Genet. Res., Camb. (1966), 7, *pp.* 76–85
With 1 *text-figure and* 2 *plates*
Printed in Great Britain

The multiple electrophoretic bands of mouse haemoglobins

By J. R. MORTON
School of Agriculture, University of Cambridge

(*Received* 8 *February* 1965)

1. INTRODUCTION

In an earlier paper (Morton, 1962) the finding of a third allele, Hb^p, at the mouse haemoglobin locus of Ranney & Gluecksohn-Waelsch (1955) was briefly reported. It was also claimed that the classification of Rosa *et al.* (1958) which listed four different haemoglobin types using the slower migrating fractions revealed by starch gel electrophoresis could not be confirmed, but that close comparison of the haemoglobins of five of the six lines which they used agreed with the classification of Russell & Gerald (1958) into two types, Hb-d and Hb-s, only. Meanwhile, Ranney, Marlowe Smith & Gluecksohn-Waelsch (1960) had shown that the most slowly migrating fraction of Hb-d was an 'aggregated' haemoglobin increasing on storage in air, but Barrowman and co-workers (Barrowman & Craig, 1961; Barrowman & Roberts, 1961) found that slowly migrating fractions distinguished foetal from adult haemoglobins in mice; Hb-p was also characterized by its slower fractions. It was thus clear that an investigation of the nature of the multiple bands of Hb-d, Hb-p and foetal haemoglobin was necessary before classification of the *Hb* genetic locus by electrophoresis could be certain. An explanation of the multiple bands good enough to assure their classification has been found; but this has involved conclusions on the nature and structure of mouse haemoglobin which are of more general interest.

2. MATERIALS AND METHODS

The mice came from a stock made by the introduction of known sources of the Hb^s allele from *C57BL/6* and of the Hb^d allele from *CBA/Cag* into the polydactylous strain in which the Hb^p allele was originally found to be segregating with Hb^d (Morton, 1962).

Blood was obtained by cutting off the terminal 2 cm. of the tail of anaesthetized adults, and by decapitating foetal mice, and was received into a 3·2% solution of trisodium citrate dihydrate as anticoagulant. Haemoglobin solutions were prepared by the method of Moretti, Boussier & Jayle (1957).

For electrophoresis strong solutions (about 10%) of HbCo were routinely used. The samples were inserted on Whatman 3MM filter papers into a gel of Connaught Starch-Hydrolysed at either 12% or the concentration recommended by the makers (whichever was the less) in a pH 8·6 buffer of Tris, EDTA and citric acid. It was

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

found that the proportions of these reagents needed to produce a buffer of pH 8·6 varied with the supplier of the Tris. Slight variations were therefore made to the standard millimolar proportions of, 40·0 mM Tris, 0·9 mM EDTA and 3·5 mM citric acid. The reservoirs of the apparatus contained 0·05 M barbitone buffer (pH 8·6) to provide a discontinuous buffer system.

Electrophoresis was carried out at as high a current as could be maintained without excessive heating, and its duration timed by the advance of the brown line (Poulik, 1957) giving an average run of 3 hours at 5mA/cm.^{2}. The gels were sliced and stained by the benzidine method for haemoglobins in starch gel (de Grouchy, 1960) and with a standard protein stain of equal proportions (0·2%) of Naphthalene Black 10B and Nigrosin dissolved in acetic acid–methanol–ethanol–water (1:1:4:4, by vol.).

[^{14}C]*N*-ethylmaleimide was coupled to the haemoglobin with the generous help of Dr Austen Riggs by the technique that he has described (Riggs, 1961). Estimates of the radioactivity of the electrophoretic bands of the haemoglobin were made as follows. After electrophoresis a slice of the gel was stained for protein and an estimate of the protein in each band found from the absorption of a narrow parallel light beam. The gel was then cut into equal vertical slices, which were dried and the radioactivity in each counted in the Nuclear Chicago gas flow counter of the M.R.C. Dept. of Molecular Biology, with the technical assistance of Eileen Blincoe. The amount of *N*-ethylmaleimide bound could then be expressed as radioactivity per arbitary protein unit.

Following Butler, Flynn, Harris & Robson (1961), 0·1 M mercaptoethanol was incorporated in some starch gels.

Sedimentations of 0·9% haemoglobin solutions in 0·09 M pH 7·8 phosphate buffer were followed in the Spinco E analytical centrifuge of the Department of Biochemistry of the University of Cambridge, with the technical assistance of Mr B. Boon.

