobin). Electric precipitation [5—7] was carried out as follows: Difco agar dissolved in 25 ml 1% veronal buffer of

1 Institute for Serobacteriological Production and Research "HUMAN".

2 Supplied by Dr. Gy. Kocsis, PHYLAXIA Veterinary Biologicals and Foodstuffs Co.

3 Supplied by Dr. J. Jákó (Postgraduate Medical School, Budapest).

Table 1. Testing of whole sera from man and various animal

Whole sera	Anti-human immune sera					
	249			375		
	IgG	alpha	alb.	IgG	alpha	alb.
Human	++++	++++	++++	++++	++++	++++
Swine	++++	+	++	—	+	—
Cow	++++	+++	—	—	++	—
Goat	++++	++	—	—	++	—
Rabbit	+	++	—	—	+++	—
Chicken	—	—	—	—	—	—

pH 8.2 was spread on a glass plate, 26 × 7.6 cm in size. The wells punched into the agar were 2 mm in diameter and pairs of wells were spaced equidistant, 6 mm away from one another. The test material was placed in an electric field for 30 min, during which the electric tension was maintained at 40 mA. Subsequently the plates were placed in a moist chamber for 1 hr, immersed in saline for 2 hrs, dried and stained (Ponceau S).

Blood stains were prepared from whole blood of man, swine, horse, cow, goat and roe deer on a linen cloth, dried at room temperature and tested 7 days after drying.

Results and Discussion

The behaviour of whole sera from 6 species towards 4 different immune sera prepared in horse is shown in Table 1. All 4 anti-human sera gave a strong reaction, designated with 4 crosses (++++), with the human serum. Cross reactions were obtained only with IgG immunoglobulin, certain proteins migrating with the alpha-1 and alpha-2 fractions and albumin. The intensities of reactions with the different protein fractions were designated with an appropriate number of crosses. Immune serum No. 249 cross-reacted especially intensively with porcine, bovine and caprine IgG, and slightly even with rabbit IgG. It also reacted with proteins

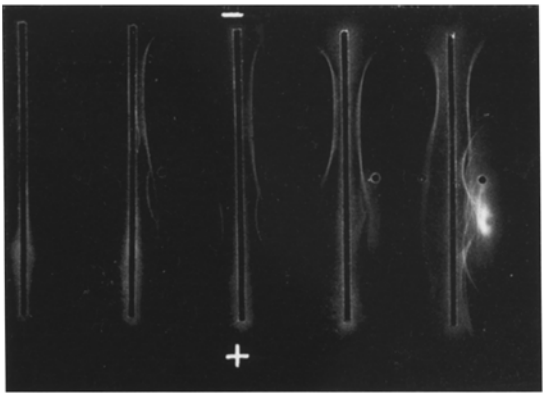

Fig. 1. The reactions with immuno serum No. 249. In the outside start-hole is human serum, to the left: immunochemically purified human IgG, swine serum, purified swine IgG, cow serum, rabbit serum, goat and chicken sera

species for cross reactions by immunoelectrophoretic analysis

L-111			L-172		
IgG	alpha	alb.	IgG	alpha	alb.
++++	++++	++++	++++	++++	++++
++	+	++	+	+	+
+	++	+	+	++	+
++	++	—	+	+	—
—	++	+	—	++	+
—	—	—	—	—	—

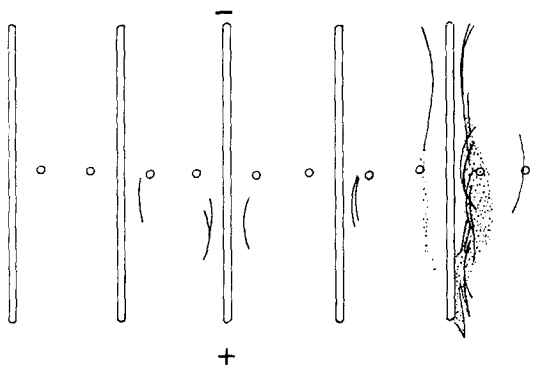

Fig. 2. Schematic outline of reactions with immune serum No. 375 (sera are similar to Fig. 1)

of the alpha-1 and alpha-2 fractions and formed a well visible precipitation arch with porcine albumin. Immune serum No. 375 proved to be the least cross-reactive, forming precipitation arches only with a few alpha proteins. None of the immune sera reacted with chicken serum (Figs. 1 and 2).

