

GROUPED VARIATIONS IN PIG SERA DEMONSTRATED BY AN IMMUNO-ELECTRO- PHORETIC TECHNIQUE

By

*E. Brummerstedt-Hansen*¹⁾ and *J. Hirschfeld*²⁾

With immunoelectrophoresis (*Grabar & Williams, 1953*) according to a slightly modified technique (*Hirschfeld, 1960*), sera from humans (*Hirschfeld, 1959*), monkeys (*Beckman, Hirschfeld & Söderberg, 1961*) and rabbits (*Hirschfeld, 1959*) can be divided into different types depending on the existence of electrophoretically variable proteins with varying occurrence in individual sera. Thus, in humans at least three protein systems, the haptoglobins (Hp) (*Smithies, 1955*), the transferrins (Tf) (*Smithies, 1957*) and the group-specific components (Gc) (*Hirschfeld, 1959*) have been found to give rise to electrophoretically different and genetically determined patterns demonstrable by starch-gel electrophoresis (Hp and Tf) (*Smithies, 1955, 1957*) and immunoelectrophoresis (Hp, Tf and Gc) (*Hirschfeld, 1959; Beckman, Hirschfeld & Söderberg, 1961*).

Immunoelectrophoretic investigations of pig sera against anti-pig immune sera prepared in rabbits have until now not shown any systematic variation between individual animals (*Brummerstedt-Hansen, 1961*). With a slightly modified technique, described elsewhere (*Hirschfeld, 1960*), sera from 37 pigs belonging to Danish Landrace could, however, be divided into several qualitatively distinct patterns. 3 main types were distinguished in the

¹⁾ Dept. of physiology, endocrinology and bloodgrouping, The Royal Veterinary and Agricultural College, Bülowsvej 13, Copenhagen, Denmark.

²⁾ State Institute for Blood Group Serology, Statens Rättskemiska Laboratorium, Stockholm 60, Sweden.

α_2 -globulin region depending on the existence of a fast (α_2F), a slow (α_2S) or a two-peaked (α_2F+S) precipitate with its peaks corresponding to the electrophoretic positions of the fast (α_2F) respectively slow (α_2S) normally arc-shaped precipitates found in certain pig sera. A mixture in equal proportions of the α_2F and α_2S types gave in immunoelectrophoresis precipitates indistinguishable from the normally occurring two-peaked precipitate found in sera of type(α_2F+S) (Fig. 1). Thus, these

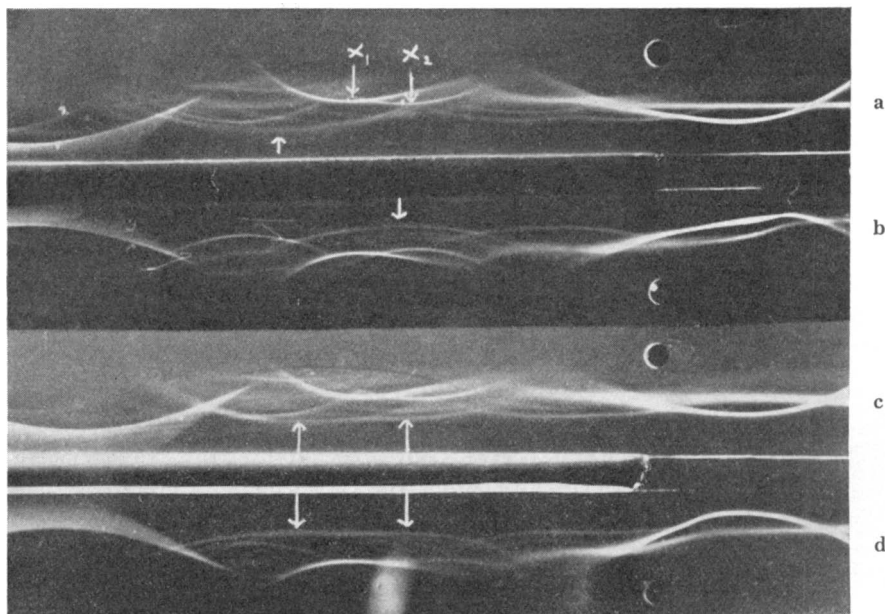


Fig. 1. The different α -globulin types. 1a: pig. no. 1, α_2F ; 1b: pig no. 2, α_2S ; 1c: mixture of pig 1 and 2, both components are visible; 1d: pig. no. 15, α_2F+S . X_1 and X_2 : see the text.

variations seem to be caused by two electrophoretically different although immunologically identical α_2 -globulins occurring either alone (α_2F and α_2S) or simultaneously (α_2F+S). Independently of these variations a precipitate situated in the β_1 -globulin region was also found to occupy different electrophoretic positions and shapes in individual sera morphologically identical to the variations observed in the α_2 -globulin region. These β_1 -globulin patterns will accordingly be called β_1F , β_1S and β_1F+S , where the type β_1F+S can also be obtained by mixing sera belonging to β_1F and β_1S in equal proportions (Fig. 2).

