Genetics of plasma transferrins in the mouse

By B. L. COHEN*

Institute of Animal Genetics, University of Edinburgh

1. INTRODUCTION

Since the development of starch-gel electrophoresis (Smithies, 1955), genetically determined differences in the electrophoretic mobility of certain plasma proteins have been observed in man, cattle (Smithies & Hickman, 1958), sheep and goats (Ashton, 1958; Ashton & McDougall, 1958). In man, such polymorphism has been found to involve at least two independent systems, namely, the haemoglobin-binding proteins or haptoglobins (Smithies, 1955), and the iron-binding β-globulins or transferrins (Giblett, Hickman & Smithies, 1959). A similar situation occurs in the pig (Cohen, unpublished observations).

None of the species in which these polymorphisms have so far been observed is convenient for experimental breeding, and this prompted a search for similar variations in the mouse. Plasmas from individuals of six inbred strains of mice have so far been examined by starch-gel electrophoresis. Of these, five strains (A, C57BL, JU, KL, RIII) were indistinguishable in plasma protein pattern, whilst the sixth strain (CBA) differed by having a group of β-globulins with a raised electrophoretic mobility. Crosses between CBA and C57BL inbred mice indicated that the difference in β-globulin type between these two strains is under genetic control and is determined by a pair of allelomorphic autosomal genes. An Fe^{59} labelling experiment showed that these β-globulins are transferrins.

The work of Thompson, Foster, Gowen & Tauber (1954) came to my notice after the start of the present investigation. These workers, using moving-boundary electrophoresis, examined the serum proteins of seven inbred strains of mice. They described the presence in one strain (E) of a β_1-globulin not present in the remaining six strains (BALB/Gw, LGW, K, RI, S, Z) and presented evidence that this β_1-globulin could be detected in the F_1 progeny of crosses between E and S strain mice. Because of the differences between moving-boundary and starch-gel electrophoresis, an exact comparison of the results of Thompson *et al.* (1954), and those presented here, is not possible. However, from the results of two-dimensional electrophoresis (Fig. 3) it appears that the transferrins are the major constituents of the β-globulin fraction of mouse plasma. It is, therefore, a reasonable assumption that the β-globulins which Thompson *et al.* (1954) found to show hereditary variation were, in fact, transferrins.

The symbol *Trf* is adopted here for the mouse transferrin locus because *Tf*, as used by Smithies & Hiller (1959) for the human transferrin locus, might be con-

* Present address: Department of Genetics, The University, Glasgow W.2.

fused with *tf*, which is used in the mouse for the locus 'tufted'. The transferrin phenotype observed in CBA animals will be referred to as TrfA, whilst that in the other strains will be called TrfB. The phenotype of heterozygous animals, such as those derived from matings between CBA and C57BL animals, will be called TrfAB. The corresponding alleles will be called Trf^A and Trf^B.

Table 1. *The phenotypes and their corresponding genotypes*

Phenotype	Corresponding genotype	Reference
Transferrin A (TrfA)	Trf^A/Trf^A	
Transferrin AB (TrfAB)	Trf^A/Trf^B	Present communication
Transferrin B (TrfB)	Trf^B/Trf^B	
Haemoglobin d (Hbd)	Hb^2/Hb^2	
Haemoglobin ds (Hbds)	Hb^2/Hb^1	Gluecksohn–Waelsch, cited in Russell & Gerald (1958)
Haemoglobin s (Hbs)	Hb^1/Hb^1	
Agouti	A^+/A^+ and A^+/a	Grüneberg (1952)
Non-agouti	a/a	

2. MATERIALS AND METHODS

(a) Mice

The following five standard strains of inbred mice (Report of International Committee on Standardized Nomenclature for Inbred Strains of Mice, 1952) were examined:

A/Fa	F?	+36
CBA/Fa	F?	+31
C57BL/Fa	F?	+31
RIII/Fa-*ro*	F?	+29
JU/Fa	F	25

A sixth strain, known as KL, was made in this laboratory from a four-way cross of the A, CBA, C57BL and RIII strains, and it had been inbred by thirty-six brother-sister matings.

