on A and B substances, these determinants could be the expression of independent LW genes.

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Variation of Red Cell Acid Phosphatase in Two Species of Kangaroos

INHERITED variation has been recently demonstrated in the transferrin of an Australian marsupial, *Macropus rufus* (=*Megaleia rufa*)[1]. So far no other variation has been shown in the blood of this or other marsupials. The present communication reports the investigation of red cell acid phosphatase in two species of Australian marsupials, *M. rufus* and *M. giganteus*, the former commonly known as the red kangaroo and the latter as the grey kangaroo.

The animals used in the present study were kept in captivity at the C.S.I.R.O. Division of Wildlife Research, Canberra. Blood was collected by puncture of the caudal vein. The method for determining acid phosphatase patterns was that described by Hopkinson, Spencer and Harris[2] with minor modifications. The haemolysates were prepared by macerating clotted blood, washing the resultant cells three times with physiological saline and lysing them with an equal volume of distilled water. The haemolysates were stored at −15° C until use. Horizontal starch-gel electrophoresis using thick filter paper inserts (Whatman No. 17) was carried out with a potential gradient of 5·5 − 6·0 V/cm for 16–17 h at +5° C and at *p*H 6·0 (gel buffer: 0·005 M succinic acid and 0·0092 M *tris*, bridge buffer: 0·41 M citric acid/sodium hydroxide). The gels were prepared with an 8·5 per cent starch concentration rather than with the 9·7 per cent recommended by the manufacturers (Connaught Laboratory hydrolysed starch, lot 209–1). After electrophoresis the gels were sliced horizontally and placed in flat glass dishes with covers. The cut surfaces were then covered with a solution of 0·005 M phenolphthalein diphosphate in 0·05 M citrate buffer at *p*H 6·0 and incubated for 2–3 h at 37° C. After incubation the reagent was decanted and about 3 ml. of concentrated ammonium hydroxide poured over each gel. Red zones appeared in areas where phenolphthalein had been liberated from phenolphthalein diphosphate by acid phosphatase.

Twenty-six out of fifty-four red kangaroos showed a pattern which is tentatively called the 'K' type (Fig. 1, 1). This consisted of two components, and the faster was slightly smaller than the slower component. Both were oval in shape. Eighteen other animals showed the 'K' pattern but with an additional small zone of acid phosphatase which was found to be moving ahead of the faster component (Fig. 1, 2). This zone is probably due to some change in the protein during storage because it was not demonstrated in haemolysates of freshly collected blood samples from three of the animals that had previously shown it. Eight of the remaining ten red kangaroos showed a pattern different from the 'K' type. In this pattern two components migrating at the same rate as the components found in 'K' type were present but the faster was smaller and less intensely stained than the slower moving component (Fig. 1, 3). However, this changed pattern could not be reproduced in the haemolysates of freshly collected blood samples from two of the ten animals but instead a normal pattern of the 'K' type was observed. This seems to suggest that the alteration in pattern is probably due to changes during storage. No phosphatase activity was detected in the red cells of the remaining two kangaroos.

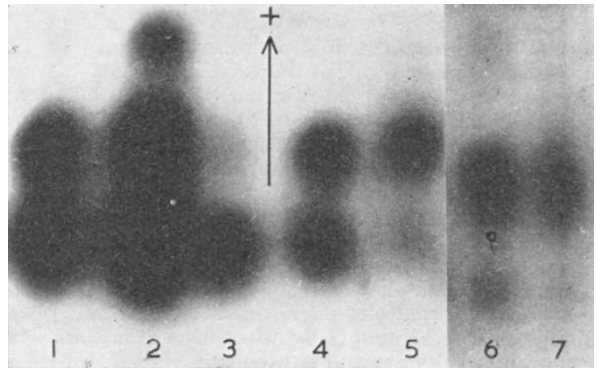

Fig. 1. Red cell acid phosphatase types found in red and grey kangaroos and some of the patterns found in man. The types are from left to right: 1, 'K'; 2, altered 'K'; 3, altered 'K'; 4, 'G'; 5, altered 'G'; 6, human B; 7, human BA

Eighteen grey kangaroos were examined and twelve showed a different pattern which is tentatively called the 'G' type (Fig. 1, 4). The two components in this type were of the same staining intensity and migration rate as the components of the 'K' type. However, the components were usually of equal size and were round in shape. Two grey kangaroos showed the typical 'K' type pattern and four showed an atypical 'G' type. In this atypical pattern there were two components with mobilities similar to the 'G' type components but the slower was less intensely stained than the faster moving component (Fig. 1, 5). This is also probably due to changes during storage and was not observed in two animals from which fresh samples were obtained.

The present work has shown difference in the pattern of red cell acid phosphatase in the two species of Macropods investigated. No evidence of polymorphism was found in the red species but the pattern was usually different from that of the grey species. Two distinct patterns were found in the grey animals and they are believed to represent a true polymorphism. Unfortunately, it was not possible to test this hypothesis because an insufficient number of animals with known pedigrees was examined.

The nature of the changes in electrophoretic mobility resulting from storage in a number of samples is being investigated. It is of interest that in the red species the fast component was presumably unstable and yielded a third area with enzymatic activity. In the grey species the slow component appeared to have deteriorated during storage.

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