HAEMOGLOBIN POLYMORPHISMS IN THE ECHIDNA, *TACHYGLOSSUS ACULEATUS*

By D. W. COOPER,* J. L. VANDEBERG,* M. E. GRIFFITHS,† and E. H. M. EALEY‡

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Abstract

Echidnas and platypuses possess two haemoglobins, HbI and HbII. HbI is in the higher concentration. Genetic variation has been found in the electrophoretic mobility of both HbI and HbII in the echidna. HbI polymorphism occurs on the mainland of Australia and HbII polymorphism in Tasmania. Rare variants of HbII occur on the mainland. There are three common HbI variants designated A, B, C, and two common HbII variants, A and B. HbI variants are in the β^{I} chain and HbII variants are in the β^{II} chain. The results allow four groups of echidnas to be defined: (1) "Tasmanian", which has HbIB, HbIIA, and HbIIB; (2) "south-eastern Australia", i.e. Victoria east of Melbourne and probably part of the south-east of New South Wales, which has HbIB and HbIIA; (3) a "hybrid" group found in western Victoria and in coastal areas of New South Wales north of Sydney, which has HbIA, HbIB, HbIC, and HbIIA; and (4) the remaining areas of the continent sampled which have HbIA, HbIC, and HbIIA.

I. INTRODUCTION

The echidna, *Tachyglossus aculeatus*, belongs to the Tachyglossidae, one of the two extant families of monotreme mammals (Griffiths 1968.) Little has been published on its genetics. The male has 63 chromosomes, the female 64, but whether the species has an XX/XO or an $X_1X_1X_2X_2/X_1X_2Y$ sex-determining mechanism is not known (Bick and Jackson 1967*a*). Its DNA content is close to that of marsupial and eutherian mammals (Bick and Jackson 1967*b*). During the course of a search for variation in the electrophoretic mobility of red cell enzymes, we have found considerable variation in haemoglobin of the echidna. It thus joins the comparatively small number of mammals shown to have this polymorphism [see Lush (1966) and Manwell and Baker (1970) for a summary]. The characteristics of the polymorphism and the information it gives about the population structure of the species are reported in this paper. The term polymorphism is used in the strict genetic sense first given it by Ford (1940), namely the occurrence together in the one population of two more discontinuous forms such that the rarest of them cannot be maintained by recurrent mutation alone.

* Department of Genetics and Human Variation, La Trobe University, Bundoora, Vic.; present address: School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113.

† Division of Wildlife Research, CSIRO, P.O. Box 84, Lyneham, A.C.T. 2602.

‡ Department of Zoology, Monash University, Clayton, Vic. 3168.

II. Materials and Methods

The places from which animals were obtained are given in Table 1 and Figure 1. With the exception of some deliberate searching on Kangaroo I. and Tasmania, organized by M.E.G., most collections were opportune. The distribution of collections is heavily biased towards the south-east of the continent, accurately reflecting the population density of echidna collectors, though not necessarily of echidnas. For some captive animals, as indicated in Table 1, the area from which the animal came could only be given as "Victoria" or "Queensland" or could not be given at all. Animals captured in urban areas such as Melbourne or Canberra are probably native to the area, but in a few cases may have been introduced from elsewhere by someone wishing to display this usually cryptic animal.

Fig. 1.—Map of south-eastern Australia and Tasmania showing where echidnas were collected. The numbers beside each solid circle represent the numbers collected there, except when only one animal was collected, in which case no number is given. Solid circles placed alongside islands (Tasmania, Flinders I., and Kangaroo I.) or beside part of the continent (Victoria, east of Melbourne) indicate that animals were caught at a number of different places in these areas. The broken lines and parentheses indicate the hybrid zone (H.Z.) in which all three HbI haemoglobins occur. The extent of the area occupied by the hybrid group is not known with any precision. Not shown on this map are the animals from central Australia (4), New Guinea (1), and western Australia (1).

Blood was collected either by allowing it to drip slowly into a bottle after cutting off the end of a claw or by cardiac puncture after anaesthetizing the animal with ether. The second method is preferable, since neither the anaesthetic nor the operation appears to hurt the animal. Up to 50 ml of whole blood can be taken from an adult animal, which can then be return unharmed to the wild or to a captive population. Chloroform is not recommended as an anaesthetic. On the one occasion it was used the animal suffered apparent lung damage and had to be killed a short time later.

