the observation that, at the more physiological temperature of 40°C, LDH-1 and LDH-5 are indistinguishable in reactivity toward substrate (*13*). Furthermore, this theory is incompatible with the observations that three distinct cell types predominantly anaerobic in metabolism—mature human erythrocytes (*15*), platelets (*16*), and bovine lens fibers (*17*)—exhibit mainly isozymes 1–4 but only traces of LDH-5. According to this theory, liver, because it contains mainly LDH-5, is considered anaerobic; however, it has a very active aerobic metabolism and, unlike skeletal muscle, maintains a positive arteriovenous difference in lactate concentration.

According to current concepts of the structure of LDH isozymes, LDH-5 is composed of four identical A subunits, whereas LDH-4 contains one B and three A subunits, LDH-3 has two A and two B subunits, LDH-2 one A and three B subunits, and LDH-1 four B subunits (*18*). Zymograms of platelet and bovine lens fibers reveal highest activity in LDH-3 and, except for diminished LDH-5 activity, approach the pattern observed on random recombination of equal amounts of A and B subunits, that is, isozymes in a proportion of 1 : 4 : 6 : 4 : 1. Prominence of LDH-4 on the zymograms indicates that decreased LDH-5 activity cannot be ascribed to insufficient synthesis of the requisite A subunit. Possibly for the anuclear cell, isozymes composed of four A subunits are constructed from constituent subunits with more difficulty or catabolized more rapidly than isozymes composed of one B and three A subunits.

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References and Notes

1. E. S. Vesell and A. G. Bearn, *Proc. Soc. Exptl. Biol. Med.* **111**, 100 (1962).
2. E. S. Vesell, K. C. Osterland, A. G. Bearn, H. G. Kunkel, *J. Clin. Invest.* **41**, 2012 (1962).
3. E. S. Vesell and A. G. Bearn, *J. Gen. Physiol.* **45**, 553 (1962); S. H. Boyer, D. C. Fainer, E. J. Watson-Williams, *Science* **141**, 642 (1963); W. E. Nance, A. Claflin, O. Smithies, *ibid.* **142**, 1075 (1963); A. P. Kraus and C. L. Neely, *ibid.* **145**, 595 (1964).
4. W. H. Starkweather, L. Cousineau, H. K. Schoch, C. J. Zarafonetis, *Blood* **26**, 63 (1965).
5. E. S. Vesell and I. A. Brody, *Ann. N.Y. Acad. Sci.* **121**, 544 (1964).
6. A. Bezkorovainy and M. E. Rafelson, Jr., *J. Lab. Clin. Med.* **64**, 212 (1964).
7. H. A. Zondag, *Science* **142**, 965 (1963).
8. J. A. Stewart and J. Papaconstantinou, *Federation Proc.* **24**, 667 (1965).
9. E. S. Vesell, *Nature* **195**, 497 (1962); N. Ressler, J. Schulz, R. R. Joseph, *J. Lab. Clin. Med.* **62**, 571 (1963).
10. E. S. Vesell, *Proc. Natl. Acad. Sci. U.S.* **54**, 111 (1965).
11. P. G. W. Plagemann, K. F. Gregory, F. Wróblewski, *J. Biol. Chem.* **235**, 2282 (1960).
12. B. W. J. Mahy and K. E. K. Rowson, *Science* **149**, 756 (1965).
13. P. G. W. Plagemann, K. F. Gregory, F. Wróblewski, *Biochem. Z.* **334**, 37 (1961).
14. G. Pfleiderer and E. D. Wachsmuth, *Klin. Wochschr.* **39**, 352 (1961); R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
15. T. A. J. Prankerd, *The Red Cell* (Blackwell, Oxford, 1961), p. 59.
16. E. W. Campbell, W. J. Small, W. Dameshek, *J. Lab. Clin. Med.* **47**, 835 (1956); H. D. Waller, G. W. Löhr, F. Grignani, R. Gross, *Thromb. Diath. Haemorrhag.* **3**, 520 (1959).
17. J. H. Kinoshita and C. Wachtl, *J. Biol. Chem.* **233**, 5 (1958).
18. E. Appella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961); C. L. Markert, *Science* **140**, 1329 (1963).
19. I thank Mrs. Ellen M. Collier and Miss Merilyn C. Hiller for technical assistance.

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Sex-Linkage of Erythrocyte Glucose-6-Phosphate Dehydrogenase in Two Species of Wild Hares

Abstract. *Glucose-6-phosphate dehydrogenase specific to the erythrocytes of each of two wild hares found in Europe was discerned by starch-gel electrophoresis at* pH *7.0 and* pH *8.6. The single, sharp band of the dehydrogenase of* Lepus europaeus *was faster than that of* L. timidus, *at both* pH *levels. The sex-linkage of this enzyme was tested through reciprocal hybrids between the two species. Each male hybrid had a single band of enzyme identical with that of its mother, while both parental types of this enzyme coexisted in female hybrids. Thus, sex-linkage of glucose-6-phosphate dehydrogenase has been suggested not only in man and in the family Equidae, but now in the family Leporidae of placental mammals as well.*

Cytological reasons for postulating the persistence of the entire primitive X-chromosome of a common ancestor, protoinsectivores, to various placental mammals of today (*1*) imply that divergent species share the same kinds of sex-linked genes. Among sex-linked traits known in man, hemophilia A and B and anhidrotic ectodermal dysplasia are also found in at least one other species of placental mammals (*2*).

