Acta vet. scand. 1963, *4*, 238—246.

From the Department of Physiology, Endocrinology and Blood-grouping, The Royal Veterinary and Agricultural College, Copenhagen, Denmark.

HAPTOGLOBIN POLYMORPHISM IN PIGS

By

Michael Hesselholt

Hemoglobin-binding proteins, haptoglobins, were first observed in human sera by *Jayle* and *Polonovski* in 1938. Later it was demonstrated by *Jayle* and *Gillard,* that some human sera contained two haptoglobins which differed from each other by their solubility in ammonium sulphate. Starch gel electrophoresis of human sera showed the presence of the two fractions. According to their mobility in the starch gel the components were designated Hp 1 and Hp 2. Hp 1 migrated faster in the gel than Hp 2. By means of the same electrophoretic technique it was further demonstrated, that the two haptoglobins occurred in sera from different human individuals in grouped variations (*Smithies 1955 a, b*). Family studies indicated, that the haptoglobin polymorphism was under simple genetic control determined by a single pair of alleles, Hp^1 and Hp^2, with codominance (*Smithies* and *Walker 1955, 1956*).

In an immunoelectrophoretic study of sera from pigs of Danish Landrace grouped variations of protein components in the α_2- and β_1-globulin field were observed, but a distinct grouping was not possible (*Brummerstedt-Hansen* and *Hirschfeld* 1961). By means of starch gel electrophoresis of sera from Yorkshire- and Landrace pigs 10 haptoglobin fractions were detected. Following the principle of the human haptoglobin nomenclature they were designated Hp 1—Hp 10. Based on the presence in sera of Hp 1, Hp 2 and Hp 3 the following six phenotypes were established: Hp 1—1, Hp 2—2 and Hp 3—3, which possessed only one of the components, and Hp 2—1, Hp 3—1 and Hp 3—2, which each possessed two of the three haptoglobins. Genetic

investigations indicated the probability, that the synthesis of the three haptoglobins was under simple genetic control determined by three non dominant alleles: Hp^1, Hp^2 and Hp^3 respectively (*Kristjansson 1961*). In a preliminary starch gel electrophoretic study of sera from pigs of Danish Landrace haptoglobin polymorphism involving Hp 1, Hp 2 and Hp 3 was later demonstrated by Danish investiagtors. At the same time a new haptoglobin component, Hp 0, was observed migrating in the gel just in front of Hp 1. Hp 0 either occurred singly or together with only one of the other components. Therefore the hypothesis was advanced, that the occurrence in sera of Hp 0, Hp 1, Hp 2 and Hp 3 was under genetic control of four alleles, but further data were necessary to support the hypothesis (*Brummerstedt-Hansen et al. 1962, a, b*). Recently a fast migrating haptoglobin, called Hp 0, has been described, but the idendity between this component and the above has not yet been established (*Kristjansson 1962*).

The purpose of the present investigations was to determine the heredity of the haptoglobin types in pigs of Danish Landrace, and further to elucidate the genetic structure of this breed with respect to the genes which control the haptoglobin synthesis.

MATERIALS AND METHODS

Serum samples from 900 pigs selected at random and from a family material comprising 71 matings with 344 piglets, all of Danish Landrace, were haptoglobintyped by means of the starch gel electrophoretic technique.

Preparation of hemoglobin: The blood samples were drawn into a citrate solution. After washing four times with a 0.9 per cent saline solution the red blood cells were packed (4500 r.p.m. for 30 min.). To 1 volume of packed erythrocytes 2 volumes of distilled water were added. After completion of lysis the stroma was removed from the solution of free hemoglobin by centrifugation (4500 r.p.m. for 90 min). The hemoglobin content was measured spectrophotometrically. The hemoglobin solution was stored at — 20 °C until used.

