THE HEREDITARY TRANSMISSION OF HEMOGLOBIN DIFFERENCES IN MICE [1]

By

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The existence, in inbred strains of mice, of two different types of electrophoretic hemoglobin patterns has been demonstrated with the method of paper electrophoresis (1). Preliminary studies of the distribution of these different patterns in the inbred strains tested indicated strongly a genetic basis of hemoglobin differences in mice. It is the purpose of the present communication to report the results of breeding experiments designed to test the assumption of a hereditary transmission of these differences and to determine the mode of inheritance.

MATERIAL AND METHODS

The determination of hemoglobin patterns was carried out by filter paper electrophoresis according to the method described (2). Two types of patterns as previously illustrated (1) were obtained, one apparently of a single homogeneous protein (Type I), and the other diffuse, apparently of more than one protein (Type II). No difficulty was encountered in distinguishing pattern I from pattern II; nevertheless, minor differences between the hemoglobin patterns of individual mice within Type I or of mice within Type II groups were noted (Figure 1). Therefore, classification of hemoglobin patterns as Type I or Type II does not necessarily imply that the hemoglobin or hemoglobins present were in each case absolutely identical.

The following inbred mouse strains with hemoglobin patterns as determined in previous studies (1) and indicated below were used for breeding experiments:

Strain No.	Strain name[2]	Hemoglobin pattern	
1	DBA/1	diffuse (d)	Type II
2	BALB/Glw	diffuse (d)	Type II
3	Fused	diffuse (d)	Type II
4	C₃H	diffuse (d)	Type II
5	Splotch	single (s)	Type I
6	Kink I	single (s)	Type I
9	Urogenital	single (s)	Type I

These strains were intercrossed in a number of different combinations as follows:

Strain Nos.	Strain names[2]	Hemoglobin pattern
1 × 4	DBA/1 × C₃H	d × d
1 × 6	DBA/1 × Kink I	d × s
2 × 5	BALB × Splotch	d × s
3 × 9	Fused × urogenital	d × s
4 × 5	C₃H × Splotch	d × s
4 × 6	C₃H × Kink I	d × s
5 × 9	Splotch × urogenital	s × s
6 × 9	Kink I × urogenital	s × s

First (F_1) and second (F_2) hybrid generations as well as backcross generations were obtained and the hemoglobin patterns of the offspring tested. All animals were at least 2 months old before their hemoglobin was examined, in order to avoid any possible admixture of fetal hemoglobin.

Altogether, 635 specimens of hemoglobin were tested and distributed as follows:

F_1 generation:	140
F_2 generation:	210
Backcross of F_1 generation to parent:	102
Backcross of F_2 generation to parent:	183
	635

RESULTS

The results obtained are summarized in Tables I to IV.

As shown in Table I, crosses of strains with "diffuse" type of hemoglobin pattern with those of "single" type (experiments 1 through 5) were made up in five different combinations. Altogether, 101 offspring were tested; the hemoglobin of all of them showed the "diffuse" type of pattern.

Crosses between different strains with the "diffuse" type of hemoglobin pattern (experiment 6)

TABLE I

Results of intercrosses between d and s strains—
F_1 generation

Exp.	Parents	Strain numbers	Parents' hemoglobin pattern	Offspring's hemoglobin pattern	
				d	s
1	DBA × Ki	1 × 6	d × s	22	0
2	BALB × Spl	2 × 5	d × s	17	0
3	Fu × ur	3 × 9	d × s	19	0
4	C₃H × Spl	4 × 5	d × s	19	0
5	C₃H × Ki	4 × 6	d × s	24	0
	All F_1 d × s			*101*	*0*
6	DBA × C₃H	1 × 4	d × d	23	0
	All F_1 d × d			*23*	*0*
7	Spl × ur	5 × 9	s × s	0	10
8	Ki × ur	6 × 9	s × s	0	6
	All F_1 s × s			*0*	*16*

gave 23 offspring with the "diffuse" type of hemoglobin pattern.

Finally, crosses were made between different strains with "single" type hemoglobin pattern (experiments 7 and 8) and all 16 offspring tested gave the "single" pattern.

Thus "diffuse" when crossed by "diffuse" gives "diffuse," and "single" by "single" gives "single," in the first hybrid generation. "Diffuse" when crossed by "single" gives "diffuse" which by paper electrophoresis is indistinguishable from "diffuse" of the original parent with the "diffuse" type of hemoglobin pattern.