The glucose-6-phosphate dehydrogenase (G-6-PD) present in the erythrocyte, known to be produced by a sex-linked gene in man, is sex-linked in the horse and donkey (*3*). In that the species-specific G-6-PD of these two interbreeding species had different electrophoretic mobilities, an opportunity was afforded to test the sex-linkage of this enzyme through reciprocal hybrids, mules and hinnies, in the family Equidae.

A parallel situation in the family Leporidae permitted the test for sex-linkage of erythrocyte G-6-PD. One of us (I.G.) successfully obtained reciprocal hybrids between two species of wild hares of Europe, *Lepus europaeus* and *L. timidus*. Both species have 48 chromosomes in their diploid complements, and, unlike mules and hinnies, male as well as female interspecific hybrids are fertile (*4*).

Vertical starch-gel electrophoresis was carried out at *p*H 8.6 in borate buffer and at *p*H 7.0 in phosphate buffer with resulting sharp delineation of G-6-PD bands. Electrophoresis was continued for 14 hours at 4°C with a gradient of 4 volt/cm (*5*).

From twice-washed erythrocytes, a hemolyzate (the cells were disrupted by freezing and thawing) of 1-to-100 dilution was prepared in 0.01*M* potassium phosphate buffer, *p*H 7.0, which contained ethylenediaminetetraacetate (EDTA) and β-mercaptoethanol. Each starch-gel plate contained 500 mg of EDTA and 5 mg of triphosphopyridine nucleotide (TPN). The staining solution was prepared by a modification of the spot-test reagent for G-6-PD (*6*). Ten milligrams of glucose-6-phosphate dipotassium salt (Sigma), 2 mg of 3(4,5-dimethylthiazolyl-1, 2)-2, 5-diphenyltetrazolium bromide, 2 mg of phenazine methosulfate, and 2 mg of TPN were dissolved in 10 ml of 0.1*M* tris-HCl buffer, *p*H 8.0, and poured over the sliced gel. The gel was then kept covered at room temperature for 2 hours.

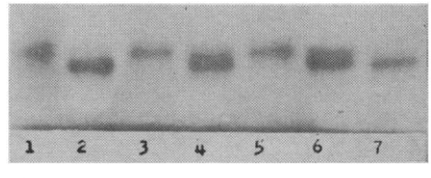

Fig. 1. Starch gel (*p*H 7.0) showing erythrocyte glucose-6-phosphate dehydrogenase of parental species and interspecific hybrids. Anodal direction is upward, and the distance between the slow-moving band of slots 2 and 4, and the starting point, measures 15 mm. Slot 1: female *Lepus europaeus*, the mother of four hybrids shown in slots 3, 4, 5, and 6. Slot 2: male *L. timidus*. Slots 3 and 5: hybrid sons. Slots 4 and 6: hybrid daughters of the mother shown in slot 1. Slot 7: male hybrid with the *L. timidus* mother.

First, in order to determine whether species-specific G-6-PD of these interbreeding hares can be distinguished from each other by electrophoresis, two males and three females of *Lepus europaeus* were compared with two males and one female of *L. timidus*. Regardless of sex, each animal of both species demonstrated a single sharp band of G-6-PD at both *p*H's, and that of *L. europaeus* consistently migrated further toward the anode than that of *L. timidus*. The difference in rate of migration was comparable to the difference that exists between A and B variants in man.

Next, all available F_1 hybrids were examined. A cross between a male *L. timidus* and a female *L. europaeus* was represented by two males and two females of the same litter. The sire of this litter was no longer available, but hemolyzate of the dam was used for comparison. The reciprocal cross to the above was represented by one male. Neither his sire nor his dam was alive.

As shown in Fig. 1, the hemolyzates of each of the two hybrid males with the *L. europaeus* mother revealed a single fast-moving band of G-6-PD identical with the maternal band. One male hybrid of the reciprocal cross had a single slow-moving band of G-6-PD inherited from the *L. timidus* mother. Each of the two female hybrids showed two bands of G-6-PD, the fast-moving band corresponding to that of their mother *L. europaeus*, the slow-moving band corresponding to that of their father *L. timidus*. Thus, sex-linkage of this enzyme was suggested.