Addition of hemoglobin to the serum sample prior to electrophoresis: Addition of increasing amounts of pig hemoglobin to a serum sample showed that approximately 100 mg % hemoglobin was required to saturate the haptoglobins. Individual quantitative differences in the haptoglobin content made it neces-

sary to add hemoglobin in excess in routine haptoglobin typing to ensure, that all hemoglobin-binding proteins were identified. This resulted in a staining of free hemoglobin besides the staining of the hemoglobin-haptoglobin zones. The position of free pig hemoglobin was just anodic to the hemoglobin-haptoglobin zones under the electrophoretic conditions in this study. Therefore the reading of the stained gels was very difficult and inaccurate. Experiments, in which hemoglobins from other animal species were used in stead of pig hemoglobin, demonstrated that chicken hemoglobin was able to combine in a complex formation with pig haptoglobin. After electrophoresis excess of chicken hemoglobin will be located cathodic to the hemoglobin-haptoglobin bands. Chicken hemoglobin in amounts of 250—300 mg % was used in the experiments. Prior to electrophoresis the hemoglobin-serum mixture was incubated at 37°C for 30 minutes.

Starch gel electrophoresis: Horizontal starch gel electrophoresis in a discontinuous system of buffer solutions was used. The gels were made with a 0.076 M tris (hydroxymethyl) aminomethane — 0.005 M citric acid buffer solution. The electrode vessels and filter paper bridges contained a 0.30 M boric acid — 0.06 M sodium hydroxide buffer solution (*Poulik 1957*). The gels were prepared using 16 g hydrolysed potato starch per 100 ml gel buffer. Size of gel: (20×13×0.3 cm). Insertion material: Whatmann no 3 filterpapir (0.6×0.3 cm). Voltage gradient: 15—20 v/cm. Electrophoresis duration: 2 hours. Electrophoresis was carried out at 1—3°C in a refrigerator.

Staining procedure: On completion of electrophoresis the gels were sliced horizontally and stained for 2 hours in the following benzidine solution (*Smithies 1955*):

Benzidine	mg 200
Distilled water	ml 100
Glacial acetic acid	ml 0.5
Hydrogen peroxide	ml 0.2

Zones in which peroxydase activity appeared such as hemoglobin and hemoglobin-haptoglobin zones become visible as blue bands after the gels have remained in this solution for 1 hour. After 2 hours the gels were read and then they were washed several times in distilled water. The gels were stored at 4°C until the next day, when new observations were made.

RESULTS

By means of starch gel electrophoresis of sera from 900 adult pigs, selected at random and from a family material comprising 71 matings with 344 piglets, ten general types of pattern with respect to Hp 0, Hp 1, Hp 2 and Hp 3 were obtained. The ten phenotypes are shown in fig. 1. Haptoglobin polymorphism involving Hp 4—Hp 10 was not investigated.

If the samples contained no pig hemoglobin beforehand, the addition of chicken hemoglobin caused a complex formation between chicken hemoglobin and Hp 5. This complex was separated during electrophoresis into three fractions as demonstrated in fig. 1. Individual quantitative variations were found in these three fractions. Hp 4 was present in different amounts in the sera investigated. In some cases Hp 4 was absent. None of the sera analysed was devoid of all four haptoglobins.

