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Characterization of the enzymatic lesion in inherited phosphofructokinase deficiency in the dog: An animal analogue of human glycogen storage disease type VII

(isozymes of phosphofructokinase/animal model of human enzymopathy/hemolytic anemia/gene regulation and compensation)

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ABSTRACT **Mammalian phosphofructokinase (PFK; ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) exists in multimolecular forms, which result from random tetramerization of three distinct subunits, M (muscle-type), L (liver-type), and P (platelet-type), each under a separate genetic control. Human muscle and liver contain homotetramers M_4 and L_4, respectively, whereas erythrocytes contain a mixture of M_4, M_3L, M_2L_2, ML_3, and L_4 isozymes. Homozygous deficiency of the M subunit in man results in glycogen storage disease (GSD) type VII, which is characterized by exertional muscle weakness and compensated hemolysis; the residual erythrocyte PFK consists of isolated L_4 isozyme. Recently, PFK deficiency associated with isolated hemolytic anemia has been identified among English springer spaniel dogs. We investigated the genetic control of the dog PFK system and the nature of the enzymatic defect in two PFK-deficient animals, using chromatographic and immunological techniques. Our studies indicate the existence of a trilocus isozyme system for the dog, as is the case with other mammals. Muscle PFK consists of M_4 isozyme, whereas the predominant species of liver and platelet consists, respectively, of the L_4 and P_4 isozyme; erythrocyte PFK consists of a three- or four-membered set composed of M and P subunits. PFK deficiency in the dogs was found to result from a total and universal lack of the M subunit, as is the case in man. However, the probands consistently exhibited L_4 isozyme in their muscle; P_4, L_4, and hybrids thereof in their erythrocytes; and an increase in the L-containing isozymes in their platelets, indicating a generalized anomalous presence of the L subunit. The apparent absence of muscle disease in these animals is most likely accounted for by both the well-known high oxidative potential of the canine muscle in general and the presence of liver PFK in the M-deficient muscle in particular. In contrast, presence of hemolysis despite residual P_4 and hybrids of P and L in the erythrocytes may be inferred to result in severe glycolytic handicap under existing intraerythrocytic conditions.**

Phosphofructokinase (PFK; ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the key regulatory enzyme of glycolysis, exists in isozymic forms in man and several other vertebrate species. Extensive studies of the rabbit, rat, and human enzyme have conclusively established the genetic control of PFK in these species (1–5). In each species, the enzyme is under the control of three structural loci that encode muscle (M or A), liver (L or B), and platelet (P or C)-type subunits, which are differently expressed by various tissues. Random tetramerization of the resultant subunits produces various isozymes, which are distinguishable from one another by the differences in their physicochemical, immunochemical, and kinetic-regulatory properties (4–12).

Inherited deficiency of the muscle-type PFK in man is reported to be associated with clinically heterogeneous syndromes characterized by muscle weakness on exertion or hemolysis, or both, or by no symptoms; the most common syndrome, glycogen storage disease (GSD) type VII is associated with myopathy and hemolysis (3, 13, 14). Recently, inherited PFK deficiency has been identified among English springer spaniel dogs (15). Although the deficient dogs manifest chronic hemolytic anemia punctuated by acute hemolytic crises and hemoglobinuria, clinically they appear not to suffer from muscle weakness or myoglobinuria on exertion. In order to precisely define the nature of the enzymatic defect and, therefore, the puzzling symptomatology of these dogs as well as to assess the experimental usefulness of this animal model of a human glycogenosis, we investigated in this study the PFK isozyme system from both normal and PFK-deficient springer spaniels.

Our results show that the inherited PFK deficiency in the dog also results from a total and universal lack of the M subunit, as is the case in human patients with glycogenosis type VII. However, the PFK-deficient dogs exhibit a generalized anomalous presence of the L subunit, most likely secondary to a compensatory gene expression. The apparent lack of muscle disease in these dogs most likely results from both the high oxidative potential of the canine muscle in general and the presence of liver PFK in the M-deficient muscle in particular. The presence of hemolysis despite residual erythrocyte enzyme is interpreted to indicate that the platelet-type PFK isozymes must be dysfunctional in the existing intraerythrocytic conditions. Thus, our results characterize not only this animal analogue of a human glycolytic enzymopathy but also the dog PFK system and a novel compensatory mechanism.