The mice were bled ($\gg 0.6$ ml.) from a small incision in the caudal artery. The blood was collected in a capillary tube and emptied into a heparinized polyethylene container. Plasma was removed after centrifugation. All mice were more than 6 weeks old when bled.

(b) Electrophoresis

(i) Horizontal, one-dimensional starch-gel electrophoresis was performed using, with minor modifications, the method described by Smithies (1955). 'Starch-hydrolysed for gel electrophoresis' prepared by the Connaught Medical Research Laboratories, Toronto, Canada, was employed.

The discontinuous buffer system described by Poulik (1957) was found to be the most useful, since in this system the mouse haptoglobin moves more slowly than any of the transferrins, whilst in the continuous borate buffer system (Smithies, 1955) it overlaps the fastest transferrin components. A piece of Whatman 3-mm. chromatography paper was soaked in each plasma sample and inserted into the gel. Sample-slits were always separated from one another by a short length of unbroken gel, and were placed 5 cm. from the edge of the cathodic wick. A stabilized d.c. potential of 6 V/cm. length of gel was applied for about 6 hours, giving an initial current density of about 5 mA/cm² of gel cross-section.

(ii) Two-dimensional filter paper/starch-gel electrophoresis (Poulik & Smithies, 1958) was done on specimens of each phenotype.

The gels were stained for protein with Naphthalene Black 12B. An *o*-dianisidine reagent (Owen, Silberman & Got, 1958) was used to stain free and bound haemoglobin.

3. RESULTS

(a) *Electrophoresis*

(i) *Plasma proteins*

Plasmas from at least six, and in the cases of CBA and C57BL many more, animals from each of the six inbred strains were examined. In no case was any consistent difference observed between animals of similar age and sex within any one strain. Comparison of plasma samples from animals of different strains showed that the CBA strain differs from the other five, which are all alike.

Two-dimensional electrophoresis showed that the difference affects a group of β-globulins. I am indebted to Dr H. Harris of The London Hospital Medical College for an experiment in which Fe⁵⁹ was added to pooled plasmas of each β-globulin phenotype. Autoradiography subsequent to electrophoresis showed that the Fe⁵⁹ was bound only to the variable β-globulins, thus demonstrating that these are transferrins (cf. Giblett *et al.*, 1959). The results of this, together with the results of one- and two-dimensional electrophoresis of CBA (TrfA), C57BL (TrfB) and CBA × C57BL F₁ (TrfAB) plasmas, are presented in Figs. 1, 2 and 3. The TrfA electrophoretic pattern comprises three components visible as bands in the stained gels. Of these bands, the fastest-moving is very faint, the second-fastest is fairly strong, and the slowest one is strong. The TrfB electrophoretic pattern also comprises three components, all of which migrate more slowly than their TrfA counterparts. Thus the fastest TrfA band is faster than any of the TrfB bands, whilst the second-fastest TrfA band moves at the same speed as the fastest TrfB band. The electrophoretic pattern of the heterozygote (TrfAB) is consistent with that to be expected from a mixture of equal parts of TrfA and TrfB plasma, i.e. four bands are visible, of which the faster two are faint and the slower two fairly strong, all four corresponding in mobility with bands present in the two homozygotes (Figs. 1 and 3).

The Fe⁵⁹ autoradiographic results, however, give no evidence of iron binding by the fastest (faint) component in any of the three phenotypes. Whether this is

due to the relatively small amount of this protein which is present, or to it being a protein other than transferrin, and therefore unable to bind iron, remains to be determined.

No evidence of haptoglobin differences was found among the mouse strains examined.

(ii) *Haemoglobins*

Gluecksohn-Waelsch, Ranney & Sisken (1957) demonstrated by filter-paper electrophoresis the existence of a pair of alternative phenotypes of mouse haemoglobin. These phenotypes were called 'diffuse' (Hbd) and 'single' (Hbs), and were shown to be determined by allelic genes (see Table 1 for nomenclature). Russell & Gerald (1958) have shown that, phenotypically, CBA mice are Hbd, and C57BL mice, Hbs. In the present work, using starch-gel electrophoresis, these findings have been confirmed and extended. The diffuse phenotype has been resolved into three haemoglobin components, and the single phenotype into two. It has proved possible to distinguish the heterozygote (Hbds), the electrophoretic pattern of which is similar to that given by a mixture of equal parts of Hbd and Hbs haemoglobin preparations.