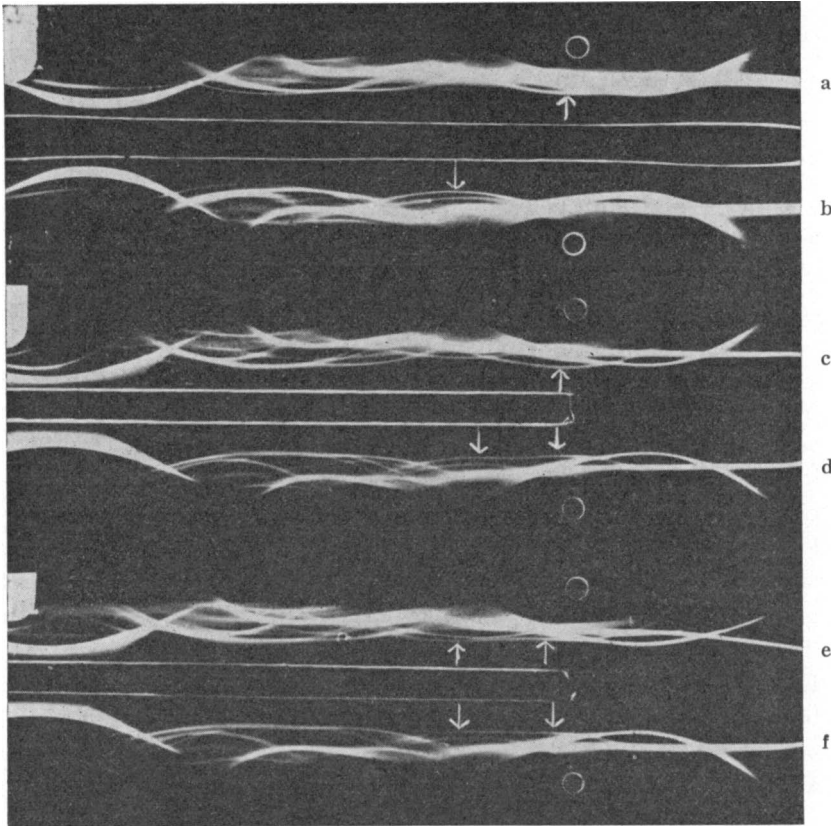


Fig. 2. The different β -globulin types. 2a and 2c: pig. no. 3, β_1S ; 2b: pig no. 4, β_1F ; 2d and 2f: pig. no. 15, β_1F+S ; 2e: mixture of pig 3 and 4, both components are visible.

In Table 1, the distribution of the different α_2 - β_1 -globulin types is given for the 37 pigs investigated. The determinations were made independently by two observers. Disagreeing results were obtained for some sera due to the existence of certain intermediary α_2 - or β_1 -precipitates and/or poorly resolved patterns due to the other variable precipitates in certain pig sera. These sera were classified as $\alpha_2(?)$ or $\beta_1(?)$. Repeated examinations of two pig sera in more than 17 independent immuno-electrophoretic experiments gave identical results as regards their α_2 - β_1 -globulin types when independently determined by two observers and thus seem to show that these variations have a high degree of technical reproducibility.

Table 1.
Distribution of different α_2 - β_1 - globulin types.

	β_1 F	β_1 F+S	β_1 S	$\beta_1(?)$	Total
α_2 F	2	2	0	5	9
α_2 F+S	1	2	4	2	9
α_2 S	4	5	1	3	13
$\alpha_2(?)$	3	2	1	0	6
Total	10	11	6	10	37

In a few instances, the determinations were verified by certain mixture experiments. If e.g. the unknown serum mixed with a serum belonging to the Fast type gave a two-peaked precipitate indistinguishable from the normally occurring two-peaked precipitate occurring in certain unmixed pig sera, whereas the unknown serum upon mixture with a serum belonging to the Slow type gave a normally arc-shaped precipitate identical with that given by the unmixed serum belonging to the Slow type, then this unknown serum was regarded as belonging to the Slow type. For some of the sera classified as $\alpha_2(?)$ or $\beta_1(?)$ such "verification tests" as well as repeated examinations of these sera unmixed seem to show that components intermediary to the Fast and Slow α_2 - and β_1 -components exist and are technically reproducible. Similar very rare intermediary types have also been found for the Gc-system in man and monkeys (*Hirschfeld, J.*, to be published).

Addition of hemoglobin to the sera prior to immunoelectrophoresis did not give any detectable changes in the immunoelectrophoretic patterns when compared with the same sera to which hemoglobin had not been added. Benzidine staining showed that the variable β_1 -globulins have the capacity to bind and transport hemoglobin. These components are also electrophoretically very similar to the human hemoglobin-binding β_1 -globulin although in man no variations in electrophoretic position or shape of this precipitate have yet been observed (*Hirschfeld, 1960*). Furthermore two other α_2 -globulins (X_1 and X_2) (Fig. 1) were in some experiments stained by the benzidine reagent. No variations in electrophoretic positions, shapes or sites between diffusion centres have been observed for these benzidine stainable α_2 -components in the pig sera investigated by means of immuno-

electrophoresis agar-gel electrophoresis and starch-gel electrophoresis. Both these precipitates are situated close to the antigen diffusion centres and one of them (X_1) has an asymmetrical shape. This precipitate is thus very similar to the human α_2 -macroglobulin precipitate as regards electrophoretic position, shape and site between diffusion centres.