MATERIALS AND METHODS

Materials from Normal and PFK-Deficient Dogs. The freshly harvested specimens of blood, skeletal muscle, and liver from normal English springer spaniels were donated by the local Animal Control and County Veterinary authorities. Venous blood samples on the two PFK-deficient dogs, Gaal and Fiver, were obtained on several occasions after informed consent of their owners. Muscle biopsy specimens were obtained under local anesthesia from Fiver only. The spec-

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Abbreviations: PFK, ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11; M, P, and L, muscle, platelet, and liver-type subunit, respectively; GDS, glycogen storage disease.

imens of internal organs were frozen immediately (−80°C), whereas the blood specimens were stored at 4°C until further use. The cell separations were carried out within 24 hr of blood collection as described earlier (12), whereas chromatographic and immunological studies were performed over the next 3–4 days. The clinical and hematological profiles of these dogs have been described in detail elsewhere (15).

PFK Activity Assays and Isozymic Complements of Various Organ/Cell Types. PFK assays were performed with a Gilford model 260 spectrophotometer at 26°C as described (10). One unit of enzyme is defined as that amount of enzyme that converts 1 μmol of fructose 6-phosphate to fructose 1,6-bisphosphate in 1 min.

Chromatographic separation of PFK isozymes was carried out by using DEAE-Sephadex A-25 equilibrated with 0.1 M Tris/PO_4 buffer (pH 8.0) as described (10, 12, 16).

Enzyme Immunoprecipitation Assays Using Subunit-Specific Antibodies. The production and characterization of a rabbit anti-human muscle PFK antiserum (10) and those of mouse monoclonal antibodies against human M and L subunits (17) have been described previously. Since rabbit anti-M and monoclonal anti-L (V44-08) antibodies were known to react with the dog PFKs, they were used in the immunoprecipitation studies. The technique of enzyme immunoprecipitation has been described in detail (17). Each organ or cell type was tested in duplicate on four to eight separate occasions.

Identification of the Pigment in the Urine of the PFK-Deficient Dogs. The presence of myoglobin in grossly bloody specimens of urine from Fiver was investigated by using DEAE-Sephadex A-50 column chromatography as described (18). This technique is quite sensitive and detects ≈5% myoglobin from a mixture of the two hemoproteins.

Histochemical Stain for Glycogen. Muscle biopsy was taken from the longissimus lumborum muscle of Fiver. Muscle was fast frozen in liquid nitrogen, fixed, sectioned, and stained with periodic acid/Schiff (PAS) stain as described (19).

Protein Determinations. These were performed as described by Lowry *et al.* (20) with bovine serum albumin as a control.

RESULTS

PFK Activity Levels in the PFK-Deficient Dogs and Humans. As reported earlier (15), Gaal and Fiver exhibit ≈10% of normal erythrocyte PFK activity (Table 1) in contradistinction to the human patients, who manifest ≈50–66% of residual PFK activity in their erythrocytes (13, 14). Fiver also shows ≈6% residual enzyme activity in his skeletal muscle, in contradistinction to human probands, who generally show an almost total lack of enzyme activity but may occasionally possess 1–3% of residual enzyme activity in their skeletal muscle (Table 1).

Table 1. PFK activity levels in the erythrocytes and skeletal muscle of the normal and muscle PFK-deficient dogs

	Phosphofructokinase activities	
Dog*	Erythrocytes,† units/g of hemoglobin	Skeletal muscle, units/g of protein
Fiver (2)	0.6 (9%)	17.8 ± 1.7 (6%)
Gaal (2)	0.7 (11%)	ND
Controls (4)	6.6 ± 1.6	295.3 ± 94

Data are means ± SD. Percentage values in parentheses indicate percentages of normal values. ND, not done.

*Numbers in parentheses indicate the number of separate determinations for each proband and the number of separate control animals investigated.

†Values were previously reported in ref. 15; values are normalized here for comparison.