Blood was put into sterile Alsever's solution (20·5 g glucose, 8·0 g trisodium citrate dihydrate, and 4·2 g sodium chloride per litre—no adjustment of pH is made) in which it can be stored at 4°C for several weeks without observable cell lysis. Cells stored in our usual blood preserving fluid,

citrated saline (17·4 g trisodium citrate dihydrate and 5·0 g sodium chloride per litre) lysed over a period of a few days. An experiment was performed in which Alsever's solution was modified by varying the amount of NaCl between 3·7 and 4·7 g per litre and citrated saline was modified by varying its NaCl concentration between 4·5 and 5·5 g per litre. Little or no lysis was observed

TABLE 1

HAEMOGLOBIN I POLYMORPHISM IN THE ECHIDNA

Population	Electrophoretic type						Total	Gene frequencies		
	A	C	AC	AB	BC	B		p^A	p^C	p^B
1. Tasmania						15	15			1·0
2. Flinders I.						2	2			*
3. Eastern Victoria										
(a) Melbourne						(6)				
(b) Victoria east of Melbourne (mainly Gippsland)						(16)				
Total						22	22			1·0
4. Near Sydney										
(a) North Ryde						(1)				
(b) Jenolan Caves						(1)				
Total						2	2			*
5. Australian Capital Territory	1			1		23	25	0·06		0·94
6. Victoria West of Melbourne										
(a) Horsham	(1)			(2)						
(b) Grampians						(1)				
(c) Little Desert	(1)					(1)				
(d) Tower Hill						(1)				
Total	2			2		3	7	*		*
7. Adelaide and Fleurieu Peninsula	4			1‡			5	*		*
8. Kangaroo I.	18		4				22	0·91	0·9	
9. Northern New South Wales and Queensland										
(a) Gosford				(2)						
(b) Pt. Macquarie				(1)						
(c) Narrabri					(1)					
(d) Moorinda Station (near Tenterfield)		(1)								
(e) Inverell	(1)									
(f) Tara	(2)	(1)	(1)							
(g) "Queensland"†	(1)									
Total	4	2	1	3	1		11	*	*	*
10. Central Australia, and western New South Wales										
(a) Garden Station (via Alice Springs)	(4)									
(b) Tero Creek	(1)									
(c) Rankins's Springs	(2)§									
Total	7						7	*		
11. Western Australia										
Murchison River	1						1	*		
12. New Guinea										
Baiyer River, near Mt. Hagen	1						1			
13. Adelaide Zoo†	2			2		2	6	*		*
14. Taronga Zoo†	2					4	6	*		*
15. Monash University† (Victoria but locality unknown)						4	4	*		*

* Numbers too small to make calculation of gene frequency worthwhile.

† Area from which animal came either imprecisely known or unknown.

‡ Not definitely known to be from this area.

§ These two were mother and offspring.

in any of the modified Alsever's solution after 4 days at room temperature while all cells in citrated saline showed considerable lysis. Evidently the salt concentration is not very critical in maintaining the echidna red cell intact. Rather the result suggests but does not prove that glucose is necessary for this purpose. Red cells which had been washed in 0·85% NaCl three times could be stored

frozen at −20 to −30°C for periods of at least a year in a solution of 400 ml of ethylene glycol and 60 g of trisodium citrate dihydrate made up to 1 litre with distilled water. The ratio of this solution to packed red cells can be from 1 : 1 to 3 : 1. Cells removed from the deep-freeze after storage in this solution were hard to lyse even when repeatedly frozen and thawed in the gel buffer (for formula see below). Recently this problem has been overcome by incorporating 0·01% of the neutral detergent Triton X100 in gel buffer which causes complete lysis in a few minutes.