As the routine criminological analysis of blood stains is based on electric precipitation, the experimental studies were carried out with protein components of dry blood stains. The saline-dissolved constituents differ from these of the normal blood in several respects (e.g. migration velocity, fragmentation, etc.). Immunological cross reactions were also followed up by electric precipitation,

Table 2. Analysis of saline extracts of dry blood stains by electric precipitation

Blood stain	Anti-human immune sera					
	249	375	L-111	L-172	G-26	R-129
Human	++++	++++	++++	++++	++++	++++
Swine	+++	—	++	—	—	—
Horse	—	—	—	—	—	+
Cow	++	+	+	—	—	—
Goat	+	+	—	—	—	—
Deer	++	—	+	—	—	—

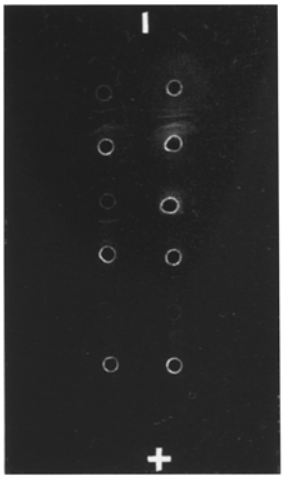

Fig. 3

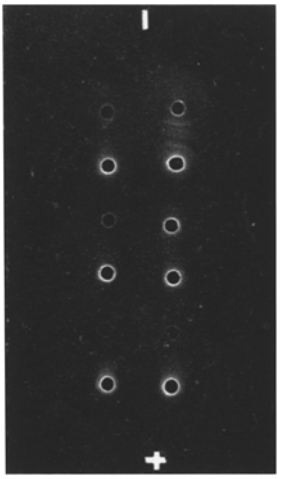

Fig. 4

Fig. 3. To the right at the top is human blood stain, to the left that of a swine (immune serum No. 249)

Fig. 4. Immune serum No. 172 reacted only with human blood stain (to the right at the top)

using several immune sera. As can be seen from Table 2, apart from the previously employed 4 horse immune sera, a polyvalent anti-human goat serum and antiserum to human red cell stroma (and haemoglobin) were also used. The immune serum No. 249 cross-reacted with extracts from all blood stains except horse blood (because the antiserum was prepared in horse), whereas immune serum No. 172 and that prepared in goat did not cross react with any test material. It is remarkable that the antihuman anti-haemoglobin (and red cell stroma) serum cross-reacted exclusively with the extract of the horse blood stain (Figs. 3 and 4).

The polypeptide chains of mammalian protein molecules possess several common antigenic determinants. Certain members of the amino acid sequence may also resemble one another between species. It is known from the literature [4] that sera from hyperimmunized animals react especially readily with blood proteins from other animal species or man. The immune serum No. 249 used in the present studies originated from a horse which has been a serum donor for several years.

It is proposed that all series of immune sera should be tested for the degree of cross reactivity with sera from other species, and those sera should preferably be used for testing with which no cross reaction occurs. It is also recommended that at least two different antihuman immune sera should be employed for blood stain analysis and that an appropriate control system should be set up with each test. For the analysis of dry blood stains, anti-human haemoglobin (and red cell stroma) immune sera should be additionally used.

References

1. Hafleigh, A. S., Williams, C. A.: Antigenic correspondence of serum albumins among the primates. Science **151**, 1530 (1966)
2. Hill, R. L., Delaney, R., Fellows, R., Lebovitz, H.: The evolutionary origins of the immunoglobulins. Proc. nat. Acad. Sci. (Wash.) **56**, 1762 (1966)

3. Singer, S. J., Doolittle, R. F.: Antibody active sites and immunoglobulin molecules. Science **153**, 13 (1966)
4. Esteves, M.-B., Binaghi, R. A.: Antigenic similarities among mammalian immunoglobulins. Immunology **23**, 137 (1972)
5. Bussard, A.: Description d'une technique combinat simultanément l'électrophorése et la précipitation immunologique dans un gel: l'électrosynérése. Biochim. biophys. Acta (Amst.) **34**, 258 (1959)
6. Culliford, B. J.: Precipitin reactions in forensic problems. Nature (Lond.) **201**, 1092 (1964)
7. Backhausz, R.: Immunodiffusion und Immunoelektrophorese. Budapest: Akadémiai Kiadó 1967
8. Ottó, Sz., Somogyi, E.: Importance of antigen-relation of mammalian-serum proteins in criminalistic. 9th Congress of the International Academy of Legal Medicine and Social Medicine, Róma, September 23—28, 1973
9. Scheidegger, J. J.: Une microméthode de l'immuno-électrophorése. Int. Arch. Allergy **7**, 103 (1955)

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