PFK Isozymes in the Normal Springer Spaniel Dogs. Fig. 1 compares the representative isozymic profiles of the informative organs and blood cells from humans with those from normal springer spaniels. The three human homotetramers are distinctly separable from one another and are distinguished by their relative positions of elution (10, 12). As shown in Fig. 1*A*, Human M_4 tetramer was eluted first at ≈214 milliosmolality (mosmol/kg of solvent) (≈40th fraction), and L_4 tetramer was eluted last at ≈480 mosmol/kg (≈75th fraction); P_4 tetramer was eluted very close to the M_4 tetramer at ≈244 mosmol/kg (≈47th fraction). Human skeletal muscle and liver (major isozyme) PFKs consist of M_4 and L_4 homotetramers, respectively (Fig. 1*A*), whereas erythrocyte PFK consists of a heterogeneous mixture of five isozymes composed of the M and L subunits—i.e., M_4, M_3L, M_2L_2, ML_3, and L_4 (Fig. 1*B*). In contrast, human platelet PFK consists of a three-membered set composed of P_4, P_3L, and P_2L_2 isozymes (Fig. 1*C*).

Unlike the human M_4 tetramer, springer spaniel muscle PFK was eluted as a single peak at ≈238 mosmol/kg (≈46th fraction). In contrast, the dog liver PFK (major peak) was eluted last at ≈480 mosmol/kg (≈75th fraction) as was the human L_4 tetramer. However, the greatest variation in the elution profile was shown by the dog platelet-type isozyme(s). The major platelet isozyme from the dog was eluted very close to the L_4 isozyme at ≈375 mosmol/kg (≈65th fraction; Fig. 1*D*), unlike the human P_4 tetramer which was eluted very close to the M_4 tetramer at ≈247 mosmol/kg (≈47th fraction) (Fig. 1*A*). The dog erythrocyte PFK consists of an incomplete set of isozymes (three or four-membered) whose first and most prominant species was coeluted with the dog M_4 (Fig. 1*E*), suggesting that the erythrocyte PFK at least contains the M-type subunit. However, the nature of the hybrid isozymes from the dog erythrocyte could not be deduced from the chromatographic data alone, since they could be either the hybrids of M/L or M/P or those of M/P/L subunits. The dog platelet PFK consistently showed two or three isozymes; the major peak is interpreted to be P_4, and the hybrids are interpreted to be P_3L and P_2L_2 species (Fig. 1*F*) by analogy with the subunit structures of human platelet PFK isozymes (Fig. 1*C*).

PFK Isozymes in the PFK-Deficient Springer Spaniel Dogs. Unlike the single early-eluted peak present in normal muscle PFK (Fig. 2*A*), the residual muscle PFK from Fiver consisted exclusively of liver-type isozyme (Fig. 2*B*), indicating a total lack of the M subunit, as was the case with the human probands; the normal liver PFK is shown in Fig. 2*C* for comparison. The absence of the M subunit was then sought in the probands' erythrocytes. Unlike the M_4 and M-containing PFK isozymes present in normal dog erythrocytes (Fig. 2*D*), the erythrocyte PFK from the deficient animals consisted exclusively of platelet-type isozymes, with the P_4 tetramer predominating (Fig. 2*E*); the normal dog platelet isozymes are illustrated in Fig. 2*F* for comparison. These results suggested that the normal erythrocyte PFK in the dog consists of M and P subunits, in contrast to humans, where it consists of M and L subunits.

Enzyme-Immunoprecipitation Studies. These interpretations of our chromatographic data were confirmed by immunochemical analysis using strictly subunit-specific anti-M and anti-L antibodies (Table 2). The rabbit anti-M antibody reacted only with the PFKs from normal muscle (M_4) and erythrocytes ($M_4/M_3P/M_2P_2$) of both the control animals but not with those from their liver (L_4) and platelets (P_4/P_3L). These data indicate that the antibody is M subunit-specific, that the M subunit contributes exclusively and significantly to the PFKs of muscle (100%) and erythrocytes (≈80–90%), respectively, and that it is normally absent from dog liver and platelets. The residual muscle (L_4) and erythrocyte (P_4/P_3L)

