Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6091–6095, October 1983
Medical Sciences

A canine model of human α-L-iduronidase deficiency

(mucopolysaccharidosis I/Hurler–Scheie syndrome/correction/mannose 6-phosphate)

ELIZABETH SPELLACY*†, ROBERT M. SHULL‡, GEORGE CONSTANTOPOULOS§, AND ELIZABETH F. NEUFELD*¶

*Genetics and Biochemistry Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and §Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205; and ‡Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37901

Contributed by Elizabeth F. Neufeld, June 30, 1983

ABSTRACT **A disease discovered in three Plott Hound littermates was found to be associated with a profound and specific deficiency of α-L-iduronidase (mucopolysaccharide α-L-iduronohydrolase; EC 3.2.1.76) in fibroblasts and leukocytes. The pedigree was consistent with autosomal recessive inheritance. A markedly increased amount of dermatan sulfate and heparan sulfate was excreted in urine. Fibroblasts cultured from the skin of the affected dogs accumulated excessive ^{35}S-labeled mucopolysaccharide; this accumulation could be decreased to a normal level by exogenous human high-uptake α-L-iduronidase (Hurler corrective factor) as well as by secretions of normal human or canine fibroblasts. The correction was inhibited by mannose 6-phosphate. Maturation of α-L-iduronidase in normal canine fibroblasts followed the pathway previously observed in human fibroblasts; no cross-reactive material was observed in the cells or in secretions from the fibroblasts of the affected dogs. The canine disorder thus resembles mucopolysaccharidosis I in all biochemical parameters tested; the clinical appearance of the animals is closest to Hurler–Scheie syndrome, a form of α-L-iduronidase deficiency of intermediate severity. The animal model should prove valuable for therapeutic experiments.**

Research on inherited disease is often hampered by scarcity of appropriate material for study and by ethical constraints on experimental procedures. When the defect is expressed in cell culture, as is the case for lysosomal storage disorders, the scarcity problem is solved; unlimited material becomes available for elucidating the biochemical defect and for developing potential therapeutic approaches. But application of knowledge gained from such studies *in vitro* to therapy of patients remains difficult, and animal models are almost essential to bridge the gap.

Through the collaboration of veterinary and medical investigators, a number of lysosomal storage disorders have been identified in animal species in recent years (1). They include bovine α-mannosidosis (2) and Pompe disease (3); caprine β-mannosidosis (4); feline GM_1 gangliosidosis (5), GM_2 gangliosidosis (6), Niemann–Pick disease (7), α-mannosidosis (8), mucopolysaccharidosis I (9), and mucopolysaccharidosis VI (10); canine GM_1 gangliosidosis (11), Niemann–Pick disease (12), Gaucher disease (13), and Krabbe disease (14); and murine Krabbe disease (15) and Niemann–Pick disease (16).

We describe here the biochemical features of a disease, observed in a Plott Hound family, that appears analogous to human mucopolysaccharidosis I, an α-L-iduronidase deficiency disease (17). Mucopolysaccharidosis I covers a wide clinical spectrum, ranging from the very severe Hurler syndrome (with skeletal malformations, retardation of physical and mental growth, corneal clouding, cardiovascular disease, and early death) to the relatively benign Scheie syndrome (with corneal clouding as the major problem). The spectrum includes a syndrome of intermediate severity, called Hurler–Scheie syndrome. The clinical features of the affected dogs place them in this intermediate category. A preliminary report of the clinical and pathological findings has been presented (18).

MATERIALS AND METHODS

Animals. A pedigree of the affected Plott Hound family is presented in Fig. 1. Plott Hounds are a breed of hunting dogs registered with the United Kennel Club. The three affected dogs (VI-2, -3, and -4) were donated to the College of Veterinary Medicine, University of Tennessee (Knoxville, TN), which also has custody of dogs VII-1 and -2. Other dogs in the pedigree are in the custody of their owners, to whom we are grateful for information and permission to take blood samples. Blood samples and skin biopsies from normal unrelated Plott Hounds were obtained through the courtesy of White Hollow Kennels (Knoxville, TN).