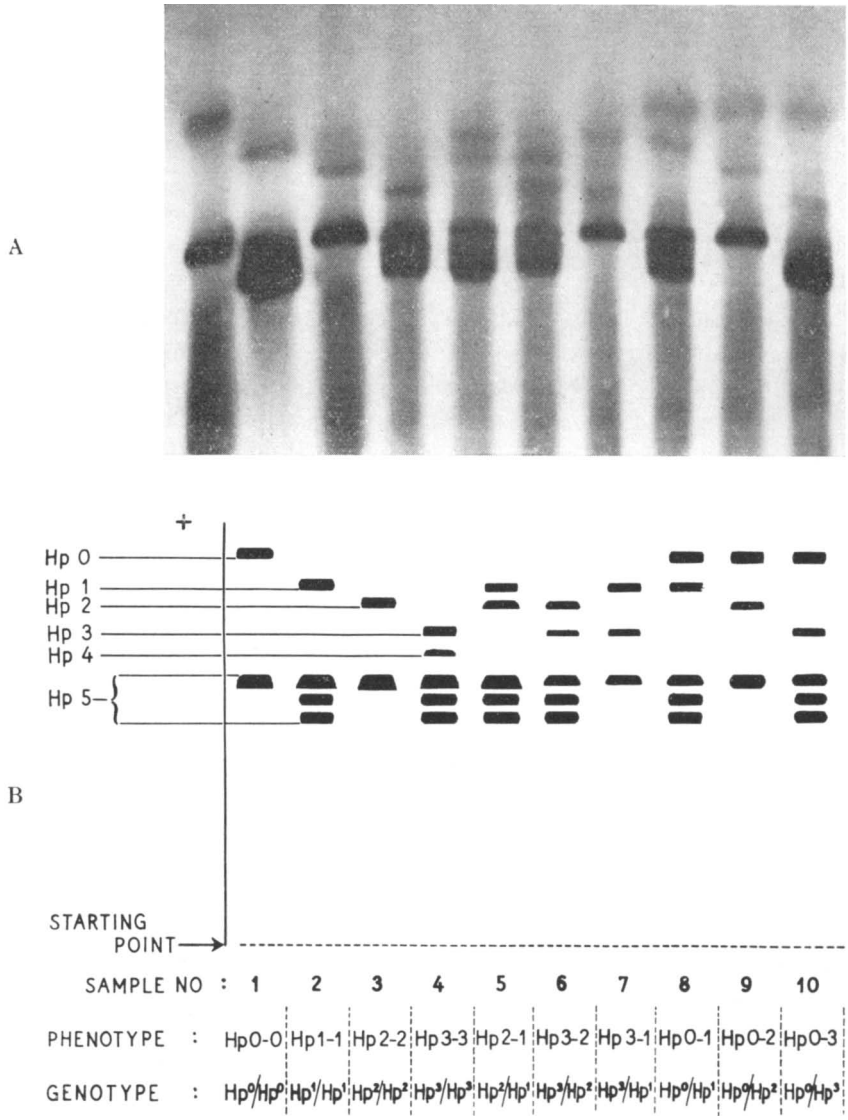
Having the same mobility in the gel as free pig hemoglobin, Hp 0, the component with the highest migration velocity, was very difficult to identify after addition of excess pig hemoglobin under the prevailing electrophoretic conditions.

Hp 0 either occurred singly or together with only one of the other haptoglobins investigated. The same was the case with Hp 1, Hp 2 and Hp 3. Therefore the hypothesis was advanced, that the occurrence of Hp 0, Hp 1, Hp 2 and Hp 3 in sera from pigs of Danish Landrace was genetically controlled and determined by four alleles: Hp^0, Hp^1, Hp^2 and Hp^3. The phenotypes shown on the figure thus represent animals with the genotypes shown on the figure. Animals which possess only one of the four haptoglobins in their sera are homozygous for the allele, and animals possessing two of the components are heterozygous.

To support the hypothesis the family material was haptoglobintyped. The result is seen in table 1. Table 1 indicates, that the progeny phenotypes observed in every mating class are only those to be expected from the hypothesis. Further good agreement was found between the expected distribution of the progeny calculated on the basis of the above hypothesis and the distribution observed.

Under the hypothesis given above, the gene frequencies among 900 pigs selected at random were determined according to the formula:

$$q_{Hp^0} = \frac{2 \times \text{Hp } 0\text{—}0 + \text{Hp } 0\text{—}1 + \text{Hp } 0\text{—}2 + \text{Hp } 0\text{—}3}{2\,N}$$



F i g. 1. A: Starch gel electrophoresis of pig sera demonstrating the 10 different haptoglobin phenotypes. Chicken hemoglobin has been added before electrophoresis. Only the anodic part of the gel is shown. Benzidine staining. B: Diagram of the haptoglobin phenotypes in pigs of Danish Landrace.

Table 1. Distribution of progeny haptoglobin phenotypes from various mating classes in pigs of Danish Landrace.