3. RESULTS

(i) *Adult haemoglobin electropherograms*

The patterns, under the electrophoretic conditions described, of two of the homozygous adult haemoglobins, Hb-d and Hb-p, have been previously reported (Morton, 1962); that of Hb-s is a single band normally indistinguishable from the main bands of Hb-d and Hb-p. The relationships of the three patterns are shown in diagrammatic form in Text-fig. 1. The bands are numbered according to their mobility towards the anode in alkaline conditions.

In Hb-d, bands 1–3 represent the typical diffuse pattern. Band 4, previously described as 'very weak', has since been noticed to be of variable intensity, sometimes absent, and always to stain more strongly with a general protein stain than with benzidine. It is now thought to represent in Hb-d a variable association of haemoglobin with another protein constituent of the red cell. Band 5 is the 'aggregated haemoglobin' of Ranney, Marlowe Smith & Gluecksohn-Waelsh (1960).

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

In Hb-p, bands 1 and 2 are electrophoretically identical with those of Hb-d, but bands 3 and 5 are absent and are apparently replaced by the further fractions 4 and 6.

In Hb-s, the whole haemoglobin usually migrates to approximately the same position as band 2 of Hb-d and Hb-p, but occasionally a forward fringe to the main band is observed, suggesting that there may be a minor fraction of very slightly different mobility in this haemoglobin.

Hb-dp, Hb-ds and Hb-ps, the haemoglobins of heterozygous adults, are indistinguishable from mixtures of equal parts of the haemoglobins of the appropriate homozygotes.

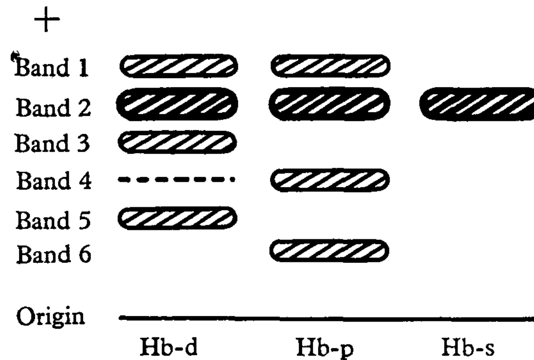

Fig. 1. Diagram of the three haemoglobin electropherograms of homozygous adult mice.

(ii) *Segregation data*

Segregation data of matings between mice varying at the haemoglobin locus are shown in Table 1. They confirm the proposition (Morton, 1962) that Hb^p is allelic to Hb^d and Hb^s, although there is throughout some deficiency of the genotype Hb^d/Hb^s.

(iii) *Binding of* N*-ethylmaleimide*

The amount of radioactive *N*-ethylmaleimide bound by each band of Hb-d was estimated by the method described above. No great accuracy is claimed for this method, but the difference between band 5 and bands 2 and 3 was striking. The values found of radioactivity per arbitrary protein unit were 1·0 for band 1, 1·7 for band 2, 1·6 for band 3 and 0·4 for band 5. Although not wholly conclusive this suggests (Riggs, 1961) that band 5 has many less free sulphydryl groups available to form sulphur bridges than has either bands 2 or 3.

(iv) *Electrophoresis with mercaptoethanol*

Mercaptoethanol is a reducing agent, acting at low concentrations to reduce S–S bridges preferentially (Deutsch & Morton, 1957). The patterns of the three adult haemoglobins after electrophoresis in the presence of mercaptoethanol are