TABLE II

Intercrosses between d and s strains—F_2 generation

Exp.*	Parents' origin	Parents' hemoglobin pattern	Offspring's hemoglobin pattern	
			d	s
1	F₁ from DBA (d) × Ki (s)	d × d	26	10
2	F₁ from BALB (d) × Spl (s)	d × d	21	7
3	F₁ from Fu (d) × ur (s)	d × d	17	7
4	F₁ from C₃H (d) × Spl (s)	d × d	19	11
5	F₁ from C₃H (d) × Ki (s)	d × d	21	7
	All F_2 from d × s	*d × d*	*104*	*42*
6	F₁ from DBA (d) × C₃H (d)	d × d	26	0
	All F_2 from d × d	*d × d*	*26*	*0*
7	F₁ from Spl (s) × ur (s)	s × s	0	20
8	F₁ from Ki (s) × ur (s)	s × s	0	18
	All F_2 from s × s	*s × s*	*0*	*38*

* Numbers of experiments correspond to original F₁ crosses as listed in Table I.

Table II contains the data from further breeding tests, in which individuals of the first hybrid generation (F_1) obtained from crosses of "diffuse" by "single" were crossed *inter se* (experiments 1 through 5); 146 animals of the second hybrid generation (F_2) of all five such experiments segregated into 104 with the "diffuse" and 42 with the "single" hemoglobin pattern, a ratio approximating the expected 3 : 1. F_2 animals from experiment 6 (d × d) did not segregate: all the offspring gave the "diffuse" hemoglobin pattern. Absence of segregation was found also in the F_2 animals of experiments 7 and 8 where "single" was crossed by "single": all 38 offspring gave the "single" pattern.

Table III shows the results of backcrosses of F_1 animals, derived from crosses of "diffuse" by "single," to their parents with "single" hemoglobin patterns; the offspring segregated for "diffuse" and "single" with a good fit to a 1 : 1 ratio.

In Table IV are summarized the hemoglobin patterns of another group of backcrosses. Here, F_2 animals, from crosses of "diffuse" by "single," who showed the "diffuse" type (experiments A 1 through 5, B 1 through 5) or the "single" type (experiments C 1 through 5) of hemoglobin pattern were backcrossed to their respective grandparents with "single" hemoglobin patterns. Of the 19 F_2 animals with "diffuse" hemoglobin pattern, 7 failed to segregate and gave "diffuse" offspring only (experiments A 1 through 5). The other twelve F_2 animals (experiments B 1 through 5) when backcrossed to the grandparent with "single" hemoglobin pattern gave a segregation of "diffuse" and "single" compatible with a 1 : 1 ratio. The 19 animals of the second hybrid generation thus fell into 2 groups: 12 carrying the factors for both "diffuse" and "single" hemoglobin patterns, and 7 carrying the factor for "diffuse" pattern only, a segregation fitting well a 2 : 1 ratio of carriers to non-carriers. The F_2 animals with the "single" type hemoglobin pattern (Table IV, experiments 1 through 5), derived from the same crosses of F_1 animals as their "diffuse" siblings, were backcrossed to animals of the parent strain with "single" pattern and gave offspring with "single" hemoglobin pattern only.

In all experiments, females and males were distributed equally among the different classes of offspring.

DISCUSSION

All the results reported here fit the assumption that factors for d (diffuse) and s (single) hemoglobin patterns segregate as simple Mendelian autosomal alleles. The results of crosses of d × s (Table I) in the first hybrid (F_1), in the second hybrid generation (F_2) (Table II) as well as in the backcross generations (Tables III and IV) might be interpreted as indicating that d is dominant over s. Although the method of paper electrophoresis employed did not permit distinction of hemoglobin patterns of animals carrying the factors for both the d and the s type from the hemoglobin pattern of animals carrying the factor for d only, it is conceivable that the hemoglobin composition of d/s animals might be distinguished from that of animals homozygous for d by other methods. Further experiments are planned to elucidate this point. The decision between dominance and co-dominance of d and s can therefore not be made at present.

The distribution of hemoglobin patterns of F_2 animals and of backcrosses of both F_1 and F_2 animals to the parent strain with the "single" type hemoglobin pattern is entirely consistent with the

TABLE III

Results of backcrosses of F_1 generation from d × s to s strains

Exp.*	Origin of F_1 parent	BC parent	Offspring	
			d	s
2	from BALB (d) × Spl (s)	Spl (s)	25	21
3	from Fu (d) × ur (s)	ur (s)	16	23
5	from C₃H (d) × Ki (s)	Ki (s)	8	9
	All F_1 from d × s backcrossed to s		49	53

* Numbers of experiments correspond to original F_1 crosses as listed in Table I.

assumption that d and s behave as alleles segregating in the expected ratios.