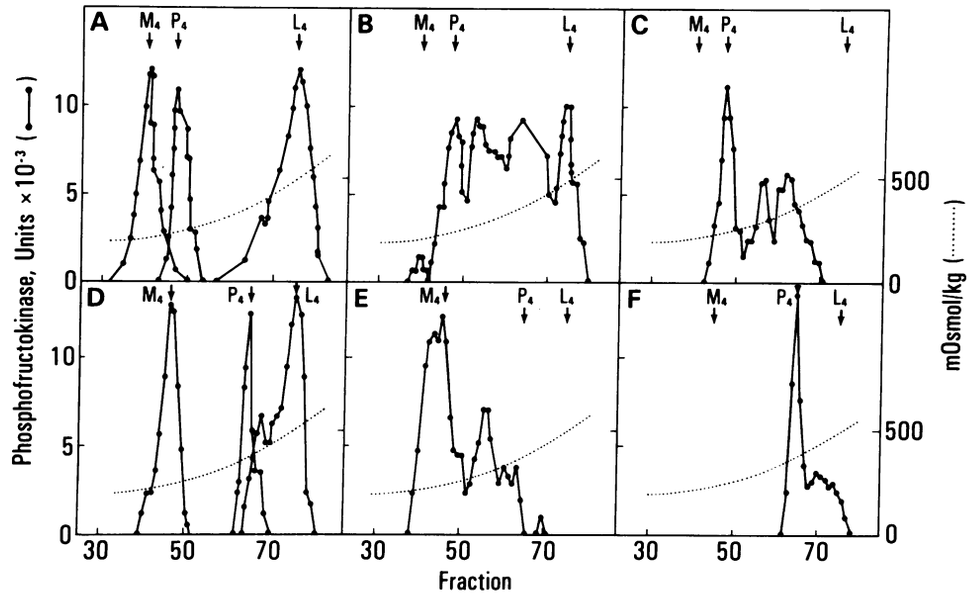

FIG. 1. Representative chromatographic profiles of PFK isozymes from man and the normal springer spaniel dogs. (*A*) Resolution of a mixture of three human homotetramers, M_4, P_4, and L_4. (*B*) Human erythrocyte PFK showing five-membered set of M/L subunits. (*C*) Human platelet PFK showing a three-membered set of P/L subunits. (*D*) Resolution of a mixture of three dog homotetramers. (*E*) Dog erythrocyte PFK showing a three- or four-membered set of M/P subunits. (*F*) Dog platelet PFK showing a two- or three-membered set of P/L subunits.

PFKs from the deficient animals were totally nonreactive with the anti-M antibody, indicating a generalized deficiency of the M subunit in these animals.

The monoclonal anti-L antibody reacted exclusively with the normal dog liver (L_4) and to a small extent with normal platelet (P_4/P_3L) PFKs but not with normal muscle (M_4) and erythrocyte ($M_4/M_3P/M_2P_2$) enzymes, indicating that the antibody is L subunit-specific and that the L subunit is normally absent in dog muscle and erythrocyte PFKs. However, as expected from the chromatographic data, the residual muscle PFK from Fiver (L_4) was almost entirely precipitated. Thus, these data confirm the presence of the L_4 isozyme in the M-deficient muscle.

Identification of the Pigment in the Urinary Specimens. No myoglobin was detectable in the heavily pigmented urinary specimens from Fiver; the pigment consisted exclusively of hemoglobin.

Glycogen Content of Muscle Biopsy Specimens. The staining with periodic acid/Schiff reagent revealed an apparent absence of increase in glycogen content of muscle biopsy specimen from Fiver.