Cell Culture. Canine skin fibroblasts were obtained from skin biopsies of dogs VI-2 and VI-3 (Fig. 1), from four normal unrelated Plott Hounds, and from one poodle dog. Cultures were maintained in Eagle's minimal essential medium, with nonessential amino acids and antibiotics, in 5% CO_2/95% air, essentially as described for cultures of human fibroblasts (19) except for an increase in fetal bovine serum to 15%. The fibroblasts used for enzyme assays were grown at 35°C, but subsequently the incubator temperature was raised to 39°C, with improvement in growth and morphological appearance of the cells. Cells were detached from the flasks with 0.15% trypsin (GIBCO). Waymouth MAB 87/3 (20) was prepared with components as described in the GIBCO catalogue but with a decreased amount of leucine for use in labeling experiments.

Leukocytes. Mononuclear leukocytes were separated by centrifugation through Ficoll-Paque (21) obtained from Pharmacia Fine Chemicals. Preparations from healthy dogs, whether normal or affected, yielded >90% mononuclear cells; however, many of the dogs kept in the countryside had intestinal parasites, and leukocytes prepared from their blood contained up to 30% eosinophils.

Enzyme Assays. Fibroblasts were harvested as described (19) and lysed by three cycles of freeze–thawing. Leukocytes were lysed by four cycles of freeze–thawing, followed by brief sonication (3 × 30 sec, in an ice-cold bath of an ultrasonic cleaner). α-L-Iduronidase (mucopolysaccharide α-L-iduronohydrolase; EC 3.2.1.76) was assayed fluorimetrically as described by Rome

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

† Present address: Division of Inherited Metabolic Diseases, Medical Research Council Clinical Research Centre, Harrow, Middlesex, U.K.
¶ To whom reprint requests should be addressed.

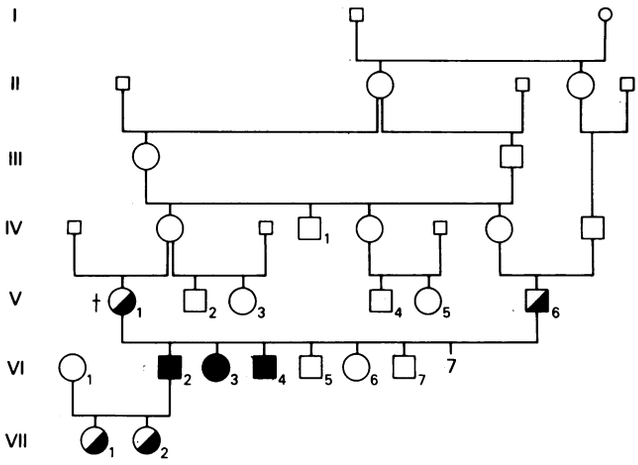

FIG. 1. Pedigree of the affected Plott Hounds, showing inbreeding in three generations. Closed symbols refer to affected dogs; half-closed symbols indicate obligate heterozygotes, assuming a recessive mode of inheritance; small symbols refer to dogs of unknown breeding. †, Deceased animal.

et al. (22); 40 μg of protein and a 3-hr incubation were used for the leukocyte lysate, and 20 μg of protein and a 1-hr incubation were routinely used for the fibroblast assays. Samples with low activity were also assayed with more protein and longer incubation periods.

β-Glucuronidase, β-galactosidase, α-mannosidase, α-L-fucosidase, and β-hexosaminidase were assayed fluorometrically as described (23) with omission of Triton X-100. α-*N*-Acetylglucosaminidase, arylsulfatase B, iduronate sulfatase, and heparan *N*-sulfatase were assayed by the methods of Hall *et al.* (19), with reduction of the incubation period in the iduronate sulfatase assay to 4 hr.

Biosynthetic Labeling and Isolation of α-L-Iduronidase. Fibroblasts of normal and affected dogs were grown in 100-mm Petri dishes for 1 wk to a density of about 0.5 mg of protein per dish. Labeling with L-[4,5-^{3}H]leucine (Amersham, 62 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) was performed as described (24) with minor modifications. The Waymouth MAB 87/3 medium was supplemented with 10% dialyzed fetal bovine serum and the leucine concentration was decreased to 2.5 μg/ml. Cells were extracted without trypsinization, by use of 1% Nonidet P-40 in Dulbecco's phosphate-buffered saline (25). Medium was concentrated with 70% $(NH_4)_2SO_4$ and dialyzed thoroughly.