The variable α_2 -globulins are very similar to the human Gc-system as regards their variations, electrophoretic positions site between diffusion centres and shapes. It is also interesting to note that the anti-pig immune serum used in the present study gave strong precipitate formation with the human Gc-precipitates. So far, no absolute proof for the immunological relation of these variable α_2 -globulins with the human Gc-system has, however, been obtained.

In conclusion, pig sera can be divided into 9 main patterns depending on the existence of two electrophoretically different protein systems each consisting of at least two electrophoretically identical main components which might occur either alone or simultaneously. The existence of further electrophoretically different although immunologically identical components within each of these systems seems highly probable. The β_1 -globulin system has been tentatively identified as a hemoglobin-binding β_1 -globulin electrophoretically similar to the human hemoglobin-binding β_1 -globulin whereas the variable α_2 -globulins do not take the benzidine stain. Instead, two other α_2 -globulins (X_1 and X_2) have been found to bind hemoglobin in some experiments and these components are constant in all sera hitherto investigated.

A genetical basis for these variations does seem highly probable and further studies are in progress as regards the constancy and inheritance of these patterns.

REFERENCES

- Beckmann, L., Hirschfeld, J. & Söderberg, U.:* Acta path. microbiol. scand. 1961, 51, 132.
- Brummerstedt-Hansen, E.:* Acta vet. scand. 1961, 2, 254.
- Grabar, P. & Williams, C. A.:* Biochim. biophys. Acta 1953, 10, 193.
- Hirschfeld, J.:* Acta path. microbiol. scand. 1959, 47, 160.
- Hirschfeld, J.:* Acta path. microbiol. scand. 1959, 46, 229.
- Hirschfeld, J.:* Science Tools 1960, 7, 18.
- Hirschfeld, J.:* Acta path. microbiol. scand. 1960, 49, 255.
- Smithies, O.:* Nature 1955, 175, 307.
- Smithies, O.:* Nature 1957, 180, 1482.

SUMMARY

With a slightly modified immunoelectrophoretic technique (lactate-buffer, 1 % agar gel) 37 sera from pigs of the Danish Landrace are investigated as regards specific variations. The investigations show that it is possible to distinguish between 3 different types both in the α_2 -field and in the β_1 -field.

In these two regions individual sera present either a fast, a slow, or a two-peaked precipitate, and in consequence it is possible to group sera in 9 different main types the distribution of which is shown in Table 1. Under α_2 (?) and β_1 (?) are indicated the number of sera for which it was not possible to group strictly in the 9 main types. This is probably due to the existence of certain intermediary α_2 - or β_1 -precipitates and/or poorly resolved patterns due to other variable precipitates in pig sera.

ZUSAMMENFASSUNG

Mittelst immunoelektrophoretischer Technik gruppierte Variationen in Schweineseren.

Mittelst einer modifizierten immunoelektrophoretischen Technik (Laktatpuffer, 1 % Agargel) wurden 37 Sera von Schweinen der dänischen Landrasse auf spezifische Variationen untersucht. Die Ergebnisse zeigen, dass sich sowohl im α_2 - als auch im β_1 -Gebiet 3 verschiedene Typen unterscheiden lassen, da das betreffende Serum in jedem der beiden Gebiete eine schnell wandernde, eine langsam wandernde Komponente oder beide Komponenten besitzt, was die Möglichkeit von 9 verschiedenen Typen ergibt. Die Anzahl der Schweine, die sich auf jeden der 9 Typen zurückführen liess, geht aus der Tabelle 1 hervor, ausserdem findet sich aber in dieser Tabelle unter α_2 (?) und β_1 (?) die Anzahl der Sera, die sich vermutlich wegen intermediärer Typen oder anderer variabler Komponenten nicht unter diese 9 Haupttypen streng einordnen liess.

RESUMÉ

Grupperede variationer i svinesera påvist med en immunoelektroforetisk teknik.

Med en modificeret immunoelektroforetisk teknik (laktatstødpude, 1 % agargel) er 37 sera fra svin af dansk landrace undersøgt med henblik på specifikke variationer. Undersøgelserne viser, at der i såvel α_2 - som β_1 -området kan skelnes mellem 3 forskellige typer efter om pågældende serum i hvert af de to områder har en hurtigt vandrende komponent, en langsomt vandrende eller har begge komponenter, hvilket giver mulighed for 9 forskellige typer. Antallet af svin, der har kunnet henføres til hver af de 9 typer, er vist i Tabel 1, men desuden rummer tabellen under α_2 (?) og β_1 (?) antal sera, der antagelig på grund af intermediære typer eller på grund af andre variable komponenter ikke har kunnet indordnes strengt under de 9 hovedtyper.

(Received August 8, 1961).