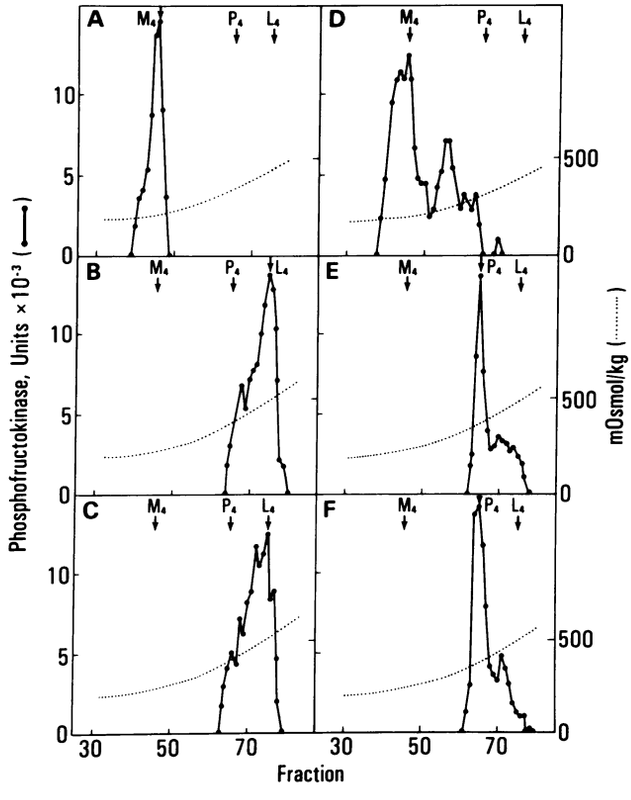

FIG. 2. Representative chromatographic profiles of PFK isozymes from the normal and PFK-deficient springer spaniels. (*A*) Normal muscle PFK. (*B*) Residual muscle PFK from a deficient dog. (*C*) Normal liver PFK. (*D*) Normal erythrocyte PFK. (*E*) Residual erythrocyte PFK from a deficient dog. (*F*) Normal platelet PFK.

DISCUSSION

Inherited PFK deficiency is rare in humans; only 35 cases from 27 unrelated families have been reported thus far (3, 14, 22–25). Homozygous deficiency of the muscle isozyme results in GSD type VII characterized by easy fatigability, muscle weakness, and myoglobinuria on exertion and a moderately severe but well-compensated hemolysis begin-

Table 2. Immunoprecipitation values of PFK from various organs and cell types of the normal and PFK-deficient dogs

	% precipitation*			
	Anti-M antibody		Anti-L antibody	
Organ/cell type	Controls (2)†	Propositi (2)‡	Controls (2)†	Propositi (2)‡
Muscle	99 ± 2	2 ± 5	0	77 ± 6
Liver	0	ND	75 ± 6	ND
Platelets	0	0	15 ± 17	37 ± 10
Erythrocytes	83 ± 10	0	0	40 ± 13

* Values given are the percent of enzyme activity precipitated by the respective antibody compared with that of the concurrent control assay from which antibody was omitted. Each value represents the mean ± SD of four to eight determinations carried out in duplicate on separate occasions; values < 7.2% are considered not significant (21).

†Numbers in parentheses are the number of separate donors investigated.

‡Values represent the mean ± SD of two separate determinations carried out in duplicate on muscle specimens from Fiver. ND, not done.

ning in early childhood[§]; dysfunction of other organ systems is conspicuously absent. The selective involvement of muscle and erythrocytes results from the fact that the M subunit contributes exclusively (100%) and substantially (≈50%) to PFKs from the skeletal muscle and erythrocyte, respectively (10, 12). As expected, these patients exhibit total and partial lack of the muscle and erythrocyte PFK, respectively; the residual erythrocyte PFK consists exclusively of the L_4 isozyme (13, 14). The exertional nature of myopathy is explained by the fact that at-rest muscle uses mitochondrial respiration to generate ATP but uses glycolysis during exercise-induced anaerobiosis. In contrast, erythrocytes rely entirely on glycolysis for energy generation, and the lack of the M subunit results in a block in glycolysis and hence hemolysis. The low erythrocyte 2,3-bisphosphoglycerate levels result in a compensatory erythropoietic drive and produce near-normal hemoglobin levels despite hemolysis (13, 14). In contrast, presence of hemolysis despite half-normal erythrocyte PFK level is explained by the unique kinetic-regulatory properties of the L_4 isozyme, which must be largely dysfunctional in the existing intraerythrocytic conditions to cause the observed block in glycolysis (14).

Our studies demonstrate that the dog PFK is also under the control of three structural loci, as is the case with other mammals. Dog muscle and liver (major species) appear to consist of isolated M_4 and L_4 homotetramers, respectively, as is the case in humans (Fig. 1 *A* and *D*). However, in contradistinction to the human erythrocyte enzyme, which consists of a complete five-membered set of M/L subunits, the dog erythrocyte enzyme is composed of a three-membered set of M/P subunits (Fig. 1 *B* and *E*). The platelet isozymes in both species appear to consist of a three-membered isozyme set of P/L subunits (Fig. 1 *C* and *F*). The dog M_4 and P_4 isozymes are more acidic, as compared to their human counterparts and are eluted later during ion-exchange chromatography (Fig. 1 *A* and *D*). These interpretations of the chromatographic data were confirmed by active enzyme-immunoprecipitation studies using subunit-specific antibodies (Table 2).