Immunoprecipitation was carried out as described (26), by using 5 μl of antiserum and 0.5 μg of human urinary α-L-iduronidase as carrier. However, the cell extracts and medium concentrates were first precleared with 40 μl of Immunoprecipitin (a 10% suspension of fixed protein A-bearing *Staphylococcus aureus* from Bethesda Research Laboratories), and the immunoprecipitates were washed more extensively by incorporating a detergent wash (10 mM Tris·HCl/0.6 M NaCl/0.1% $NaDodSO_4$/0.05% Nonidet P-40/0.02% NaN_3) and a phosphate-buffered-saline wash before the acetone step.

Immunoprecipitates were solubilized and subjected to polyacrylamide gel electrophoresis and fluorography, as described (24).

^{35}S-Labeled Mucopolysaccharide Accumulation and Correction. Cells were labeled with $^{35}SO_4$ (New England Nuclear). ^{35}S-Labeled mucopolysaccharide accumulation was determined and media concentrates were prepared by procedures described by Cantz *et al.* (27). Urinary corrective factor had been prepared by Barton and Neufeld (28).

Urinary Mucopolysaccharides. The procedures used for isolation and analysis of urinary mucopolysaccharides (glycosaminoglycans) have been described in detail (29). The composition of the mucopolysaccharides was estimated from carbazole/orcinol ratios and from susceptibility to degradation by chondroitinases AC and ABC (30).

RESULTS

α-L-Iduronidase Activity in Leukocytes. Mononuclear leukocyte lysates of the affected dogs had essentially undetectable α-L-iduronidase activity (0–1% of the control mean), as shown in Fig. 2. Leukocyte lysates from relatives of the affected dogs showed great scatter, four of the values were one-half or less of the normal mean, and five were clearly in the normal range. The α-L-iduronidase activity in leukocytes of V-6, the father, was higher than in the lowest normal control. Because the father is an obligate heterozygote (assuming autosomal recessive inheritance), the results indicate either an overlap of normal and heterozygous enzyme levels or the presence of a heterozygote among the unrelated control dogs. Dogs whose leukocyte activity was lower than the father's and who, therefore, are presumed to be heterozygotes, include IV-1, V-5, and VI-5, as well as the two offspring of an affected male, VII-1 and VII-2, who are obligate heterozygotes. Dogs V-2, V-3, V-4, VI-6, and VI-7 had leukocyte α-L-iduronidase in the normal range and are therefore presumed to be of normal genotype.

Specificity of α-L-Iduronidase Deficiency. α-L-Iduronidase activity was also profoundly deficient in fibroblast homogenates from the affected dogs. Eight other lysosomal enzyme activities measured in fibroblast homogenates were comparable to those of the control animals (Table 1). Of five other glycosidase activities measured in leukocytes, β-galactosidase, α-mannosidase, and β-glucuronidase were somewhat altered in the affected dogs (66%, 42%, and 147% of the normal mean, respectively), whereas α-L-fucosidase and β-hexosaminidase were in the control range.

Urinary Mucopolysaccharide Excretion. Analysis of urinary mucopolysaccharides showed a 6- to 25-fold increase over the normal mean (Table 2). The increase occurred in dermatan sulfate and heparan sulfate, with the former predominating. The mucopolysaccharidiuria was similar to that observed in human patients deficient in α-L-iduronidase (Table 2).

Mucopolysaccharide Accumulation and Correction in Cultured Fibroblasts. When incubated in the presence of $^{35}SO_4$, cells from the affected dog, VI-3, accumulated more radioactive mucopolysaccharide than did the cells of a control dog (Fig. 3). The kinetics of the accumulation (linear increase for the af-

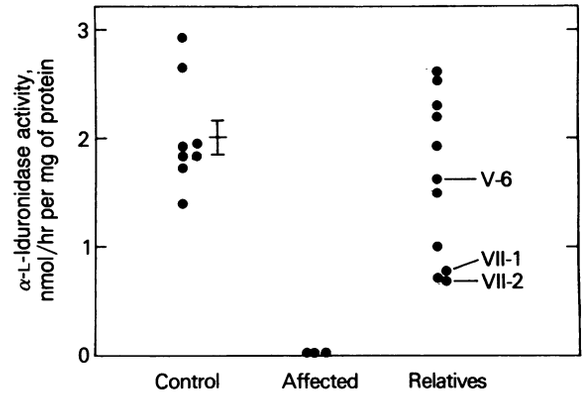

FIG. 2. α-L-Iduronidase activity in mononuclear leukocyte lysates from the 3 affected dogs, 8 unrelated Plott Hounds, and 11 Plott Hounds related by pedigree. Mean and SEM are indicated for the control group. The relatives, in order of descending enzyme activity, are VI-7, V-4, VI-6, V-3, V-2, V-6, V-5, IV-1, VII-1, VI-5, and VII-2. The relatives identified on the diagram are obligate heterozygotes, if autosomal recessive inheritance is assumed.

