chromosome is a small acrocentric and the Y chromosome is a very small telocentric. Thus the sex is controlled genetically by an XY/XX mechanism.

The results for the murine opossum were consistent between cells, and suggest that the diploid number is 14 and that the karyotype is very similar to that of the woolly opossum.

The metaphase spread and karyotype from a male four-eyed opossum (Fig. 2) indicate that the diploid number for this species is 22. However, the karyotype is different from that of the common opossum. The four-eyed opossum has three pairs of large telocentric autosomes (group A) and seven pairs of medium telocentric autosomes (group B). The X chromosome is a small telocentric and the Y chromosome is a very small telocentric, sex being controlled by an XY/XX mechanism. Our observations on the karyotype of the common opossum, which confirm those of Shaver (*4*), show that there are six pairs of subtelocentric autosomes, and four pairs of telocentric autosomes. The X chromosome is a medium sub-metacentric and the Y chromosome is a small telocentric.

Our observations show that the Didelphidae are not characterized by the uniform diploid number of 22 assumed by Sharman (*2*), and suggest that the variation in diploid number among American marsupials may be as great as that observed in Australasian marsupials, the numbers 14 and 22 predominating in both groups. Moreover, our results indicate that within the group of American species characterized by 22 chromosomes the karyotype may nevertheless vary.

Among the many questions which remain is that of whether the Didelphidae are a closely related group. Simpson (*11*) stated that only one subfamily of the Didelphidae, namely, the Didelphinae, is still extant. However, in 1955 Reig (*12*) presented osteological evidence that the living genera *Caluromys, Dromiciops*, and *Glironia* are in fact surviving members of the subfamily Microbiotheriinae usually regarded as extinct. Other comparative anatomical studies have shown that the male and female reproductive organs of the genus *Caluromys* have features quite distinctive from those of *Didelphis* and many similar forms. These features are the median vagina (*13*), the cleft glans penis (*14*), and the morphology of the spermatozoa (*15*). The little evidence available at present indicates that although one genus of the possibly extant Microbiotheriinae has a diploid number of 14 chromosomes, it may not be unique in this aspect since *Marmosa* also has a diploid number of 14 chromosomes. However, the diploid number of chromosomes and the karyotypes of *Dromiciops* and *Glironia* must be studied before further comments can be made on this matter. In addition, detailed studies are required on the chromosomes of the genus *Marmosa*, a very extensive group of the didelphids which are taxonomically divided into five subgeneric categories (*16*).

At present we have no information on the chromosomes of the family Caenolestidae which lives in the Andes Mountains of South America (*17*). This very rare family is of particular interest from an evolutionary point of view because of its alleged affinities with the superfamily Phalangeroidea of Australasia (*18*), which is characterized by a range of chromosome numbers (*2*).

Sharman and Barber (*19*) suggested that during the evolution of marsupials the chromosome numbers have been reduced by some type of Robertsonian chromosomal change. Various plausible patterns of translocation can be postulated whereby 22 chromosomes can be reduced to 14. However, the suggestion that a diploid number of 22 is the primitive number of chromosomes for marsupials can no longer be sustained by the argument that American marsupials, considered evolutionarily primitive on other grounds, are characterized by a diploid number of 22. If the Robertsonian change has occurred in marsupials, then it has probably occurred independently in the American and Australasian stock.

J. D. BIGGERS, H. I. FRITZ
W. C. D. HARE, R. A. MCFEELY
School of Veterinary Medicine,
University of Pennsylvania, Philadelphia