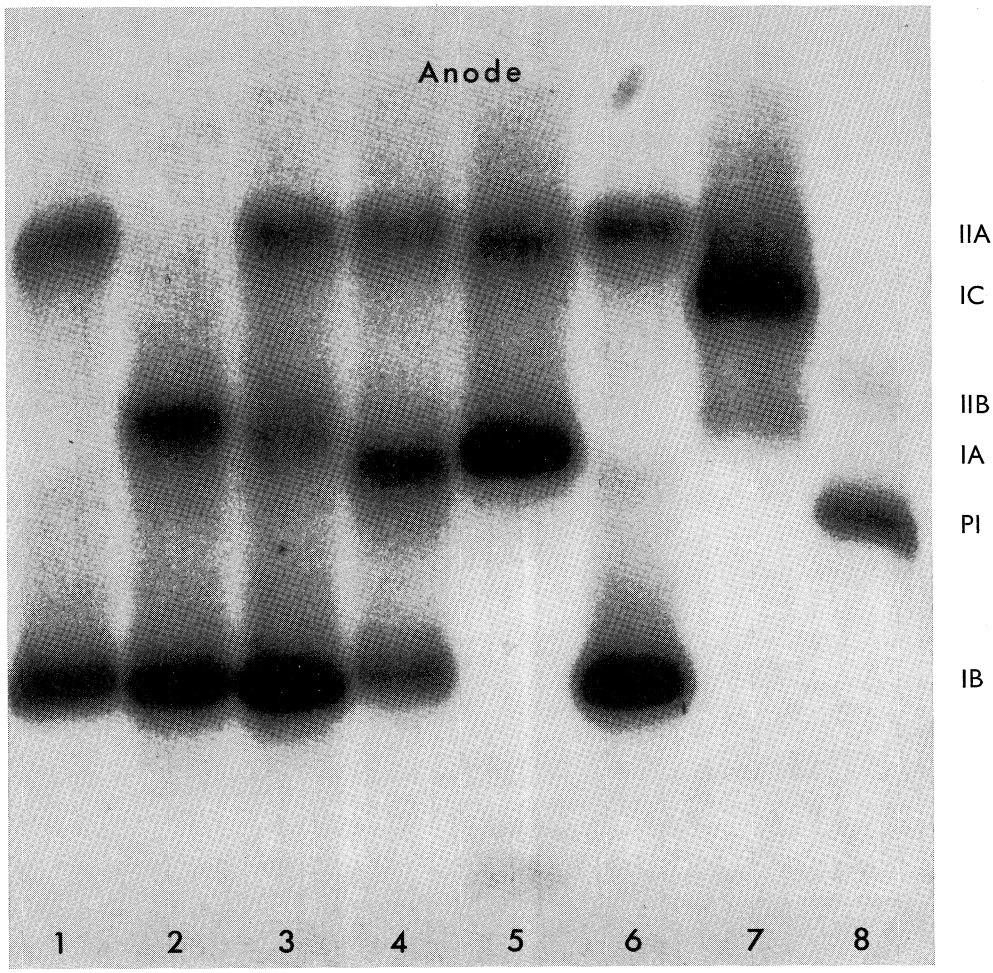

Fig. 2.—Starch-gel electrophoresis of echidna (channels 1–7) and platypus (Pl, channel 8) haemoglobin, under conditions described in the text. *1*, HbIB, HbIIA; *2*, HbIB, HbIIB; *3*, HbIB, HbIIAB; *4*, HbIAB, HbIIA; *5*, HbIA, HbIIA; *6*, HbIB, HbIIA; *7*, HbIC, HbIIAB; *8*, platypus HbI and HbII. The slow-moving band in channel 5 is a non-haemoglobin protein whose nature was not identified. The trace component in channel 6 near the region of HbIA and HbIIB is a haemoglobin component which was sometimes found in this position. Its nature and significance are unknown.

Lysates made up of one part cells to four parts gel buffer were spun at 20,000 *g* for 30 min before electrophoresis. Electrophoresis was carried out in 12% starch gel (Electro-Starch, Otto Hiller, Madison, Wisconsin) for 15–18 hr at 4–7 V cm. Two stock buffers were used. The first was 0·9M trishydroxyaminomethane, 0·5M borate, 0·02M EDTA (Smithies 1964, 1965) which gave

a pH in the gel of 8·5 and the second a modification of this with 0·1M EDTA giving a final pH in the gel of 8·0 (Cooper *et al.* 1969). Both were used at 9 in 80 dilutions in the electrodes and 1 in 20 dilutions in the gel and both gave essentially the same pattern except that the mobilities of the bands were slower in the second system. The patterns could be recorded by photographing the cut surface of the sliced gel without further treatment or else after staining with 1% napthalene black and washing with napthalene black solvent, i.e. methanol–water–glacial acetic acid (5 : 5 : 1%).