Table 1. Lysosomal enzyme activities in fibroblasts and leukocytes

Enzyme	Fibroblasts		Leukocytes		
	Affected, $n = 2$	Control, $n = 2$	Affected, $n = 3$	Control, $n = 8$	Relatives, $n = 11$
α-L-Iduronidase	0.12	23	0.01 (0.0–0.02)	2.1 (1.4–3.0)	1.6 (0.6–2.6)
β-Glucuronidase	520	370	940 (770–1,200)	640 (500–780)	670 (280–920)
β-Galactosidase	530	410	190 (150–230)	290 (230–390)	330 (230–430)
α-Mannosidase	290	160	370 (340–400)	880 (420–1,300)	730 (300–1,600)
α-L-Fucosidase	270	280	140 (130–150)	180 (100–220)	165 (120–260)
β-Hexosaminidase	1,700	1,100	970 (180–1,200)	1,300 (900–1,800)	970 (520–1,600)
Arylsulfatase B	94	87	ND	ND	ND
Iduronate sulfatase	250	320	ND	ND	ND
Heparan *N*-sulfatase	2.3	2.0	ND	ND	ND

Enzyme activities are expressed as nmol of substrate hydrolyzed per hr per mg of protein, except for iduronate sulfatase and heparan sulfatase; for these, activity is expressed as % substrate hydrolyzed per hr per mg of protein and % substrate hydrolyzed per hr per 50 μl of homogenate, respectively (19). Numbers in parentheses give the range of enzyme activity. ND, not determined.

fected, a plateau for the normal) were similar to those displayed by analogous human cells (31).

Addition of human urinary "Hurler corrective factor" (partially purified high-uptake α-L-iduronidase) decreased the accumulation by the affected cells to a normal level without changing it in normal cells. Secretions of normal dog fibroblasts were corrective for α-L-iduronidase-deficient cells of both human and canine origin. Correction was inhibited by 2 mM mannose 6-phosphate, an inhibitor of lysosomal enzyme uptake (32–34) but not by 2 mM mannose 1-phosphate (Fig. 3 *Inset*). Secretions of normal or affected canine fibroblasts had no effect on the low ^{35}S-labeled mucopolysaccharide accumulation by normal cells (data not shown).

Synthesis and Maturation of α-L-Iduronidase. The natural history of the α-L-iduronidase in canine fibroblasts was similar to that described in human cells (26). The enzyme was made as a protein of apparent M_r 71,000 and converted during a 24 hr chase to M_r 67,000. There followed a slow conversion to M_r 62,000, seen after a 72-hr chase (Fig. 4). The α-L-iduronidase secreted into the medium was slightly larger than the cellular precursor form (M_r, 75,000). The presence of 10 mM NH_4Cl in the medium caused a quantitative secretion of precursor enzyme. However, it can be seen in Fig. 4 that the canine cells secreted a considerable amount of newly made α-L-iduronidase, even in the absence of NH_4Cl. This is in contrast to the retention of nearly all the newly made enzyme within fibroblasts of human origin (26).

Table 2. Urinary mucopolysaccharide excretion

Subjects	Uronic acid/ creatinine, μg/mg	Carbazole/ orcinol	Composition, % CS	DS	HS
Canine					
VI-4	221	0.58	5	70	25
VI-3	51	0.62			
VI-2	123	0.55			
Normal controls (7)	8.8 ± 5.5	0.90	60	20	20
Human					
Hurler, children (4)	200 ± 55	0.66 ± 0.06	10	60	30
Scheie, adults (3)	58 ± 20	0.69 ± 0.07	10	55	35
Normal controls					
Children (15)	25.5 ± 16.9	1.04 ± 0.1*	85*	<5	10
Adults (10)	5.2 ± 2.7				

Numbers in parentheses indicate number of subjects. Data are given as mean ± SD. The data for human patients and controls are taken from ref. 29. Urine from dog VI-3 was obtained by cystocentesis. CS, chondroitin 4/6 sulfate; DS, dermatan sulfate; HS, heparan sulfate.
* Data pooled for children and adults.