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

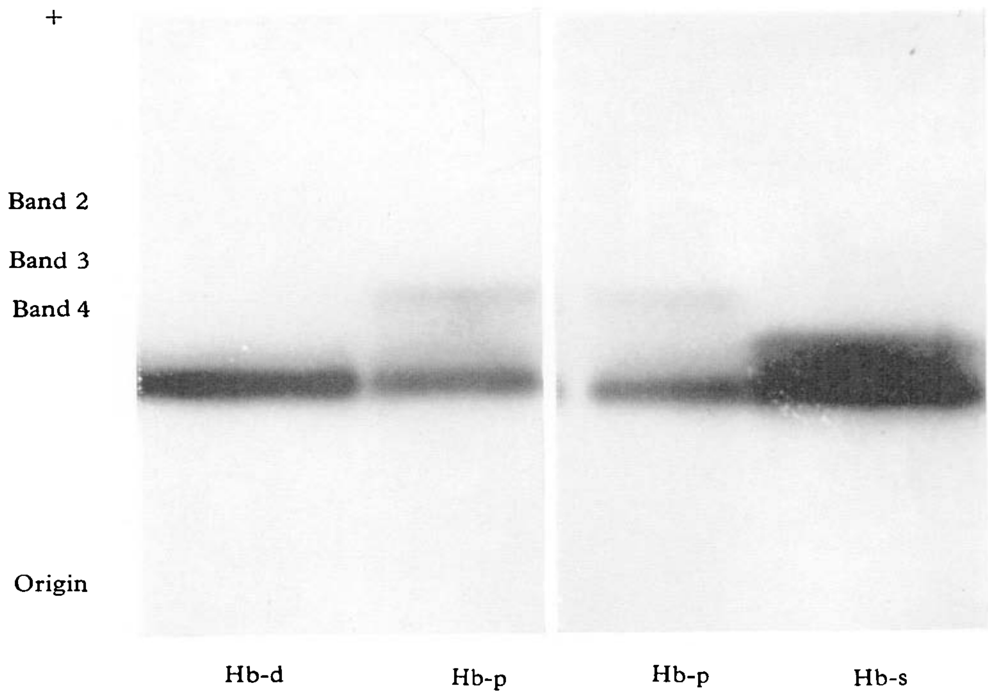

Fig. 2. Comparison of the reduced monomeric forms of (left) Hb-d and (right) Hb-s with Hb-p. Electrophoresis at 4·8 mA/cm². for 190 min. in pH 8·6 discontinuous buffer with 0·75% mercaptoethanol. Photographed unstained.

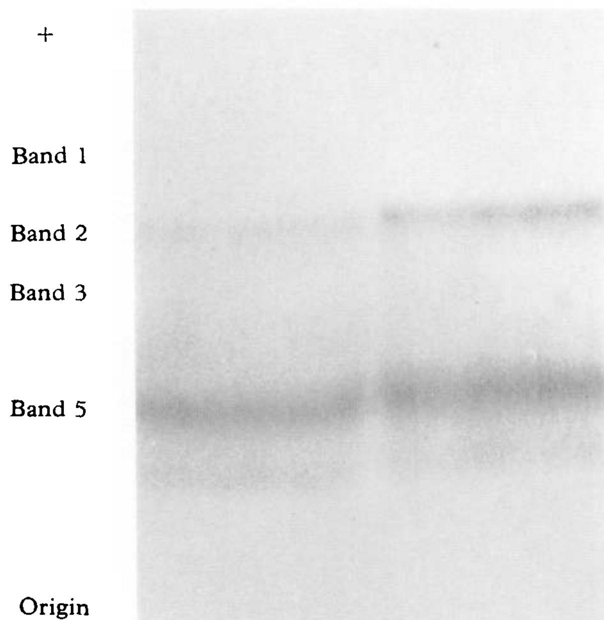

Fig. 3. A second electrophoresis (0·05 M discontinuous buffer pH 8·6, 4·9 mA/cm.² for 130 min.) after 12 days' storage at 4°C. of two aliquots of band 2 of Hb-d excised from a first electrophoresis. Aliquot 1 (left) stored open to the air, aliquot 2 (right) stored under paraffin oil. Stained for general protein.

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

Table 1. *Genetic segregation of* Hb^{p} *with* Hb^{s} *and* Hb^{d}

Mating type	Mating number	d	dp	p	s	'p'	'd'
p × d	P5		6				
	P6		4				
s × dp	HP3					4	2
ps × ps	HSP1				2	9	
dp × d	H16	20	21				
	H17	3	4				
	P1	5	4				
	P2	1	2				
	P3	2	3				
	P4	4	3				
p × dp	H15		8	3			
d × 'p'	H11		10				4
	H12		27				4
dp × 'p'	H22		0			4	1
	H23		8			17	
	H24		10			6	
	H26		2			4	4
	H27		1			3	1
	H30		5			11	
	H31		10			5	
	H32		4			4	

d, dp, p and s are used for haemoglobins Hb-d, Hb-dp, Hb-p and Hb-s. 'p' and 'd' are used for cases in which Hb-p and Hb-ps, and Hb-d and Hb-ds, cannot be distinguished with certainty.

greatly simplified (Fig. 2, Plate I). Hb-s remains a single fraction; Hb-d exhibits a similar major fraction with a second band just posterior to it; and Hb-p again shows the same major fraction but with an even more slowly migrating minor band. Allowing for the retardation of migration always found with mercapto-ethanol, the mobilities of these bands suggest that they are band 2 in Hb-s, bands 2 and 3 in Hb-d and bands 2 and 4 in Hb-p. Taken with the experiments with $[^{14}\mathrm{C}]$*N*-ethylmaleimide these results suggest that band 5 and probably also bands 1 and 6 differ from bands 2 and 3 or 4 by the formation of S–S bridges.