(b) *Segregation data*

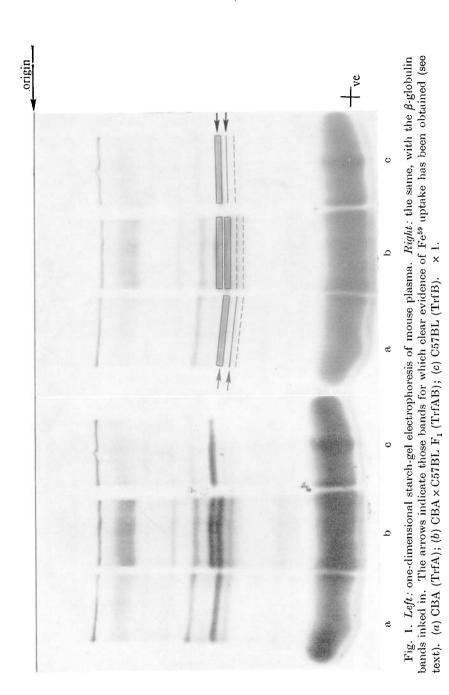The CBA and C57BL strains differ in the following relevant ways:

	CBA	C57BL
	agouti	non-agouti
	Hbd	Hbs
	TrfA	TrfB

By crossing CBA males with C57BL females, an F_1 generation of twenty-nine animals was reared. These were all phenotypically agouti, Hbds, TrfAB. Thus, since all the F_1 males were heterozygous, sex-linkage of the transferrin locus is excluded. Using the maximum likelihood estimation procedure described by

Table 2. *Classification of 128 F_2 mice for sex, coat colour, haemoglobin and transferrin phenotypes*

Transferrin phenotypes and sex	Haemoglobin and coat-colour phenotypes						Totals
	Hbd		Hbds		Hbs		
	Agouti	Non-agouti	Agouti	Non-agouti	Agouti	Non-agouti	
TrfA ♂	4	2	6	3	0	1	16 ⎫ 40
TrfA ♀	5	3	8	3	5	0	24 ⎭
TrfAB ♂	5	0	14	6	8	2	35 ⎫ 62
TrfAB ♀	2	2	9	3	6	5	27 ⎭
TrfB ♂	3	2	7	0	1	1	14 ⎫ 26
TrfB ♀	2	0	7	1	2	0	12 ⎭
Totals	21	9	51	16	22	9	128
	30		67		31		

Fig. 1. *Left*: one-dimensional starch-gel electrophoresis of mouse plasma. *Right*: the same, with the β-globulin bands inked in. The arrows indicate those bands for which clear evidence of Fe^{59} uptake has been obtained (see text). (*a*) CBA (TrfA); (*b*) CBA × C57BL F_1 (TrfAB); (*c*) C57BL (TrfB). × 1.
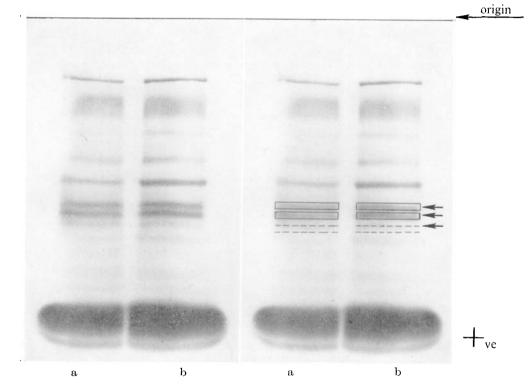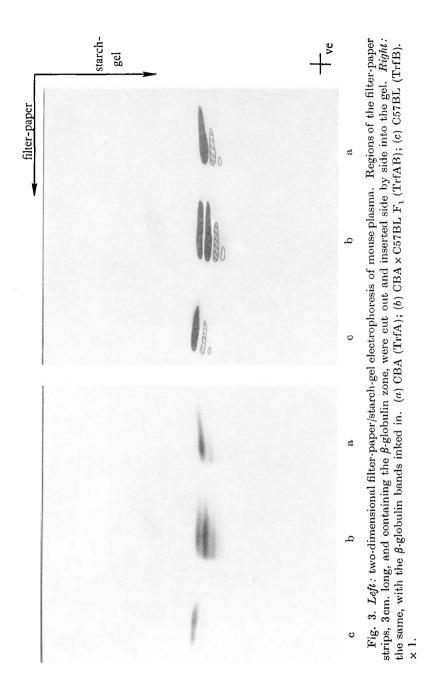
B. L. COHEN