No crossreactive protein was observed in the corresponding areas of the gels for the affected dog (VI-3). A control experiment showed that these cells synthesized and processed an unrelated lysosomal enzyme, β-hexosaminidase, in the same manner as cells of a normal dog (data not shown).‖

DISCUSSION

The metabolic defect in the iduronidase-deficient Plott Hound is similar to that of human patients with mucopolysaccharidosis

‖The natural history of β-hexosaminidase in canine fibroblasts differed somewhat from that described for the enzyme in human cells (24). The α chain was converted from M_r 60,000 to M_r 51,000; the β chain was converted from M_r 63,000 to M_r 49,000. Some smaller bands also occurred (a doublet of M_r 29,000 and 28,000, and a triplet of M_r 23,000, 21,000, and 20,000); their origin has not been identified. The α and β chains were found in the medium primarily in the larger precursor form, but a small amount of processed protein could also be detected.

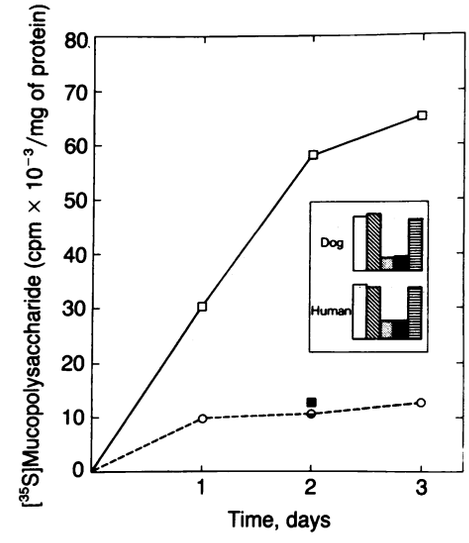

FIG. 3. Accumulation of ^{35}S-labeled mucopolysaccharide by fibroblasts from an affected dog (□) and from a normal Plott hound (○); the closed symbols indicate that human corrective factor was present during the labeling period. (*Inset*) The effect of various additions to the medium on a 2-day accumulation of ^{35}S-labeled mucopolysaccharide in canine and human (Hurler) fibroblasts deficient in α-L-iduronidase. □, Baseline accumulation; ▨, with fibroblast secretions from an affected dog; ▩, with fibroblast secretions from a normal dog; ■, with fibroblast secretions from a normal dog plus 2 mM mannose 1-phosphate; ▤, with fibroblast secretions from a normal dog plus 2 mM mannose 6-phosphate. Bar heights represent accumulation relative to the baseline value.