(v) *Ultracentrifuge analyses*

The sedimentation constants calculated from the ultracentrifuge analysis of Hb-d, Hb-dp and Hb-p are shown in Table 2. These three haemoglobins apparently behave in the same way. In fresh samples run within 2 days of lysis of the red blood cells a single peak is observed with the normal sedimentation constant for mammalian haemoglobins of about 4·2 Svedberg units, as noted by Ranney, Marlow Smith & Gluecksohn-Waelsch (1960), and consistent with a molecular weight of 63,500 (Riggs, 1963). Ranney *et al.* also noted the formation of a more rapidly sedimenting fraction which, together with band 5 of the electrophoretic pattern, increased when Hb-d was stored; this they termed 'aggregated haemo-

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

Table 2. *Sedimentation constants, in Svedberg units, of haemoglobin solutions*

Experiment number	Type and age of sample	Monomer*	Dimer*	Trimer*
890	Hb-d 10 days	4·26	6·08	
	Hb-dp 10 days	4·17	6·01	
892	Hb-d 2 days	4·23		
	Hb-d 6 days	4·32	6·51	
922	Hb-dp 1 day	4·05		
	Hb-p 1 day	4·06		
1045	Hb-d 80 days	4·04		8·25
	ditto + mercaptoethanol	4·16		
1050	Hb-d 13 days	4·12	6·45	
1057	Hb-d 29 days	4·16	6·46	
	Mean values	4·157 ± 0·029	6·302 ± 0·105	8·25

* Monomer, dimer and trimer refer to the whole haemoglobin molecule and thus represent 4, 8 and 12 polypeptide chains respectively.

globin'. The same observation was made here for Hb-d and extended to Hb-dp and Hb-p. In all but one of the samples stored in corked tubes at 4°C. a fraction with a sedimentation constant of 6·3 Svedberg units was observed. This increased with time compared to the 4·2 S fraction at least up to the twenty-ninth day of storage. Similar increases were observed in bands 5 and 6 as appropriate of Hb-d, Hb-dp and Hb-p after electrophoresis of the stored samples. Since a haemoglobin dimer will no doubt be less spherical than the monomer, the value of 6·3 S is consistent with the view that 'aggregated haemoglobin' is a dimer of the 4·2 S molecule, and with the values found by Riggs, Sullivan & Agee (1964) in a similar situation in the bull frog and turtle species. By dimers is here meant the bonding together of two whole haemoglobin molecules, giving in all eight polypeptide chains.

Ranney *et al.* (1960) further noted that on prolonged storage solutions of Hb-d tend to precipitate. Such partial precipitation was observed here in a sample of Hb-d that had been stored for 80 days. When the supernatant was run on the ultracentrifuge a normal and a fast peak were again observed, but in this case the faster fraction had a sedimentation constant of 8·25 Svedberg units, suggestive of a trimer of the 4·2 S molecule. The electropherogram of this solution showed band 2 and a somewhat diffuse fraction migrating between the normal positions of bands 5 and 6. When mercaptoethanol was added to this aged sample, most of the precipitate dissolved and subsequent ultracentrifuge analysis showed only the 4·2 S peak.

Taken with the results of the two previous sections this indicates the formation of S–S bonded dimers, and occasionally higher polymers in stored solutions of the multiple haemoglobins.

(vi) *Instability of electrophoretic fractions*

With the initial intention of finding out which of the monomeric electrophoretic bands formed the polymeric, or conversely which monomeric bands could be

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

reconstituted from the polymers, all bands of the electrophoretic patterns of Hb-d and Hb-p were cut from the gel, extracted by freezing, thawing and spinning in a basket centrifuge tube, and subjected to electrophoresis a second time. The resultant patterns were often very faint and over many experiments the results were not always comparable on different occasions for reasons that were not clear in every case. Nevertheless there is no doubt of the general conclusion that the different electrophoretic bands do not represent permanently discrete haemoglobins but are able reversibly to convert one into another.