(Facing p. 434)

Fig. 2. *Left:* one-dimensional starch-gel electrophoresis of mouse plasma. *Right:* the same, with the β-globulin bands inked in. The arrows indicate those bands for which clear evidence of Fe^{59} uptake has been obtained (see text). (*a*) Artificial mixture of equal parts of CBA and C57BL plasma; (*b*) CBA × C57BL F_1 (TrfAB) plasma.   × 1.

B. L. COHEN

Fig. 3. *Left*: two-dimensional filter-paper/starch-gel electrophoresis of mouse plasma. Regions of the filter-paper strips, 3 cm. long, and containing the β-globulin zone, were cut out and inserted side by side into the gel. *Right*: the same, with the β-globulin bands inked in. (*a*) CBA (TrfA); (*b*) CBA × C57BL F₁ (TrfAB); (*c*) C57BL (TrfB). × 1.

B. L. COHEN

Carter & Falconer (1951), no evidence for partial sex-linkage was obtained. A total of 128 F_2 and 90 backcross animals were reared and classified. Of the backcross animals, 66 were from $F_1 \times CBA$, and 24 from $F_1 \times C57BL$ matings. The numbers of animals observed in each phenotypic class are presented in Tables 2 and 3.

Table 3. *Classification of backcross mice for sex, coat colour, haemoglobin and transferrin phenotypes*

Transferrin phenotypes and sex		$F_1 \times CBA$			$F_1 \times C57BL$				
					Hbds		Hbs		
		Hbd Agouti	Hbds Agouti	Totals	Agouti	Non-agouti	Agouti	Non-agouti	Totals
TrfA	♂	5	12	17	0	0	0	0	0
	♀	14	6	20	0	0	0	0	0
TrfAB	♂	7	10	17	2	1	2	2	7
	♀	7	5	12	2	0	2	2	6
TrfB	♂	0	0	0	2	0	1	2	5
	♀	0	0	0	1	1	2	2	6
Totals		33	33	66	7	2	7	8	24

From these data χ^2 values were obtained to test the significance of the differences between observed and expected numbers in the segregation of the transferrin phenotypes on the hypothesis that they are determined by allelic genes. Similar calculations were made with respect to the haemoglobin and coat-colour segregations. None of these χ^2 values is significant at the 5% level of probability (Table 4).

Table 4. χ^2 *tests of the segregation ratios*

	Transferrin phenotypes				Haemoglobin phenotypes				Coat-colour phenotypes		
	TrfA	TrfAB	TrfB	Total	Hbd	Hbds	Hbs	Total	Agouti	Non-agouti	Total
F_2 generation											
Obs.	40	62	26	128	30	67	31	128	94	24	128
Exp.	32	64	32	128	32	64	32	128	96	32	128
	$\chi^2 = 3\cdot2, P > 0\cdot2$				$\chi^2 = 0\cdot3, P > 0\cdot8$				$\chi^2 = 0\cdot2, P > 0\cdot5$		
Backcross $F_1 \times CBA$											
Obs.	37	29	0	66	33	33	0	66	66	0	66
Exp.	33	33	0	66	33	33	0	66	66	0	66
	$\chi^2 = 1\cdot0, P > 0\cdot3$										
Backcross $F_1 \times C57BL$											
Obs.	0	13	11	24	0	9	15	24	14	10	24
Exp.	0	12	12	24	0	12	12	24	12	12	24
	$\chi^2 = 0\cdot2, P > 0\cdot5$				$\chi^2 = 1\cdot5, P > 0\cdot2$				$\chi^2 = 0\cdot7, P > 0\cdot7$		

The scoring method of Finney (1949), with trial values of 0·5 for p in each case, gives maximum likelihood estimates of the recombination fractions, based on the combined F_2 and backcross data as:

$$p \pm \text{S.E.}_p$$
$$Trf : Hb \quad 0·47 \pm 0·034$$
$$Trf : A^+ \quad 0·55 \pm 0·048$$

In each case the estimate of p differs from the null value of 0·5 by rather less than its standard error. Thus there is no indication of linkage.