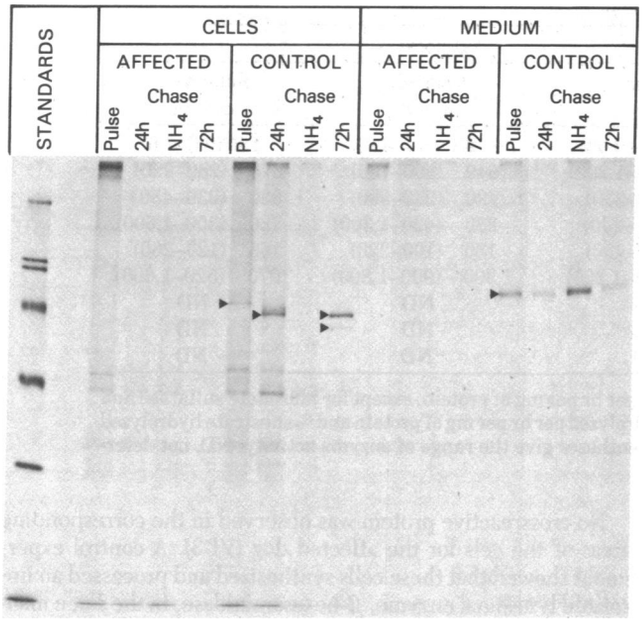

FIG. 4. Pulse–chase labeling of α-L-iduronidase in fibroblasts from normal and affected dogs. Four 100-mm culture dishes were used for each cell strain. Cells were labeled with [^{3}H]leucine. After 3 hr, one dish of each set was harvested (pulse), and the remainder were incubated for the length of time specified in unlabeled medium enriched with 0.1 mg of leucine per ml (chase). NH_4Cl (10 mM) was present where indicated during the pulse and a 24-hr chase. Fluorographic exposure was for 8 days. The radioactive standards used were myosin (M_r, 200,000), phosphorylase B (M_r, 93,000), bovine serum albumin (M_r, 69,000), ovalbumin (M_r, 46,000), carbonic anhydrase (M_r, 30,000), and lysozyme (M_r, 14,300).

I with respect to all parameters examined so far. Cells and tissues (unpublished results) of the dog are almost totally devoid of α-L-iduronidase activity and consequently store excess mucopolysaccharide. No crossreactive protein appears to be synthesized by the deficient fibroblasts of either species (26); however, this finding should be interpreted with caution, because it may reflect merely a lack of reactivity of the available antibody (35) with an altered α-L-iduronidase protein.

The natural history of α-L-iduronidase in normal canine fibroblasts is also similar to that of its human counterpart (26) in several respects, including the molecular size of precursor and mature enzyme and the slow rate of processing. Secretions of canine cells were corrective for iduronidase-deficient cells of a Hurler patient, showing that canine α-L-iduronidase has the mannose 6-phosphate recognition marker required for uptake of enzymes in human fibroblasts (32–34). Reciprocal correction experiments showed that canine cells also have the mannose 6-phosphate receptor.

Thus the affected Plott Hound ought to be a good model for therapeutic trials. It is thought that transplantation of normal tissue may be the easiest way to provide a continuous supply of enzyme for uptake by the patient's cells. The transplantation of fibroblasts (36, 37), bone marrow (38), and amniotic epithelial cells (39) has been tried or suggested; the prospect for therapeutic results appears greatest for bone marrow transplantation but the risks of this procedure are also very great. Some questions cannot be properly studied in human subjects; the most important concerns the ability of donor enzyme to penetrate through the blood–brain barrier and decrease lysosomal storage within cells of the central nervous system. Because bone marrow transplantation in dogs is a well-established procedure (40), we hope that the canine model, once developed into a colony, will help to provide the answer.

We thank Mrs. Clara Hall for valuable suggestions. This work was supported in part by a U.S. Public Health Service International Fellowship to E.S.