Table 3. *Equilibria between electrophoretic bands of* Hb-d

	Number of times band recognized in second electrophoresis					
Band excised	1	2	3	5	Mercaptoethanol 2	Mercaptoethanol 3
1	3	1				
2	18	20	6	10	2	1
3	11	13	10	9	2	2
5		1		1	2	2
Mercaptoethanol 2					8	2
Mercaptoethanol 3					4	3

The results are combined and summarized in Tables 3 and 4 which show for Hb-d and Hb-p the number of occasions that each band has been definitely recognized in the second electrophoresis from each individual band excised after the first electrophoresis.

One of the causes of the variability is easily recognized. The amounts of the dimeric bands 5 and 6 formed depend on the availability of oxygen in the conditions under which the sample is stored between the two electrophoreses: for example, Fig. 3 (Plate I) shows the electrophoresis of two extracted samples of one band 2 of Hb-d which had been further stored at 4°C. for 12 days, one open to air, and the other covered with paraffin oil. Both show the full pattern of 4 bands of Hb-d but in the open sample band 5 is clearly stronger.

At the present time the remaining variation between occasions cannot be explained, but the results clearly show that for Hb-d each band can convert into every other directly or indirectly, and that the same may be said for Hb-p with the possible exception that its dimer, band 6, is more stable than band 5 of Hb-d.

Table 4. *Equilibria of electrophoretic bands of* Hb-p

	Number of times band recognized in second electrophoresis					
Band excised	1	2	4	6	Mercaptoethanol 2	Mercaptoethanol 4
1	1	4				
2	3	7		3	1	
4	1	4	4			
6				3	2	2
Mercaptoethanol 2					3	2
Mercaptoethanol 4						2

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

(vii) *Foetal haemoglobins*

Craig & Russell (1963) have shown that the electrophoretic patterns of foetal haemoglobins from mice of Hb-d and Hb-s strains are similar, the strains differing only in the time of changeover to the adult pattern. Foetal mice from Hb-p parents showed a haemoglobin pattern again indistinguishable from that of Hb-d foetal mice. It is proposed to call this haemoglobin Hb-f.

The typical electropherogram of mouse foetal haemoglobin is 5-banded as shown in the first sample of Fig. 4 (Plate II). In the presence of mercaptoethanol the pattern simplifies to bands 2, 3 and 4 only. Band 5 of Hb-f thus appears analogous to band 5 of Hb-d, but it differs from the latter in that it is easily seen in fresh preparations, as noted by Barrowman & Roberts (1961). This may be seen in the remaining samples in Fig. 4 (Plate II) which show that even when the foetus has almost achieved the full adult haemoglobin pattern, the degree of dimerization of the foetal haemoglobin after storage for 1 day is approximately equivalent to the degree in true adult Hb-d after storage for 10 days.

It should be noted, finally, that band 4 of Hb-f must differ from band 4 of Hb-p or Hb-dp since no band 6 is observed in the pattern of the foetal protein.

4. DISCUSSION

Band 1 of the electrophoretic patterns of Hb-d and Hb-p is the least clearly characterized. It is not found in gels incorporating mercaptoethanol, suggesting that it must be an oxidation product possibly as a result of sulphur–sulphur bridging. It was at first thought that it might represent a sulphur-bridged combination with glutathione, as had been proposed by Rosa & Labie (1962), for the structure of human Hb-A^3, subsequently called Hb-A_1 (Atassi, 1964*b*). But no increase in band 1 was found when the glutathione was added to Hb-d and Hb-p before electrophoresis, and it is difficult on this theory to understand the formation of band 1 in second electrophoreses of the excised bands 2 and 3 of Hb-d and 2 and 4 of Hb-p.

Band 5 of Hb-d and band 6 of Hb-p are dimers, that is molecules of molecular weight about 127,000, of bands 2 or 3 and bands 2 or 4 respectively, most probably joined by disulphide bands. Whether band 5 for example is better considered the dimer of 2 or 3 will be discussed below. Bands 5 and 6 both increase with storage in oxygenated conditions parallel with the increase of the peak in the ultracentrifuge analysis having a sedimentation constant typical of the dimer of the main fraction. Both can be converted by mercaptoethanol into the monomeric state, band 5 into bands 2 and 3 and band 6 into bands 2 and 4. It has also been shown that the rate of formation of band 5 of Hb-d can be checked by covering the solution with paraffin oil. For Hb-d it was demonstrated that further polymerization occurs yielding a soluble trimer and a precipitate of an unknown degree of polymerization. Riggs, Sullivan & Agee (1964) have reported similar dimerization of haemoglobins of the bull frog and several species of turtle.