References and Notes

1. A. Cabrera, *Rev. Museo. Arg. Cienc. Nat. "Bernandino Rivadavia"* No. 4 (1957).
2. G. B. Sharman, *Australian J. Zool.* **9**, 38 (1961).
3. *Didelphis azarae* Temminck: F. A. Saez, *Amer. Nat.* **65**, 287 (1931); *Didelphis marsupialis aurita* Wied: A. Dreyfus and J. E. Campos, *Bol. Fac. Filos. Cienc. Sao Paulo* **17**, 3 (1939); *Didelphis marsupialis virginiana* Kerr: T. S. Painter, *J. Exp. Zool.* **35**, 13 (1922); W. E. Hoy, Jr., and W. C. George, *J. Morphol. Physiol.* **47**, 201 (1929); S. Ohno, W. O. Kaplan, R. Kinosita, *Exp. Cell Res.* **19**, 417 (1960); *Lutreolina crassicaudata* Desmarest: F. A. Saez, *Physis B. Aires* **18**, 153 (1939).
4. E. L. Shaver, *Canad. J. Genet. Cytol.* **4**, 62 (1962).
5. J. H. Tjio and J. Whang, *Stain Technol.* **37**, 17 (1962).
6. P. R. Basrur, V. R. Basrur, J. P. W. Gilman, *Exp. Cell Res.* **30**, 229 (1963).
7. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *ibid.* **20**, 613 (1960).
8. J. D. Biggers, R. B. L. Gwatkin, S. Heyner, *ibid.* **25**, 41 (1961).
9. P. Morrison and J. H. Petajan, *Physiol. Zool.* **35**, 52 (1962).
10. The karyotypes are arranged in accordance with the recommendations made at the 3rd Mammalian Cytology and Somatic Cell Genetics Conference, San Juan, Puerto Rico, 1964.
11. G. G. Simpson, *Bull. Amer. Museum Nat. Hist.* **85**, 1 (1945).
12. O. A. Reig, *Invest. Zool. Chilenas* **2** (No. 2), (1955).
13. J. P. Hill and E. A. Fraser, *Proc. Zool. Soc. London* **189** (1925).
14. J. D. Biggers, *Symp. Zool. Soc. London*, in press.
15. ——— and E. D. DeLamater, in preparation.
16. G. H. H. Tate, *Bull. Amer. Museum Natur. Hist.* **66**, 1 (1933).
17. W. H. Osgood, *Fieldiana: Zool.* **14**, 1 (1921).
18. W. D. L. Ride, in *The Evolution of Living Organisms*, C. W. Leeper, Ed. (Melbourne Univ. Press., Melbourne, Australia, 1962), p. 281.
19. G. B. Sharman and H. N. Barber, *Heredity* **6**, 345 (1952).
20. We thank Dr. D. A. Abt, Miss Ellen Satterthwaite, and Mrs. Shiela Lessick for skilled technical assistance. Supported in part by NSF grant G-17658 and PHS grant 1 PO6 FR-00120. Dr. H. I. Fritz is a postdoctoral fellow of the Institute of Arthritis and Metabolic Diseases. Dr. R. A. McFeely is a postdoctoral fellow of the Institute of Child Health and Human Development.

1 April 1965

Sex-Linkage of Glucose-6-Phosphate Dehydrogenase in the Horse and Donkey

Abstract. *Distinctly different electrophoretic patterns of red cell glucose-6-phosphate dehydrogenase were resolved from the hemolyzates of horse and donkey erythrocytes. Examination of their reciprocal hybrids, mules and hinnies, showed that the red cells of female mules and female hinnies contain both horse and donkey G-6-PD; the male mule with an X chromosome from its horse mother contained pure horse G-6-PD, whereas the male hinny with the donkey X chromosome contained pure donkey G-6-PD. These findings on the male reciprocal hybrids suggest X-linkage.*

The production of glucose-6-phosphate dehydrogenase (G-6-PD) in erythrocytes is governed in humans by a gene linked to the X chromosome, and genetic variants detectable by starch-gel electrophoresis are known (*1, 2*). Usually the presence of wild-type alleles is revealed by finding their deficient mutants; chance plays a great role in detection of such mutants. For instance, electrophoretic variants of

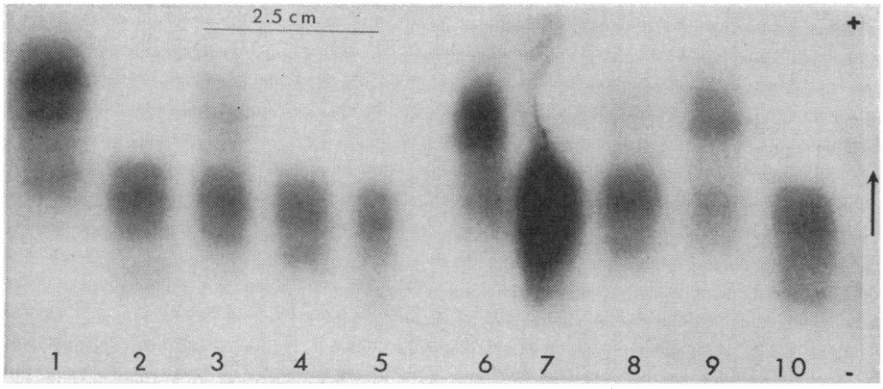

Fig. 1. Starch-gel electrophoresis patterns of red cell G-6-PD. 1, male donkey; 2, male horse; 3, female mule; 4, male mule; 5, male horse; 6, male hinny; 7, male horse; 8, female hinny; 9, female mule; 10, male horse. The arrow shows the direction of migration.

the G-6-PD system in the fruit fly, *Drosophila melanogaster* (*3*), have also been shown to follow an X-linked pattern.

On the basis of the observation that the X chromosomes of many placental mammals, including man, are nearly identical in absolute size and comprise about 5 percent of the genome (*4*), Ohno *et al.* postulated that diverse species of placental mammals share the same kind of X-linked genes (*5*). Indeed, hemophilia A and hemophilia B are X-linked in the human as well as in the dog (*6*), and anhidrotic ectodermal dysplasia, X-linked in man, is also X-linked in cattle (*7*).

As another example, if the G-6-PD of the horse and the donkey should show distinctly different electrophoretic mobilities, the possible X-linkage of the enzyme in these species could be tested by examining the distribution of this enzyme in the red cells of reciprocal hybrids between the two, the mule and the hinny. Accordingly, we have undertaken the study of this particular mammalian system.