In light of these data, we investigated the nature of the enzymatic lesion in the PFK-deficient dogs. The residual enzyme activity (≈6% of normal) in the muscle of Fiver was found to consist exclusively of liver-type isozyme both chromatographically (Fig. 2*B*) and immunologically (Table 2). As expected from the subunit structure(s) of the dog erythrocyte PFK (M/P), the residual enzyme in the erythrocytes of the probands consisted predominantly of the P_4 isozyme (Fig. 2*E*). However, in addition, the M-deficient erythrocytes now exhibited L-containing isozymes; thus, their PFK profile resembled that of platelet PFK (Fig. 2*F*). These results were consistent with a generalized anomalous presence of the L subunit in the probands. Since the muscle and erythrocytes from springer spaniels normally do not express the L subunit, these data are interpreted to suggest anomalous expression of the L subunit in the face of a total lack of the M subunit in the probands. Since the normal erythrocyte PFK does not contain the L subunit, blood contamination of the PFK-deficient muscle biopsy specimen cannot be invoked to explain the presence of liver-type isozyme. In addition, anti-L antibody also precipitated significant amounts of erythrocyte and platelet PFKs from the probands, suggesting a generalized anomalous presence of the L subunit.

Our results readily explain why a genetically identical lesion (i.e., a total lack of the M subunit in the dog) results in a phenotypically distinct clinical syndrome as compared to that in humans (i.e., severe hemolytic anemia but neither muscle weakness nor glycogen deposition). This phenotypic variation results from a number of factors, including differential dependence of muscle and erythrocytes on glycolysis to generate energy. The M subunit contributes ≈40–50% to the human erythrocyte PFK, whereas it contributes ≈80–90% to the dog enzyme. Therefore, a total lack of the M subunit results in a greater degree of PFK deficiency in the dog erythrocyte and presumably a greater severity of hemolytic syndrome. The apparent absence of the muscle disease most probably results from a combination of the unusual physiology of the canine muscle and the presence of liver PFK in the muscle of the deficient dogs.

Normally, dogs exhibit an extraordinary exercise tolerance (27), which probably reflects the unusual biochemical, metabolic, and physiologic properties of the canine skeletal muscle (28–30). Although the dog produces lactic acid under extreme physical stress (27, 28), the contribution of the anaerobic metabolism is only ≈5% to the increase in total metabolic rate under such a situation (28). The higher exercise endurance of the dog is correlated with the higher succinate oxidase activity, maximum oxygen consumption, and blood flow of the canine muscle (29). These data are consistent with the presence of only slow-twitch oxidative (type I) and fast-twitch oxidative–glycolytic (type IIA) fibers and the replacement of the classical fast-twitch glycolytic (type IIB) fibers by an unusual type of high oxidative–glycolytic fibers in the canine muscle (30). Thus, in the dog muscle, all fibers are considered to exhibit high oxidative as well as glycolytic potential. It is likely that the higher oxidative potential of the canine muscle may largely account for the lack of muscle disease in the probands, especially during mild to moderate exertion, whereas ≈6% of normal PFK activity may afford protection during severe physical stress.

From the differential involvement of muscle and erythrocytes in the canine probands, it may be safely inferred that the residual erythrocyte PFK does not permit an adequate glycolytic flux. However, it is unclear at the moment whether or not the residual muscle PFK permits adequate glycolysis in the PFK-deficient muscle. Although clinically these dogs exhibit no muscle symptoms, they consistently exhibit higher-than-normal muscle creatine kinase (ATP:creatine *N*-phosphotransferase, EC 2.7.3.2.) levels that rise moderately after vigorous exercise (15), indicating some muscle involvement. It is conceivable that there exists a threshold of physical activity permitted by the L_4 isozyme, beyond which muscle glycolysis is not supported and muscle dysfunction ensues. If this were the case, then a full-fledged muscle disease might become apparent in the probands after very severe exercise.