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References and Notes

1. S. Ohno, W. Beçak, M. L. Beçak, *Chromosoma* **15**, 14 (1964); S. Ohno, *Ann. Genet.* **8**, 3 (1965).
2. V. A. McKusick, *Quart. Rev. Biol.* **37**, 69 (1962).
3. C. K. Mathai, J. M. Trujillo, S. Ohno, E. Beutler, *Fed. Proc.* **24**, 440 (1965); J. M. Trujillo, B. Walden, P. O'Neil, H. B. Anstall, *Science* **148**, 1603 (1965); C. K. Mathai, S. Ohno, E. Beutler, *Nature*, in press.
4. I. Gustavsson, in preparation.
5. We thank E. Beutler for advice.
6. V. F. Fairbanks and E. Beutler, *Blood* **20**, 591 (1962).
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Polonium-210 Analyses of Vegetables, Cured and Uncured Tobacco, and Associated Soils

Abstract. *Analysis of the edible portion of vegetables and samples of green leaf tobacco failed to show polonium-210. The cured samples of leaf tobacco and the soils that were analyzed all contained small quantities of the element. Muck soils contained three times as much Po^{210} as did mineral soils. Solutions used commonly to extract "available" forms of many mineral elements failed to extract a detectable amount of Po^{210}. Indications are that Po^{210} or its radioactive precursors are not taken up from the soil directly by plant roots but rather by sorption in dead, moist plant materials at the atmosphere-plant interface.*

Mayneord *et al.* (*1*) suggest that Po^{210} in plants is derived from the soil, the air or from both. Po^{210} may originate from the radioactive decay of in precursors such as Pb^{210}, Rn^{222}, or Ra^{226}. It may also originate from radioactive decay of the daughters of Rn^{222} deposited on the leaves (*1*). Radford and Hunt (*2*) suggest that Po^{210} in tobacco may initiate neoplasms in the bronchial epithelium of cigarette smokers. Po^{210}, an alpha emitter, is volatile above 500°C—well below the temperature of a burning cigarette (*2*). It binds rapidly and strongly to surfaces and thus may be firmly attached to smoke particles (*2*). In cats, significant amounts of added Po^{210} were absorbed from the stomachs (*3*), and, since the amounts of Po^{210} in vegetables passing through the body could be vastly greater than that which enters by way of cigarette smoke into the lungs, it seemed imperative, that the amounts of Po^{210} in vegetables be determined.

Air-cured leaf tobacco, grown in the summer of 1964 in Wisconsin, was dried at 50° to 60°C and ground to pass through an eight-mesh screen. Samples of three different tobacco varieties and several soil types were analyzed for Po^{210}.

Edible portions of fresh vegetables were collected in the summer of 1964 and stored under dry ice for 6 months. Samples of these were then dried and analyzed for Po^{210}. Duplicate samples were stored for 5 more months and then analyzed.

For analyses, 2-g samples of plant tissue (dry weight) were digested with 20 ml of 12*N* HCl for 40 minutes at 60° to 70°C; 2-g samples of soils were digested with 10 ml of 12*N* HCl for the same period. After digestion, the solution was quantitatively transferred to a plating flask by washing with 0.5*N* HCl until the final plating volume was 75 ml. To eliminate the interference of the ferric ion, 0.1 g of ascorbic acid, a mild reducing agent, was added to the flask. The contents were then heated (90° to 100°C), continuously stirred for 4 hours, and plated on a silver planchet. The planchet was then removed, washed with distilled water, and air dried, and the Po^{210} was counted in a solid-state alpha-particle counter for 4 hours (*4*). The background was 0.02 ± 0.01 count/min and it was similar for the blank. The efficiency of the counter and the plating procedure for a known amount of Po^{210} was 10.5 ± 1.5 percent. Although digestion of plant tissue or soil was incomplete with hot, concentrated HCl, when known amounts of Po^{210} were added recovery was quantitative.

Analyses were made on 30 samples of the edible portion of each of the following plants: sweet corn, field corn (grain and leaves), cabbage, carrots, red beets, cucumbers, radishes, snap beans, and potatoes; there was no detectable Po^{210}. We then assumed that the Po^{210} present in the plant tissue was not being released upon chemical treatment with hot 12*N* HCl. When a wet-ashing method (HNO_3, H_2SO_4, and $HClO_4$) (*5*) was used to completely digest 1 g of dry sweet corn grain, no Po^{210} was detected. Using this method on 1 g of dry cigarette tobacco, we found a significant amount of Po^{210}. The wet-ashing method gave results similar to those obtained by digestion with 12*N* HCl. We then assumed that the size of the sample (1 g dry weight) was too small for the detection of Po^{210} in vegetables. However, when samples of 2-, 5-, 10-, and 100-g dry weight were used, the count was still not detectable. Known amounts of Po^{210} were added to these samples of various sizes and quantitatively recovered.

Because the difference between the number of counts in the sample and the number in the background obtained from any sample of the vegetables, either in the moist or in the dried state, was less than or equal to the standard deviation of the number of counts, we conclude that the amounts of Po^{210} in these samples are below the limits of detection (Table 1). As we mentioned, radioactivity was significant in one 1-g sample of dry cigarette tobacco and