All typings were carried out on freshly made haemolysates, either from cells taken from the Alsever's solution used for collection or from cells in the glycol–citrate solution. Typings were not made on haemolysates which had been frozen and thawed. Thus none of the bands observed were likely to have been methaemoglobin.

III. Results and Discussion

Some of the patterns found are shown in Figure 2. These patterns have been interpreted to mean that the echidna possesses two haemoglobins, HbI and HbII. The former, the more concentrated, has the polypeptide constitution $\alpha_2 \beta_2^{I}$ and the latter, $\alpha_2 \beta_2^{II}$ (Thompson, personal communication 1972). The possession of two haemoglobins in fairly high concentrations is evidently a monotreme characteristic, since the two platypuses examined also had two (see channel 8 of Fig. 2).

Electrophoretic variation has been found in both components of the echidna. By analogy with many other such polymorphisms which have been studied in animals for which genetic data can be obtained, one can reasonably assume that this variation is under genetic control. HbI polymorphism occurs on the mainland (Table 1). There are three variants labelled A, B, and C, which are determined by alleles of the β^{I} chain cistron. All Tasmanian animals are type B for haemoglobin I.

Table 2

Haemoglobin II polymorphism in the echidna

Population	Electrophoretic type						Total	Gene frequencies		
	A	AB	AC	B	A plus "Taronga"†	A plus "Queensland"†		p^A	p^B	p^C
Tasmania	2	6		7			15	0·33	0·67	
Flinders I.	1	1					2	*	*	*
Australian mainland	91	1	2		1	1	96	0·98	0·004	0·013
Kangaroo I.	21		1				22			
New Guinea	1						1	*		
Total	116	8	3	7	1	1	136			

* Total number of animals too small to make giving gene frequencies worthwhile.

† See Figure 3 for diagrams showing each of the rare variants.

HbII variation occurs mainly in Tasmania where there are two polymorphic forms, A and B (Table 2). These are determined by alleles of the β^{II} cistron of HbII. Variation in HbII on the mainland is uncommon, almost all animals being HbIIA. This statement must be made with some reserve for animals which also have HbIA, the mobility of which is close to that of HbIIB (see Figs. 2 and 3). It is possible

but not very likely that some HbIIAB heterozygotes could have been typed as HbIIA only. Only one HbIIAB type was found on the mainland (Fig. 2, channel 7; Table 2). The patterns for the rare mainland variants of HbII are shown in Figure 3. With the possible exception of that found on Kangaroo I., these are β^{II} chain variants. This Kangaroo I. variant could be a double heterozygote, i.e. HbIAC and HbIIAC —the interpretation given in Figure 3—or it could be a single heterozygote for the α chain, which is common to HbI and HbII. The fact that the HbIIAC phenotype was found in two Victorian echidnas which were also HbIB makes the former explanation more likely.