1. Desnick, R. J., McGovern, M. M., Schuchman, E. H. & Haskins, M. E. (1982) in *Animal Models of Inherited Metabolic Diseases, Progress in Clinical and Biological Research*, eds. Desnick, R. J., Patterson, D. F. & Scarpelli, D. G. (Liss, New York), Vol. 94, pp. 27–65.
2. Hocking, J. D., Jolly, R. D. & Batt, R. D. (1972) *Biochem. J.* **128**, 69–78.
3. Richards, R. B., Edwards, J. R., Cook, R. D. & White, R. R. (1977) *Neuropathol. Appl. Neurobiol.* **3**, 45–56.
4. Jones, M. Z. & Dawson, G. (1981) *J. Biol. Chem.* **256**, 5185–5188.
5. Baker, H. J., Jr., Lindsey, J. R., McKhann, G. F. & Farrell, D. F. (1978) *Science* **174**, 838–839.
6. Cork, L. C., Munnell, J. F., Lorenz, M. D., Murphy, J. V., Baker, H. J. & Rattazzi, M. C. (1977) *Science* **196**, 1014–1017.
7. Wenger, D. A., Sattler, M., Kudoh, T., Snyder, S. P. & Kingston, R. S. (1980) *Science* **208**, 1471–1473.
8. Burditt, L. J., Chotai, K., Hirani, S., Nugent, P. G., Winchester, B. G. & Blakemore, W. F. (1980) *Biochem. J.* **189**, 467–473.
9. Haskins, M. E., Jezyk, P. F., Desnick, R. J., McDonough, S. K. & Patterson, D. F. (1979) *Pediat. Res.* **13**, 1294–1297.
10. Jezyk, P. F., Haskins, M. E., Patterson, D. F., Mellman, W. J. & Greenstein, M. (1977) *Science* **198**, 834–836.
11. Read, D. H., Harrington, D. D., Keenan, T. W. & Hinsman, E. J. (1976) *Science* **194**, 442–445.
12. Bundza, A., Lowden, J. A. & Charlton, K. M. (1979) *Vet. Pathol.* **16**, 530–538.
13. van de Water, N. S., Jolly, R. D. & Farrow, B. R. H. (1979) *Aust. J. Exper. Biol.* **57**, 551–554.
14. Suzuki, Y., Austin, J., Armstrong, D., Suzuki, K., Schlenker, J. & Fletcher, T. (1970) *Exp. Neurol.* **29**, 65–75.
15. Duchen, L. W., Eicher, E. M., Jacobs, J. M., Scaravilli, F. & Teixeira, F. (1980) *Brain* **103**, 695–710.
16. Miyawaki, S., Mitsuoka, S., Sakiyama, T. & Kitagawa, T. (1982) *J. Hered.* **73**, 257–263.
17. McKusick, V. A. & Neufeld, E. F. (1983) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S. (McGraw-Hill, New York), pp. 751–777.
18. Shull, R. M., Munger, R. J., Spellacy, E., Hall, C. W., Constantopoulos, G. & Neufeld, E. F. (1982) *Am. J. Pathol.* **109**, 244–248.
19. Hall, C. W., Liebaers, I., DiNatale, P. & Neufeld, E. F. (1978) *Methods Enzymol.* **50**, 439–456.
20. Gorham, L. W. & Waymouth, C. (1965) *Proc. Soc. Exp. Biol. Med.* **119**, 287–290.
21. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, Suppl. 97, 77–89.
22. Rome, L. H., Weissmann, B. & Neufeld, E. F. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2331–2334.
23. Robbins, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1911–1915.
24. Hasilik, A. & Neufeld, E. F. (1980) *J. Biol. Chem.* **255**, 4937–4945.
25. Proia, R. L. & Neufeld, E. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6360–6364.
26. Myerowitz, R. & Neufeld, E. F. (1981) *J. Biol. Chem.* **256**, 3044–3048.
27. Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) *Methods Enzymol.* **28**, 884–897.
28. Barton, R. W. & Neufeld, E. F. (1971) *J. Biol. Chem.* **246**, 7773–7779.
29. Constantopoulos, G. & Dekaban, A. S. (1975) *Clin. Chim. Acta* **59**, 321–336.
30. Constantopoulos, G., McComb, R. D. & Dekaban, A. S. (1976) *J. Neurochem.* **26**, 901–908.
31. Fratantoni, J. C., Hall, C. W. & Neufeld, E. F. (1968) *Proc. Natl. Acad. Sci. USA* **60**, 699–706.
32. Kaplan, A., Achord, D. T. & Sly, W. S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2026–2030.
33. Sando, G. N. & Neufeld, E. F. (1977) *Cell* **12**, 619–627.
34. Sly, W. S. & Fischer, H. D. (1982) *J. Cell. Biochem.* **18**, 67–85.
35. Rome, L. H., Garvin, A. J. & Neufeld, E. F. (1978) *Arch. Biochem. Biophys.* **189**, 344–353.

36. Dean, M. F., Muir, H., Benson, P. F., Button, L. R., Boylston, A. & Mowbray, J. (1976) *Nature (London)* **261,** 323–325.
37. Gibbs, D. A., Spellacy, E., Tompkins, R., Watts, R. W. E. & Mowbray, J. F. (1983) *J. Inher. Metab. Dis.* **6,** 62–81.
38. Hobbs, J. R., Hugh-Jones, K., Barrett, A. J., Byrom, N., Chambers, D., Henry, K., James, D. C. O., Lucas, C. F., Rogers, T. R., Benson, P. F., Transley, L. R., Patrick, A. D., Mossman, J. & Young, E. P. (1981) *Lancet* **ii,** 709–712.
39. Adinolfi, M., Akle, C. A., McColl, I., Fensom, A. H., Tansley, L., Connolly, P., Hsi, B. L., Faulk, W. P., Travers, P. & Bodmer, W. F. (1982) *Nature (London)* **295,** 325–327.
40. Storb, R., Rudolph, R. H., Kolb, H. J., Graham, T. C., Mickelson, E., Erickson, V., Lerner, K. G., Kolb, H. & Thomas, E. D. (1973) *Transplantation* **15,** 92–100.