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

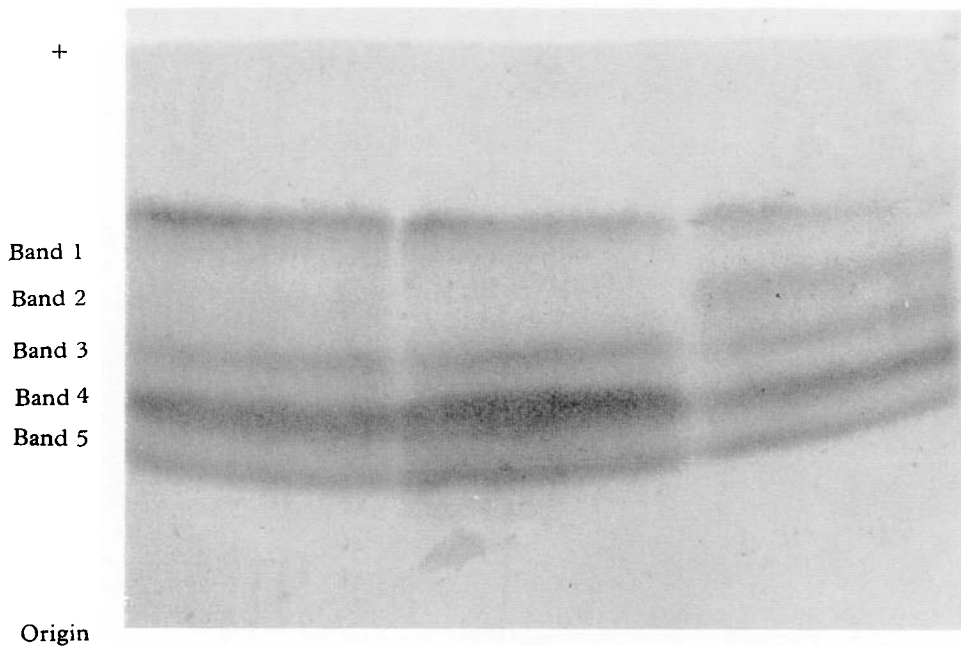

Fig. 4. Patterns, stained for general protein, of foetal and adult (Hb-d) haemoglobins after electrophoresis in 0·05 M discontinuous buffer pH 8·6, at 5·1 mA/cm.^{2} for 190 min., showing extra band in young foetuses (sample 1) and accelerated dimerization (band 5) in young and old foetuses (samples 1 and 3). Sample 1 stored for 1 day from 13-day-embryos, sample 2 stored for 10 days from adult, sample 3 stored for 1 day from 16-day-embryos.

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

Since the submission of this paper Riggs (1965) has published a preliminary report of similar work with mouse haemoglobin in which he concludes that the dimer Hb-d is joined by disulphide bridges between β-chains.

Bands 2 and 3 of Hb-d and 2 and 4 of Hb-p are monomers and represent in some way which will now be considered the genetic differences from the single band 2 of Hb-s. Hutton, Bishop, Schweet & Russell (1962*a*) separated two chromatographic peaks in Hb-d and found only one in Hb-s. They further found that the two peaks of Hb-d could be explained by the coexistence of two different β-chains in Hb-d. They not unnaturally suggested that the chromatographic peaks are related to the two main electrophoretic bands. The fact that bands 2 and 3 convert into one another adds some complexity to this suggestion. Hutton *et al.* (1962*b*) also found that a major difference between Hb-s and Hb-d lay in the presence of tryptophan in the Hb-s β-chain, but its absence in β^d. This was confirmed and extended by Popp (1962) who showed that the peptide containing tryptophan in Hb-s was absent in Hb-d, but that a peptide containing arginine in Hb-d was absent in Hb-s. Popp points out that he has not proved the homology of these two peptides nor that the tryptophan–arginine change is the only difference between them. But by analogy with the single amino-acid genetic variants in human haemoglobins it seems likely that this will prove to be their only difference. If this is so, we need to explain how Hb-d and Hb-p form dimers which Hb-s does not, and also how two different genetic changes, Hb^s to Hb^d and Hb^s to Hb^p, both produce a multibanded pattern of the monomer.