Comprehensive data on the genetic aspects of human hemoglobins have been summarized by Neel (4): the genetic aspects of hemoglobins in man are somewhat different from those found in mice. Human hemoglobins are controlled genetically by a group of factors some of which behave as alleles; it seems that each allele is responsible for the presence of one particular hemoglobin. With respect to human hemoglobins, then, an individual homozygous for any one of these genetic factors shows the presence of pre-

TABLE IV

Results of backcrosses of F_2 generation to s strains

Exp.*	Origin of F_2 parent	Hemoglobin pattern of parent	Number of animals tested	BC parent	Offspring	
A					d	s
1	F₂ from DBA (d) × Ki (s)	d	2	Ki (s)	13	0
2	F₂ from BALB (d) × Spl (s)	d	1	Spl (s)	3	0
3	F₂ from Fu (d) × ur (s)	d	3	ur (s)	17	0
5	F₂ from C₃H (d) × Ki (s)	d	1	Ki (s)	4	0
	A: *all non-segregating F_2 d*		*7 = d/d*	*s*	*37*	*0*
B						
1	F₂ from DBA (d) × Ki (s)	d	4	Ki (s)	16	13
2	F₂ from BALB (d) × Spl (s)	d	3	Spl (s)	11	9
3	F₂ from Fu (d) × ur (s)	d	1	ur (s)	11	1
4	F₂ from C₃H (d) × Spl (s)	d	1	Spl (s)	1	2
5	F₂ from C₃H (d) × Ki (s)	d	3	Ki (s)	6	8
	B: *all segregating F_2 d*		*12 = d/s*	*s*	*45*	*33*
C						
1	F₂ from DBA (d) × Ki (s)	s	6	Ki (s)	0	31
2	F₂ from BALB (d) × Spl (s)	s	2	Spl (s)	0	13
3	F₂ from Fu (d) × ur (s)	s	1	ur (s)	0	2
4	F₂ from C₃H (d) × Spl (s)	s	1	Spl (s)	0	4
5	F₂ from C₃H (d) × Ki (s)	s	5	Ki (s)	0	18
	C: *all F_2 s*		*15 = s/s*	*s*	*0*	*68*

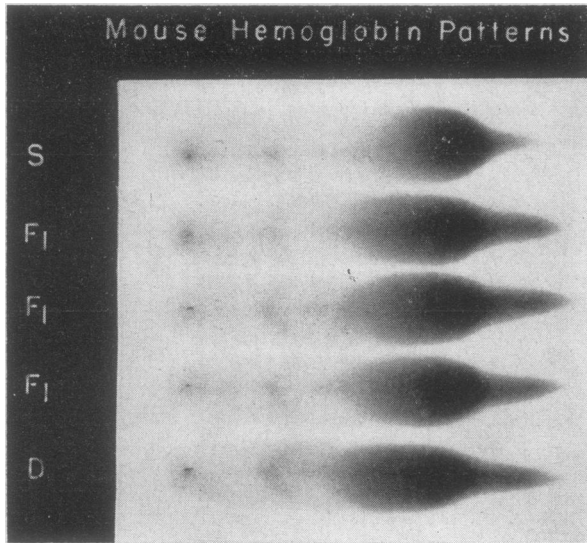* Numbers of experiments correspond to original F_1 crosses as listed in Table I.

FIG. 1. PAPER ELECTROPHORETIC PATTERN OF HEMOGLO-
BINS OF PARENTS AND OFFSPRING

S is parent with Type I (single) hemoglobin pattern;
D is parent with Type II (diffuse) hemoglobin pattern.
All three F_1's are sibs and show Type II pattern.

Paper electrophoresis pattern: 6 hour run, 450 volts,
stained with bromphenol blue.

dominantly one type of hemoglobin, whereas an
individual heterozygous for any 2 of the allelic
genetic factors will have 2 electrophoretically dem-
onstrable different hemoglobins. However, re-
cent studies by Kunkel and Wallenius (5), Mor-
rison and Cook (6), and Schapira, Dreyfus, and
Kruh (7) indicate that there may be more than
one variety of hemoglobin in the erythrocytes, even
of the normal human who is homozygous for the
genetic factor for normal human hemoglobin.

In the mouse, the presence of the s allele in
homozygous condition is accompanied by the
presence of only one type of hemoglobin pattern,
i.e., the "single" type. Animals homozygous for
the d allele demonstrate the "diffuse" type of he-
moglobin pattern which may, however, be a mix-
ture of hemoglobins rather than one hemoglobin.
The individual heterozygous for d and s shows the
same hemoglobin pattern electrophoretically as
does the d homozygote. It would appear particu-
larly interesting if a mixture of hemoglobins as is

perhaps present in the d homozygote were con-
trolled by one genetic unit; this situation would be
different from that of the abnormal hemoglobins in
man where each allele manifests itself by the pres-
ence of one hemoglobin.

SUMMARY

The hereditary transmission of hemoglobin dif-
ferences in mice has been investigated by the
method of paper electrophoresis.

Crosses between strains with two different elec-
trophoretic hemoglobin patterns, "diffuse" (d)
and "single" (s), show that the genetic factors for
the 2 patterns segregate as simple Mendelian au-
tosomal alleles.

The methods employed in this investigation do
not permit differentiation of the electrophoretic
hemoglobin pattern of mice of the first hybrid
generation, heterozygous for "diffuse" and "single"
(d/s), from the "diffuse" pattern of the parent,
homozygous for "diffuse" (d/d).

The hereditary control of hemoglobin differences
in the mouse appears to differ from that of ab-
normal hemoglobins in man.

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