Mating classes	No. of classes	No. of animals in class	Progeny Phenotypes																		χ^2	d. f.
			0-0		1-1		2-2		3-3		2-1		3-2		3-1		0-1		0-2		0-3			
			obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp		
3—3×3—3	3	12							12	12														
3—3×3—1	13	60							32	30					28	30							0.27	1
3—3×3—2	4	16							7	8			9	8									0.26	1
3—3×2—1	1	4											2	2	2	2								
3—3×2—2	1	4											4	4										
3—3×1—1	6	43													43	43								
3—3×0—3	3	12							6	6											6	6	0.00	1
3—1×3—1	12	58			13	14.5			15	14.5					30	29							0.21	2
3—1×3—2	2	8							3	2	0	2	4	2	1	2								
3—1×2—2	2	8									3	4	5	4										
3—1×2—1	7	34			8	8.5					7	8.5	11	8.5	8	8.5							1.06	3
3—1×0—1	2	8			2	2									3	2	2	2			1	2		
3—1×0—3	2	12							1	3					3	3	1	3			7	3		
3—1×1—1	5	29			15	14.5									14	14.5							0.04	1
3—2×2—1	2	8					2	2			3	2	1	2	2	2								
3—2×1—1	1	8									4	4			4	4								
3—2×0—1	1	4									0	1			0	1			3	1	1	1		
2—2×1—1	1	4									4	4												
2—1×1—1	3	12			6	6					6	6												
Total	71	344			44	45.5	2	2	76	75.5	27	31.5	36	30.5	138	141	3	5	3	1	15	12		

Table 2. The observed and on basis of gene frequencies expected distribution of haptoglobin types among 900 pigs of Danish Landrace.

Hp 0-0		Hp 0-1		Hp 0-2		Hp 0-3		Hp 1-1		Hp 2-1		Hp 3-1		Hp 2-2		Hp 3-2		Hp 3-3	
obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
3	1.5	29	24.9	5	8.4	33	36.7	99	105	76	70.7	312	309.2	14	11.7	98	104.0	231	227.1

$\chi^2 = 3.01.$

in wihch N represents the number of animals investigated. The following frequencies were found:

$$q\,Hp^0 = 0.041$$
$$q\,Hp^1 = 0.342$$
$$q\,Hp^2 = 0.115$$
$$q\,Hp^3 = 0.503$$

As indicated in table 2 good agreement between the observed and on the basis of gene frequencies expected distribution of haptoglobin types among 900 pigs was obtained. It may be assumed that the population investigated is in genetic equilibrium with respect to the genes controlling the haptoglobin synthesis in sera from pigs of Danish Landrace.

As mentioned in the introduction, protein polymorphism in the β_1-globulin field has been demonstrated by *Brummerstedt-Hansen and Hirschfeld* in an immunoelectrophoretic study of sera from pigs of Danish Landrace. The occurrence of intermediate types made a distinct grouping impossible. As the variable component in the β_1-field was hemoglobin-binding, it was assumed that the polymorphism described in the β_1-field, and the haptoglobin polymorphism demonstrated by the starch gel electrophoretic technique, were identical. Comparative investigations comprising approximately 280 samples showed good agreement between the results obtained by the two methods of electrophoresis. In two cases the results obtained were different.

ACKNOWLEDGEMENT

For valuable help and continuous interest during planning and carrying out these investigations as well as for the excellent working conditions afforded grateful acknowledgement is made to Professor, Dr. med. vet. Johs. Moustgaard, Head of the Department of Animal Physiology, Endocrinology and Bloodgrouping.

REFERENCES

Brummerstedt-Hansen, E., M. Hesselholt, B. Larsen, J. Moustgaard, I. Møller, P. Bräuner Nielsen & B. Palludan: The 8th Animal Blood Group Conference in Europe, Ljubljana, Yugoslavia, 1962.

Brummerstedt-Hansen, E. & M. Hesselholt: Haptoglobinvariationer i sera fra svin af Dansk Landrace. Den kongelige Veterinær- og Landbohøjskoles Institut for Sterilitetsforskning, Aarsberetning, 1962, 211—219.

Brummerstedt-Hansen, E. & J. Hirschfeld: Grouped Variations in Pig Sera demonstrated by an Immunoelectrophoretic Technique. Acta vet. scand., 1961, *2*, 317—322.