It is possible that normally springer spaniel muscle expresses a small amount of liver PFK, and the observed L_4 isozyme in the muscle of Fiver represents the residual isozyme rather than the reexpressed one. This appears unlikely in view of the following arguments. First, the skeletal muscle from 16 of 18 vertebrate species studied so far shows the presence of a single most basic isozyme—i.e., muscle type; only in the case of two avian species is the presence of an additional (?liver-type) isozyme demonstrated (31). There is no reason why the dog would be different. Second, because of the random tetramerization of the PFK subunits, ≈6% of the L_4 isozyme, if present, should yield ≈25% precipitation of muscle PFK with anti-L antibody (12). Clearly, this value is far above the low limit of detection of our immunoprecipitation technique (≈7%) and should have become apparent. Conversely, the sensitivity of the technique permits the detection of as little as 2% of a given subunit type in a mixture of isozymes. Not once was muscle PFK

[§]Recently, we have identified a new subtype of GSD type VII characterized by late-onset progressive limb weakness (24), which is found to result from a genetic mutation distinct from the one causing the classic early-onset GSD type VII (26).

from normal animals precipitated by the anti-L antibody during more than 10 immunoprecipitation experiments.

Of the eight human GSD types in which the enzymatic deficiency has been identified (32), spontaneous animal counterparts have been reported for GSD I (glucose-6-phosphatase deficiency) in the mouse; for GSD II (acid α-glucosidase deficiency) in the dog, the quail, and cattle; for GSD III (debrancher enzyme deficiency) in the dog; and for GSD VIII (phosphorylase kinase deficiency) in the rat and the mouse (for review, see ref. 33). In addition, experimentally induced GSD-like conditions that resemble human GSD type II, V, and VII have been described; however, corresponding enzyme defects have not been identified in these conditions. Walvoort (33) in his excellent recent review has critically appraised the relevance of these animal disorders as models of human glycogenoses. Detailed comparison of the clinicopathological, biochemical, and immunological studies of the human and nonhuman cases indicate that, except perhaps for shorthorn cattle with GSD II (34), none of these animal models are homologues but rather are analogues of their human counterparts. A similar conclusion has been drawn regarding animal models of human lysosomal storage diseases (35). Present studies further reinforce these observations.

The PFK-deficient dogs, thus, provide an animal analogue of human GSD type VII and may be categorized as a type II animal model (36) with a compatibility score of 2-1-1 (37). Both of these classification systems attempt to assess the degree of homology between the animal model and its human counterpart in terms of the etiology, pathogenesis, and clinical symptomatology of the metabolic disease under consideration. The amelioration of muscle dysfunction and glycogen deposition, if brought about by a mere 6% of normal PFK activity, is remarkable and raises some intriguing questions regarding human GSD type VII. Is it possible that the variation in the myopathic syndrome observed among human probands results from a variable expression of the L subunit in the M-deficient muscle? Could therapeutic intervention be feasible in these patients by the induction of the expression of *PFKL* or *PFKP* locus in the muscle?

Despite the differences in the phenotypic expression of muscle PFK deficiency in man and in the dog, this animal analogue may prove to be a useful model of human erythrocyte enzymopathies that cause hemolytic anemia, since the exact mechanisms of premature erythrocyte demise remain unknown (38). This model may also be used to investigate the various strategies of bone marrow transplantation, enzyme replacement, and gene replacement therapy. Finally, if under severe stress muscle dysfunction is evident, then the model will help to investigate the pathophysiology of GSD type VII.