Blood specimens obtained from seven horses (five males, two females), four donkeys (three males, one female), eight mules (three males, five females), and two hinnies (one male, one female) were collected in acid-citrate-dextrose (ACD) or heparin; and stroma-free extracts of the red cells were prepared according to Kirkman (*2*). Portions of this crude hemolyzate were made 0.057 m*M* in nicotinamide adenine dinucleotide phosphate (NADP) and adjusted to contain 0.3 to 0.9 g of hemoglobin per 100 milliliters. The hemolyzates were used within 48 hours.

Ascending starch-gel electrophoresis was carried out by the method of Shows *et al.* (*8*), which proved superior to those of Kirkman and Hendrickson (*2*) and of Porter *et al.* (*9*). Briefly, electrophoresis was continued for 16 to 18 hours at 2° to 4°C, with a gradient of 4 to 5 volt/cm and a gel-buffer system (ethylenediaminetetraacetic acid, boric acid, and tris) at *p*H 8.0. The gels were stained with a solution containing glucose-6-phosphate, NADP, nitro-blue tetrazolium, phenazine methosulfate, $MgCl_2$, and tris-HCl buffer at *p*H 8.0, and were incubated for 3 to 5 hours.

The starch-gel electrophoresis plates (Fig. 1) of preparations from donkey cells without exception showed three constant bands of enzyme activity, two fractions migrating faster and one slightly slower than the rapid component of the horse G-6-PD.

The enzyme from horse cells showed a very wide band, the forward boundary of which moved at a rate approximately midway between the intermediate and slow bands of the donkey system. Thus, the G-6-PD dehydrogenases of the two parental species were distinctly different from each other.

The enzyme of the cells of the three male mules behaved in the same manner as that of the horse. In the female mules, however, the enzyme patterns were not consistent from one animal to another, the enzyme activity appearing to be a mixture in varying degrees of components from both parental species, with a single exception which appeared similar to that of the horse enzyme system.

The enzymes in the female hinny's cells also appeared to be a mixture, though the donkey component was rather faint. In repeated experiments, the enzyme pattern of the male hinny's red cells was always identical with that of the donkey.

No sign of an intraspecies variant of G-6-PD has been found either among horses or among donkeys (*10*).

The X chromosome of the male is invariably derived from his mother. Thus, males of reciprocal interspecies hybrids offered the critical test for the X-linkage of G-6-PD. The results described here on the male mules and the male hinny are entirely consistent with the X-linkage of this enzyme in both the horse and the donkey.

Female mules as well as female hinnies derive one X chromosome from the donkey and one from the horse. Thus, the coexistence of enzymes from both parents was expected. Only one female mule deviated from this expectation. The theory of random inactivation of an individual X chromosome early in embryonic life (*11*) could explain this deviation. By chance, in the donkey this chromosome may have been inactivated in all the erythroid precursor cells of this female mule. Hence the appearance of only the horse G-6-PD.

The multiplicity of bands in the electrophoretic patterns of G-6-PD suggests the existence of isozymes within the red cell system.

JOSE M. TRUJILLO, BETTY WALDEN
PEGGY O'NEIL, HAROLD B. ANSTALL
Department of Pathology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston

References and Notes

1. S. H. Boyer, I. H. Porter, R. G. Weilbacher, *Proc. Nat. Acad. Sci. U.S.* **48**, 1868 (1962).
2. H. N. Kirkman, E. M. Hendrickson, *Amer. J. Human Genet.* **15**, 241 (1963).
3. W. J. Young, J. E. Porter, B. Childs, *Science* **143**, 140 (1964).
4. S. Ohno, W. Bečak, M. L. Bečak, *Chromosoma* **15**, 14 (1964).
5. S. Ohno, *Ann. Genet.*, in press.
6. F. B. Hutt, C. G. Rickard, R. A. Field, *J. Heredity* **39**, 2 (1948); J. F. Mustard, H. C. Roswell, G. A. Robinson, T. D. Hoeksema, H. G. Downie, *Brit. J. Haematol.* **6**, 259 (1960).
7. H. Drieux, M. Priouzeau, G. Thiery, M.-L. Priouzeau, *Rec. Med. Vet.* **126**, 385 (1950).
8. T. B. Shows, Jr., R. E. Tashian, G. J. Brewer, R. J. Dern, *Science* **145**, 1056 (1964).
9. I. H. Porter, S. H. Boyer, E. J. Watson-Williams, A. Adam, A. Szeinberg, M. Siniscalco, *Lancet* **1964-I**, 895 (1964).
10. We thank Dr. Ohno of Duarte, California, for suggestions. A similar work has been simultaneously carried out by the Duarte group, and their results agree with ours.
11. E. Beutler, M. Yeh, V. F. Fairbanks, *Proc. Nat. Acad. Sci. U.S.* **48**, 9 (196 M. F. Lyon, *Nature* **190**, 372 (1961).
12. We thank Dr. G. Luquette, Houston Zoological Gardens; Dr. J. H. Jardine, M. D. Anderson Hospital Institute, Univ. of Texas, Houston; and the staff of Texas A.&M. University Large Animal Clinic, College Station, for assistance in obtaining specimens. Supported by PHS grant CA 06939.

23 April 1965