Jayle, M. F. & P. Gillard: Précipitation saline d'une proteine définie dans un mélange héterogene. Bull. Soc. Chim. Biol., 1947, *29*, 149—157.

Kristjansson, F. K.: Genetic Control of Three Haptoglobins in Pigs. Genetics, 1961, *46*, 907—910.

Kristjansson, F. K.: The 8th Animal Blood Group Conference in Europe, Ljubljana, Yugoslavia, 1962.

Polonovski, M. & M. F. Jayle: Sur la préparation d'une nouvelle fraction des proteines plasmatiques, l'haptoglobine, C. R. Acad. Sci., 1940, *211*, 517—519.

Poulik, M. D.: Starch Gel Electrophoresis in a Discontinuous System of Buffers. Nature 1957, *180*, 1477—1479.

Smithies, O.: Zone Electrophoresis in Starch Gels, Group Variations in the Serum Proteins of Normal Human Adults. Biochem. J. 1955 a, *61*, 629—641.

Smithies, O.: Grouped Variations in the Occurrence of New Protein Components in Normal Human Serum. Nature 1955 b, *175*, 307—308.

Smithies, O.: Zone Electrophoresis in Starch Gels and its Application to Studies of Serum Proteins. Adv. in Prot. Chem., 1959, *14*, 65—113.

Smithies, O. & N. F. Walker: Genetic Control of some Serum Proteins in Normal Humans. Nature 1955, *176*, 1265—1266.

Smithies, O. & N. F. Walker: Notation for Serum Protein Groups and the Genes Controlling their Inheritance. Nature 1956, *178*, 694—695.

SUMMARY

In a starch gel electrophoretic study of sera from pigs of the Danish Landrace protein polymorphism involving four haptoglobins designated Hp 0, Hp 1, Hp 2 and Hp 3 was observed. Each component either occurred singly or together with only one of the three other components.

Each individual pig investigated exhibited one of ten serum phenotypes with respect to Hp 0, Hp 1, Hp 2 and Hp 3.

Studies within families supported the hypothesis that the polymorphism was genetically controlled and determined by four alleles, Hp^0, Hp^1, Hp^2 and Hp^3, with codominance. The phenotypes and the corresponding genotypes are shown in figure 1.

ZUSAMMENFASSUNG

Haptoglobinpolymorphie beim Schwein.

Das stärkeelektrophoretische Studium von Seren von Schweinen der dänischen Landrasse ergab Proteinpolymorphie in bezug auf 4 Haptoglobinkomponenten: Hp 0, Hp 1, Hp 2 und Hp 3. Jede Kompo-

nente kam entweder allein oder zusammen mit nur einer der anderen drei Komponenten vor.

Auf Grund des Vorkommens der 4 Haptoglobine in Seren wiesen sämtliche untersuchten Tiere *einen* von 10 Serumphänotypen auf.

Familienuntersuchungen stützten die Hypothese, dass die Polymorphie genetisch bedingt war und unter der Kontrolle von 4 Allelen, Hp^0, Hp^1, Hp^2 und Hp^3, ohne Dominanz, stand. Die zehn Phänotypen und die entsprechenden Genotypen sind in Figur 1 dargestellt.

RESUMÉ

Haptoglobinpolymorfi hos svin.

Ved et stivelsegelelektroforetisk studium af sera fra svin af Dansk Landrace blev der påvist proteinpolymorfi med hensyn til 4 haptoglobinkomponenter: Hp 0, Hp 1, Hp 2 og Hp 3. Hver komponent forekom enten alene eller sammen med kun een af de andre tre komponenter.

På grundlag af forekomsten i sera af de 4 haptoglobiner udviste samtlige undersøgte dyr een af 10 serumfænotyper.

Familieundersøgelser støttede hypotesen om, at polymorfien var genetisk bestemt og under kontrol af 4 alleler, Hp^0, Hp^1, Hp^2 og Hp^3, uden dominans. De ti fænotyper og de tilsvarende genotyper er vist på fig. 1.

(Received January 15. 1963).