One hypothesis which could account for the observations is the following. Initially the changes $Hb^s \rightarrow Hb^d$ and $Hb^s \rightarrow Hb^p$ affect amino-acid sequences deep in the globin molecule, not noticeably changing the surface electrostatic charge, hence the similar appearance of band 2 in all three electrophoretic patterns; but these two changes alter the molecule in a way which enables it to exist as two structural isomers, the new isomers revealing the electrostatic differences of Hb-d and Hb-p from Hb-s which are observed as bands 3 and 4. Finally it is supposed that the structural isomers, bands 3 and 4, have altered surface topography to permit polymerization to take place, probably by S–S bonding.

Alternative hypotheses no doubt are possible, but it is relevant that there have recently been several reports of equilibria between electrophoretic and chromatographic fractions of the globin molecule, not related to the isomerism involved in normal oxygenation (Benesch & Benesch, 1964). Lingrel & Borsook (1962) found it necessary to postulate some equilibria between fractions to account for their results with the minor fractions of rabbit haemoglobin. Atassi (1964*a*) found interchange equilibria between fractions of whale myoglobin. Atassi (1964*b*) also found an equilibrium to exist between fractions A_1 and A_0 of human haemoglobin which he interpreted as two configurational isomers, and, of particular relevance here, he related the conversion of Hb-A_0 to Hb-A_1 on storage to the increase in —SH reactivity reported by Benesch & Benesch (1962). Benesch, Benesch, Ranney & Jacobs (1962) demonstrated an equilibrium between two electrophoretic bands of human Hb-H. This last work is of particular relevance to the proposed

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

nature of the mouse multiple haemoglobins since Benesch *et al.* were able to advance convincing evidence that the two bands differ in tertiary structure.

This work on the multiple bands of mouse haemoglobin confirms the proposition that the haemoglobins of mice so far fully investigated can be classified by electrophoresis into the products of the three genes Hb^d, Hb^p and Hb^s only. It further suggests that the mouse may prove particularly useful for the investigation of the polymerization and isomerism noted in the haemoglobins of other species, since alternatives at a single genetic locus having a small chemical effect on the molecule are responsible for the presence or absence of both these phenomena.

SUMMARY

A survey of past work shows that certainty in the electrophoretic classification of mouse haemoglobins requires further understanding of the meaning of the multiple electrophoretic bands observed.

Six electrophoretic bands are observed in all. Numbered in order of mobility they give the formulae 2 for Hb-s, 1235 for Hb-d, 1246 for Hb-p and 12345 for foetal haemoglobin (Hb-f). When mercaptoethanol is added to the electrophoretic medium these formulae become: 2 for Hb-s, 23 for Hb-d, 24 for Hb-p, 234 for Hb-f. Bands 5 and 6 and a fast ultra-centrifuge fraction both increase when samples are stored.

Bands 5 of Hb-d, 6 of Hb-p, and probably 5 of Hb-f, are dimers of the whole haemoglobin molecule almost certainly joined by sulphur bridges. The monomers are bands 2 and 3 of Hb-d and 2 and 4 of Hb-p. Within each type interconversion of bands 2 and 3 and bands 2 and 4 has been observed.

The hypothesis is suggested that Hb-d and Hb-p both exist as two conformational isomers. In each case one isomer is electrophoretically indistinguishable from Hb-s: the other permits the electrophoretic recognition of their differences in bands 3 and 4, and the formation of sulphur bridges to give bands 5 and 6.

I wish to thank Dr Austen Riggs, Mr B. Boon and Miss Eileen Blincoe for their technical help. I am particularly grateful to Dr Douglas G. Gilmour for his help and encouragement throughout this work.

REFERENCES

ATASSI, M. Z. (1964 *a*). Properties of components of myoglobin of the Sperm Whale. *Nature, Lond.* **202**, 496–498.

ATASSI, M. Z. (1964 *b*). Chemical studies on haemoglobins A_1 and A_0. *Biochem. J.* **93**, 189–197.

BARROWMAN, J. & CRAIG, M. (1961). Haemoglobins of foetal *C57BL/6* mice. *Nature, Lond.* **190**, 818–819.

BARROWMAN, J. & ROBERTS, K. B. (1961). Foetal haemoglobs of *CBA* mice. *Nature, Lond.* **189**, 409–410.