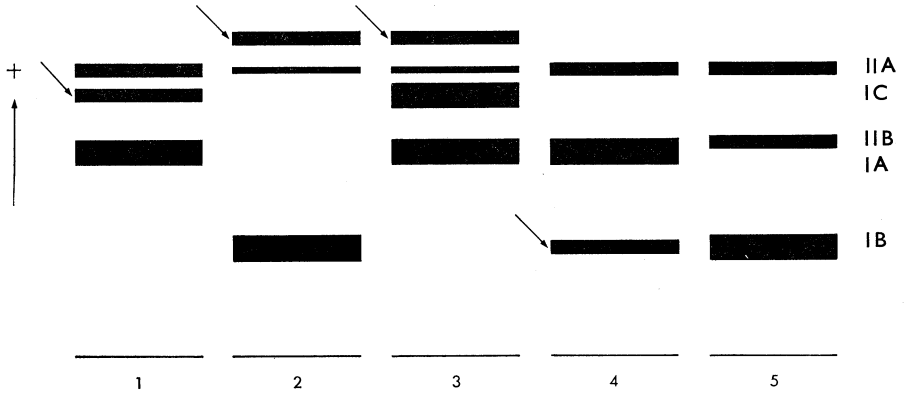

Fig. 3.—Diagram showing the mobility of the rare HbII variants on the mainland and Kangaroo I., compared with HbIIA and HbIIB. In each case the variant is arrowed. Note that in channels 2 and 3, which have HbIIAC, these appear to be more C than A. The types and their localities are as follows: *1*, HbIA, HbIIA plus HbII "Queensland"—the place in Queensland from which this animal came was not known; *2*, HbIB, HbIIAC—this phenotype was observed twice, in animals from Powelltown, Vic., and Benalla, Vic.; *3*, HbIAC, HbIIAC, observed in animals from Kangaroo I.; *4*, HbIA, HbIIA plus "Taronga"; *5*, HbIB, HbIIAB—this phenotype is common in Tasmania and on Flinders I. The designations of the common variants (but not of the rare ones) are given on the right of the figure.

The results shown in Tables 1 and 2 suggest the existence of four geographical groups of echidnas, one in Tasmania and three on the mainland. The distribution of these groups is as follows:

(1) Tasmanian, characterized by being all HbIB and being polymorphic for HbII. The two animals from Flinders I. also seem to fit into this group, which is in accord with the generally accepted view of their status (Griffiths 1968). The Tasmanian echidna has been regarded either as a separate species (Iredale and Troughton 1934) or as a subspecies (Griffiths 1968). While our results mark off the Tasmanian and Flinders I. echidnas as being distinct from mainland echidnas, they do not decide whether the difference is specific or subspecific.

(2) A "south-eastern mainland" group, characterized by a very high frequency of HbIB (Fig. 1). A larger sample size would be needed to see if HbIA or HbIC were definitely absent from this area. The limits of the area occupied by this group can only be approximately defined. At present it

seems to be in the Great Dividing Range and associated coastal plain from near Melbourne through Gippsland to near Canberra, where only two animals not homozygous for HbIB were found. The fact that one of these was homozygous HbIA makes one suspect that this animal at least had been introduced from elsewhere, since there is a low probability of the occurrence of a homozygote for a low-frequency allele in such a relatively small sample size.

(3) A "hybrid" group, also of uncertain extent, which contains all three mainland HbI variants. Near the southern coast of the continent this group is found in western Victoria. It could extend as far as Adelaide where one HbIAB and four HbIA animals were found, although we cannot be completely certain that this heterozygote was really native to the Adelaide area. Near the eastern coast this group is found around Gosford, Port Macquarie, and Narrabri.

(4) The rest of the area of the continent from which samples have been obtained, a group which has high frequencies of HbIA and HbIC and in which HbIB was not found. HbIB does not occur on Kangaroo I. Only HbIA was found in the four animals sampled from central Australia, the one from Western Australia and the two from western New South Wales. The one echidna from New Guinea also had HbIA.

Given the sparseness of the data in relation to the large area from which the samples are drawn, these divisions must be regarded as only tentative. Moreover there is no suggestion that the three mainland groups are separate, but rather they grade into one another. Overall the data suggest that there is a rather sharp increase in frequency of HbIA and HbIC as one goes westwards and northwards from eastern Victoria, and that the area occupied by the hybrid group is not large. If so, does the hybrid group represent a cline which has arisen as a result of natural selection? Or is it the result of hybridization between two formerly separated races of echidna? These two possibilities are of course not mutually exclusive. A study of other markers in the echidna might show whether one, the other, or both were correct. It is of some interest that the area in which HbIB occurs in high frequency corresponds quite closely to one of the four main zoogeographic regions of Australia, the Bassian which extends over Tasmania, the Bass Strait islands, and temperate eastern and south-eastern Australia (Rawlinson 1971). However, the presence of the HbII polymorphism in Tasmania but not in the mainland demonstrates that echidnas are not genetically homogeneous within this region.

The sequence of amino acids in the β^{I} chain from a HbIB echidna has very recently been ascertained by Thompson and his co-workers (Whittaker *et al.* 1972). This sequence shows 31 differences with respect to the human β chain, a somewhat unexpected result in view of the fact that kangaroo and human β chain show 38.

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