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1. Bloxham, D. P. & Lardy, H. A. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 8A, pp. 239–278.
2. Uyeda, K. (1979) *Adv. Enzymol. Relat. Areas Mol. Biol.* **48**, 193–244.
3. Vora, S. (1982) *Isozymes: Curr. Top. Biol. Med. Res.* **6**, 119–167.
4. Dunaway, G. A. (1983) *Mol. Cell. Biochem.* **52**, 75–91.
5. Vora, S., Oskam, R. & Staal, G. E. J. (1985) *Biochem. J.* **229**, 333–341.
6. Tsai, M. Y. & Kemp, R. G. (1973) *J. Biol. Chem.* **248**, 785–792.
7. Tsai, M. Y. & Kemp, R. G. (1974) *J. Biol. Chem.* **249**, 6590–6596.
8. Tsai, M. Y., Gonzalez, F. & Kemp, R. G. (1975) in *Isozymes*, ed. Markert, C. L. (Academic, New York), Vol. 2, pp. 819–835.
9. Kahn, A., Meienhofer, M., Cottreau, D., Lagrange, J. L. & Dreyfus, J. C. (1979) *Hum. Genet.* **48**, 93–108.
10. Vora, S., Seaman, C., Durham, S. & Piomelli, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 62–66.
11. Meienhofer, M.-C., Cottreau, D., Dreyfus, J.-C. & Kahn, A. (1980) *FEBS Lett.* **110**, 219–222.
12. Vora, S. (1981) *Blood* **57**, 724–732.
13. Vora, S., Corash, L., Engel, W. K., Durham, S., Seaman, C. & Piomelli, S. (1980) *Blood* **55**, 629–635.
14. Vora, S., Davidson, M., Seaman, C., Miranda, A. F., Noble, N. A., Tanaka, K. R., Frenkel, E. P. & DiMauro, S. (1983) *J. Clin. Invest.* **72**, 1995–2006.
15. Giger, U., Harvey, J. W., Yamaguchi, R. A., McNulty, P. K., Chiapella, A. & Beutler, E. (1985) *Blood* **65**, 345–351.
16. Davidson, M., Collins, M., Byrne, J. & Vora, S. (1983) *Biochem. J.* **214**, 703–710.
17. Vora, S., Wims, L. A., Durham, S. & Morrison, S. L. (1981) *Blood* **58**, 823–829.
18. Gondko, R., Schmidt, M. & Leyko, W. (1964) *Biochim. Biophys. Acta.* **86**, 190–191.
19. Carson, S. L., Matthews, J. L. & Pickett, J. P. (1978) in *Laboratory Medicine*, ed. Race, G. J. (Harper & Row, New York), Vol. 3, pp. 33–34.
20. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
21. Vora, S., Durham, S., de Martinville, B., George, D. L. & Francke, U. (1982) *Somatic Cell Genet.* **8**, 95–104.
22. Tani, K., Fujii, H., Takegawa, S., Miwa, S., Koyama, W., Kanayama, M., Imanaka, A., Imanaka, F. & Kuramoto, A. (1983) *Am. J. Hematol.* **14**, 165–174.
23. Chance, B., Eleff, S., Bank, W., Leigh, J. S., Jr., & Warnell, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7714–7718.
24. Danon, M. J., DiMauro, S., Bresolin, N., Shanske, S. & Vora, S. (1985) *Neurology* **35**, 207 (abstr.).
25. Shimizu, T., Kuwajima, M., Kono, N., Mineo, I., Sumi, S., Yonezawa, T., Nonaka, K. & Tarui, S. (1983) *Med. J. Osaka Univ.* **33**, 49–58.
26. Vora, S., Danon, M. J., Turchen, S. & DiMauro, S. (1985) *Clin. Res.* **33**, 332A (abstr.).
27. Dill, D. B., Edwards, H. T. & Talbott, J. H. (1933) *J. Physiol. (London)* **77**, 49–62.
28. Cerretelli, P., Piiper, J., Mangili, F. & Ricci, B. (1964) *J. Appl. Physiol.* **19**, 25–28.
29. Maxwell, L. C., Barclay, J. K., Mohrman, D. E. & Faulkner, J. A. (1976) *Am. J. Physiol.* **133**, C14–C18.
30. Snow, B. H., Billeter, R., Mascarello, F., Carpene, E., Rowlerson, A. & Jenny, E. (1982) *Histochemistry* **75**, 53–65.
31. De Faria, J. B., Crivellaro, O. & Bacila, M. (1976) *Comp. Biochem. Physiol. B* **55**, 323–329.
32. Howell, R. R. & Williams, J. C. (1983) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Bowen, M. S. (McGraw-Hill, New York), pp. 141–166.
33. Walvoort, H. C. (1983) *J. Inher. Metab. Dis.* **6**, 3–16.
34. Edwards, J. R. & Richards, R. B. (1979) *Br. Vet. J.* **135**, 338–348.
35. Desnick, R. J., McGovern, M. M., Schuchman, E. H. & Haskins, M. E. (1982) in *Animal Models of Inherited Metabolic Diseases* (Liss, New York), pp. 27–65.
36. Smith, J. E. (1982) in *Animal Models of Inherited Metabolic Diseases* (Liss, New York), pp. 421–433.
37. Gerrity, L. W. & Friedman, J. M. (1982) in *Animal Models of Inherited Metabolic Diseases* (Liss, New York), pp. 459–461.
38. Beutler, E. (1980) *Blood Cells* **6**, 827–829.