BENESCH, R. & BENESCH, R. E. (1964). Properties of haemoglobin H and their significance to function of haemoglobin. *Nature, Lond.* **202**, 773–775.

BENESCH, R., BENESCH, R. E., RANNEY, H. M. & JACOBS, A. S. (1962). Isomeric forms of haemoglobin H. *Nature, Lond.* **194**, 840–842.

BENESCH, R. E. & BENESCH, R. (1962). The influence of oxygenation on the reactivity of the —SH groups of haemoglobin. *Biochemistry*, **1**, 735–738.

BUTLER, E. A., FLYNN, F., HARRIS, H. & ROBSON, E. B. (1961). The laboratory diagnosis of macroglobulinaemia. *Lancet*, **2 (1961)**, 289–293.

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press

Craig, M. L. & Russell, E. S. (1963). Electrophoretic patterns of hemoglobin from fetal mice of different inbred strains. *Science, N.Y.* **142,** 398–399.

Deutsch, H. F. & Morton, J. I. (1957). Dissociation of human serum macroglobulins. *Science, N.Y.* **125,** 600.

Grouchy, J. de (1960). Protéines et enzymes tissulaires—une étude par électrophorèse en gel d'amidon. *Revue fr. Étud. clin. biol.* **5,** 286–290.

Holt, S. B. (1945). A polydactyl gene in mice capable of nearly regular manifestation. *Ann. Eugen.* **12,** 220–249.

Hutton, J. J., Bishop, J., Schweet, R. & Russell, E. S. (1962 *a*). Hemoglobin inheritance in inbred mouse strains, I. Structural differences. *Proc. natn. Acad. Sci. U.S.A.* **48,** 1505–1513.

Hutton, J. J., Bishop, J., Schweet, R. & Russell, E. S. (1962*b*). Hemoglobin inheritance in inbred mouse strains, II. Genetic studies. *Proc. natn. Acad. Sci. U.S.A.* **48,** 1718–1724.

Lingrel, J. B. & Borsook, H. (1962). Haemoglobin minors as possible intermediates in haemoglobin synthesis. *Nature, Lond.* **195,** 355–356.

Moretti, J., Boussier, G. & Jayle, M.-F. (1957). Réalisation technique et premières applications de l'électrophorèse sur gel d'amidon. *Bull. Soc. Chim. biol.* **39,** 593–605.

Morton, J. R. (1962). Starch gel electrophoresis of mouse haemoglobins. *Nature, Lond.* **194,** 383–384.

Popp, R. A. (1962). Studies on the mouse hemoglobin loci. V. Differences among tryptic peptides of the β-chain governed by alleles at the *Hb* locus. *J. Hered.* **53,** 142–146.

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

Ranney, H. M. & Gluecksohn-Waelsch, S. (1955). Filter paper electrophoresis of mouse haemoglobin: a preliminary note. *Ann. hum. Genet.* **19,** 269–272.

Ranney, H. M., Marlowe Smith, G. & Gluecksohn-Waelsch, S. (1960). Haemoglobin differences in inbred strains of mice. *Nature, Lond.* **188,** 212–214.

Riggs, A. (1961). The binding of *N*-ethylmaleimide by human hemoglobin and its effect upon oxygen equilibrium. *J. biol. Chem.* **236,** 1948–1954.

Riggs, A. (1963). The amino-acid composition of some mammalian hemoglobins: Mouse, Guinea Pig, and Elephant. *J. biol. Chem.* **238,** 2983–2987.

Riggs, A., Sullivan, B. & Agee, J. R. (1964). Polymerisation of Frog and Turtle hemoglobins. *Proc. natn. Acad. Sci. U.S.A.* **51,** 1127–1134.

Riggs, A. (1965). Haemoglobin polymerisation in mice. *Science, N.Y.* **147,** 621–623.

Rosa, J. & Labie, D. (1962). Change in A^3 haemoglobin due to β chain. *Nature, Lond.* **196,** 901.

Rosa, J., Shapira, G., Dreyfus, J. C., Grouchy, J. de, Mathé, G. & Bernard, J. (1958). Different heterogeneities of mouse haemoglobin according to strains. *Nature, Lond.* **182,** 947–948.

Russell, E. S. & Gerald, P. S. (1958). Inherited electrophoretic patterns among 20 inbred strains of mice. *Science, N.Y.* **128,** 1569–1570.

https://doi.org/10.1017/S0016672300009484 Published online by Cambridge University Press