The results of statistical tests of the segregation data are therefore in agreement with the hypotheses that the transferrin phenotypes TrfA and TrfB are determined by a pair of allelic genes that segregate independently of both the haemoglobin locus in linkage group I (Popp & St. Amand, 1959) and the agouti locus in linkage group V (Grüneberg, 1952).

4. DISCUSSION

The genetical results presented here are consistent with the conclusion that the difference between the TrfA and TrfB phenotypes is determined by a single pair of allelic genes. The electrophoretic results, and the findings of Thompson *et al.* (1954) (if applicable), are consistent with the conclusion that the transferrins of Trf^A/Trf^A and Trf^B/Trf^B mice differ in charge, rather than in size. The electrophoretic results also demonstrate that each transferrin allele determines the electrophoretic behaviour of three (or possibly only two) β-globulins with iron-binding activity, and that, in the heterozygote, four (or possibly only three) components with the same activity can be resolved. However, because certain of the TrfA and TrfB components migrate in starch-gel with identical mobilities, the phenotypic pattern observed in the heterozygote cannot be distinguished from that given by a mixture of equal parts of plasma from the two homozygotes. There is, therefore, no compelling reason to postulate the existence in the Trf^A/Trf^B heterozygote of any 'hybrid' transferrin substances. Whether such hybrid substances do, indeed, exist might be determined by more refined analysis of the purified proteins, e.g. by the method of Ingram (1956). Similarly, further analysis at the molecular level might furnish an explanation for the mechanism whereby each transferrin allele appears to affect the electrophoretic mobility of three (or possibly only two) iron-binding proteins, and might help to explain the molecular interrelationships of these proteins. One hypothesis which may be proposed, and for which the difference between the human haemoglobin variants HbA and HbS may serve as a model (Ingram, 1956), is as follows: that each transferrin allele controls an amino-acid substitution which affects the charge, and that the same substitution occurs in each of the iron-binding proteins determined by that gene. In this case, on refined chemical analysis, one would expect to find some sort of homology between Trf^A and Trf^B component products of similar relative position, e.g. between the slowest TrfA and TrfB bands, rather than between bands of identical electrophoretic mobilities, e.g. the slowest TrfA and the second-fastest TrfB band.

Genetically determined variations in the plasma β-globulins have now been reported in man, cattle, sheep, goats, horses, pigs and mice. At least in the cases of man, cattle, sheep, pigs and mice, these β-globulins have been found to be trans-ferrins (Giblett *et al.*, 1959; Harris, personal communication). In man and cattle, sufficient data have now accumulated to show that the frequencies of the various transferrin alleles differ in different populations (Smithies, 1957; Harris, Robson & Siniscalco, 1958; Smithies & Hickman, 1958; Ashton, 1959*a*), and these findings raise the possibility that the various alleles differ in adaptive value. Indeed, evidence has already been presented (Ashton, 1959*b*) showing some effect of trans-ferrin genotype on the fitness of cattle.

It would be of great interest to know what physiological and genetic factors underlie the present polymorphism of so many mammalian species with regard to such characters as transferrin type. Inquiry into the nature of the physiological factors involved could probably be pursued conveniently using cattle as experi-mental animals. However, research into the nature of the genetic factors would be hampered in man and cattle by their long generation-time and by the impossibility or difficulty of carrying out experimental breeding on a sufficiently large scale. Thus the findings presented here, of the existence in the mouse of transferrin differences very similar to those already found in other mammalian species, may open the way to the experimental detection and analysis of differences in fitness between animals differing in genotype at the transferrin locus.

5. SUMMARY

1. The plasma proteins of six inbred strains of mice have been studied, using starch-gel electrophoresis.

2. The existence of two alternative plasma transferrin (β-globulin) phenotypes has been demonstrated. Five of the strains have one of these and one strain has the other. Each of the two transferrin patterns comprises three (or possibly only two) electrophoretic bands. The two patterns differ in all of these bands.

3. The two transferrin types recognized are determined by a pair of allelic, auto-somal genes (designated Trf^A and Trf^B). The TrfA phenotype (CBA strain) is determined by the genotype Trf^A/Trf^A, and the TrfB phenotype (A, C57BL, JU, KL, RIII strains) by the genotype Trf^B/Trf^B. The phenotype TrfAB, of the heterozygote (genotype Trf^A/Trf^B), is distinguishable and shows four (or possibly only three) bands. In this way it closely resembles a mixture of equal parts of TrfA and TrfB plasma.

4. No linkage was detected between the Trf locus and sex, the agouti locus or the haemoglobin locus.

5. The possible molecular basis of the action of the transferrin alleles in the mouse, and the widespread distribution in mammals of polymorphism involving the transferrins, are discussed.

REFERENCES

ASHTON, G. C. (1958). Further β-globulin phenotypes in sheep. *Nature, Lond.*, **182**, 1101–1102.

ASHTON, G. C. (1959a). β-globulin alleles in some Zebu cattle. *Nature, Lond.*, **184**, 1135–1136.

ASHTON, G. C. (1959b). β-globulin polymorphism and early foetal mortality in cattle. *Nature, Lond.*, **183**, 404–405.

ASHTON, G. C. & McDOUGALL, E. I. (1958). β-globulin polymorphism in cattle, sheep and goats. *Nature, Lond.*, **183**, 945–946.

CARTER, T. C. & FALCONER, D. S. (1951). Stocks for detecting linkage in the mouse, and the theory of their design. *J. Genet.* **50**, 307–323.

FINNEY, D. J. (1949). The estimation of the frequency of recombinations. *J. Genet.* **49**, 159–176.

GIBLETT, E. R., HICKMAN, C. G. & SMITHIES, O. (1959). Serum transferrins. *Nature, Lond.*, **183**, 1589–1590.

GLUECKSOHN-WAELSCH, S., RANNEY, H. M. & SISKEN, B. F. (1957). The hereditary transmission of haemoglobin differences in mice. *J. clin. Invest.* **36**, 753–756.

GRÜNEBERG, H. (1952). *The Genetics of the Mouse*, 2nd ed. The Hague: Martinus Nijhoff.

HARRIS, H., ROBSON, E. B. & SINISCALCO, M. (1958). β-globulin variants in man. *Nature, Lond.*, **182**, 452.

INGRAM, V. M. (1956). A specific chemical difference between the globins of normal human and sickle-cell anaemia. *Nature, Lond.*, **178**, 792–794.

OWEN, J. A., SILBERMAN, H. J. & GOT, C. (1958). Detection of haemoglobin, haemoglobin-haptoglobin complexes and other substances with peroxidase activity after zone electrophoresis. *Nature, Lond.*, **182**, 1373.

POPP, R. A. & ST. AMAND, W. (1959). The mouse haemoglobin locus. *Anat. Rec.* **132**, 489.

POULIK, M. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature, Lond.*, **180**, 1477–1479.

POULIK, M. & SMITHIES, O. (1958). Comparison and combination of the starch gel and filter paper electrophoretic methods: two-dimensional electrophoresis. *Biochem. J.* **68**, 636–643.

Report of International Committee on Standardized Nomenclature for Inbred Strains of Mice (1952). *Cancer Res.* **12**, 602–613.

RUSSELL, E. S. & GERALD, P. S. (1958). Inherited electrophoretic haemoglobin patterns among 20 inbred strains of mice. *Science*, **128**. 1569–1570.

SMITHIES, O. (1955). Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. J.* **61**, 629–641.

SMITHIES, O. (1957). Variants in human serum β-globulins. *Nature, Lond.*, **180**, 1482–1483.

SMITHIES, O. & HICKMAN, C. G. (1958). Inherited variations in the serum proteins of cattle. *Genetics*, **43**, 374–385.

SMITHIES, O. & HILLER, O. (1959). The genetic control of transferrins in humans. *Biochem. J.* **72**, 121–126.

THOMPSON, S., FOSTER, J. F., GOWEN, J. W. & TAUBER, O. (1954). Hereditary differences in the serum proteins of normal mice. *Proc. Soc. exp. Biol., N.Y.*, **87**, 315–317.