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Steroid-Sulfatase Deficiency in Sex-Linked Ichthyosis

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SUMMARY

Steroid-sulfatase activity was absent in the cultured fibroblasts of nine affected members of eight families with sex-linked ichthyosis. An intermediate value of enzyme activity was found in an obligate heterozygote and a normal value in a patient with ichthyosis vulgaris. Cultured epidermal cells of an affected individual also had no enzyme activity, while normal cultured epidermal cells did.

Ichthyosis describes a group of skin diseases which share in common the presence of adherent scales that have been likened to the appearance of fish scales. Most of these disorders are inherited, but examples of sporadic or acquired forms have been reported [1]. A sex-linked type of ichthyosis has been delineated with distinctive clinical features [2–4]. Shapiro et al. [5] recently reported a steroid-sulfatase deficiency in patients with sex-linked ichthyosis in two families in which placental steroid-sulfatase activity had been abnormally low in some pregnancies [6].

This report establishes the deficiency in cultured fibroblasts of individuals with sex-linked ichthyosis who had been referred to our genetics clinic. In one individual, epidermal cells were cultured and also shown to have steroid-sulfatase deficiency.

MATERIAL AND METHODS

The patients were referred to the Dermatology Genetics Clinic at the Massachusetts General Hospital during the past 10 years for diagnosis and treatment. The diagnosis of sex-linked ichthyosis was based on the following criteria: (1) dark adherent scales most apparent in the posterior but also involving the anterior surfaces of the trunk and extremities; (2) sparing of the palms and soles; (3) absence of keratosis pilaris; (4) marked clearing in the summer; (5) negative personal and family history of eczema and other manifestation of atopy; (6) parents not involved; (7) only male family members with the disease and no male to male transmission; and (8) onset during the first few months of life. The patient with ichthyosis vulgaris was chosen for the study

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because he met the following diagnostic criteria: (1) light scales primarily on extensor surfaces; (2) involvement of the palms and soles; (3) keratosis pilaris; (4) presence of eczema; (5) an affected parent; (6) onset late in the first year of life; and (7) improvement in the summer.

The tissue culture supplies were obtained from Gibco (Grand Island, N.Y.), labeled dehydroepiandrosterone sulfate [7-^{3}H(N)] from New England Nuclear (Boston, Mass.), and dehydroepiandrosterone sulfate (DHEAS) from Sigma Chemical (St. Louis, Mo.). The isotope was mixed with carrier DHEAS to achieve a specific activity of 9.9×10^3 cpm/nm DHEAS.

To obtain fibroblast cultures, a 2 mm skin punch biopsy was obtained under local anesthesia, and the tissue was minced with a scalpel and scratched into 100 mm petri dishes. The cells were grown in Dulbecco medium with 10% fetal calf serum at 37°C. A split thickness skin biopsy was obtained from one patient and incubated in 0.25% trypsin in phosphate buffered saline (PBS) at 4°C for 18 hr. The epidermis was removed and a cell suspension prepared by shaking in Dulbecco medium with 20% fetal calf serum. This suspension was pipetted onto 3T3 cells treated with mitomycin C and grown in that medium at 37°C [7, 8].

Three 100 mm plates of fibroblasts from each individual were used to prepare the enzyme extract. The medium was removed and the cells rinsed with PBS. The cells were treated with 0.25% trypsin in PBS at 37°C for 5 min, and the resulting suspension washed once with tissue culture medium and twice with PBS. The cells were frozen, thawed, and then homogenized in 0.1 M Tris buffer, pH 7.2. A suitable volume of homogenate was added to the reaction tube and the volume brought to 0.4 ml with Tris buffer. To this was added 0.1 ml of substrate solution to make the DHEAS concentration 4×10^{-5} M, and the tube was incubated at 37°C for up to 2 hr. A 0.2 ml aliquot of the mixture was extracted with 0.4 ml of benzene and 0.2 ml benzene was added to 10 ml of aquasol which was counted by scintillation spectrophotometry. The epidermal cell cultures were handled in a similar fashion but were harvested by scraping with a rubber policeman.

An aliquot of the homogenates was centrifuged at 2500 g for 10 min and protein determined on the supernatant by the method of Bradford [9].

RESULTS

The assay for steroid-sulfatase was performed by a modification of Shapiro's method [6] using DHEAS as the substrate. This reaction rate was linear for at least 2 hr and was proportional to the amount of enzyme added.

No sulfatase activity was detected in the cultured fibroblasts or keratinocytes of affected individuals (X1-X8, XE), but it was present in both cell types of normal controls (C1, C2, and CE) as shown in table 1. A normal value was also observed in an individual with ichthyosis vulgaris. One obligate heterozygote showed an intermediate value in early passage cultures (table 2) and a much decreased value in subsequent passages. In contrast, confluent cell densities remained constant in each subculture. Neither a decrease in confluent density or enzyme activity occurred upon passage of control fibroblasts. Loss of the activity, therefore, does not appear to be related to any change in cell viability, but may be associated with lyonization.

DISCUSSION

The data confirm the earlier report that steroid-sulfatase activity is lacking or at least markedly diminished in patients with sex-linked ichthyosis, and the values obtained for enzyme activity in normal fibroblasts are in good agreement with those reported by Shapiro et al. [5]. Furthermore, nine patients representing eight families were selected on the basis of having the skin disease rather than as members of a family with placental steroid-sulfatase deficiency. In addition, they represented all the patients we

TABLE 1

SULFATASE ACTIVITY OF CULTURED FIBROBLASTS

Patient	pm DHEAS/hr/mg protein
X1	0*
X2	0
X3	0
X4	0
X5A	0
X5B	0
X6	0
X7	0
X8	0
C1	1125
C2	1039
D1	834
X1E	0
CE	986

NOTE.—Sulfatase activity is expressed as the amount of DHEAS desulfated/hr/mg of tissue protein. X-1 through X-8 = fibroblasts from patients with sex-linked ichthyosis, C1 and C2 = fibroblast controls, and D1 = fibroblasts from the patient with ichthyosis vulgaris. X1E = epidermal cells from an affected patient, and CE = control epidermal cells. A and B refer to two brothers.

* "0" = below the lower limit of the assay which is 5–10 pm DHEAS/hr/mg protein.

had seen except two who could not be located. This indicates that the deficiency is not just present in a subgroup of patients with sex-linked ichthyosis, but rather is characteristic of the disease as a whole.

The demonstration that enzyme activity is present in normal cultured keratinocytes but deficient in cultured affected keratinocytes suggests that sex-linked ichthyosis could result from disturbed steroid metabolism in the epidermis rather than in other tissues. The altered levels of sulfated and nonsulfated steroids could influence the control of normal metabolic processes, and this could modify keratinization. Thyroid deficiency, for example, can lead to an ichthyotic-like state through altered lipid metabolism. It is even possible, however, that the enzyme acts on other sulfated substrates whose metabolism is essential for the normal function of the epidermis.

TABLE 2

SULFATASE ACTIVITY AND CONFLUENT DENSITIES OF CULTURED FIBROBLAST OF MOTHER PATIENTS X5A AND X5B.

Patient		pmDHEAS/hr/mg protein	Cell No. (X10-7)
2nd Passage:			
Heterozygote	5	660	1.4
Control	1	1020	1.4
8th Passage:			
Heterozygote	5	30	1.5
Control	1	1124	1.3

The deficiency of this enzyme in cultured fibroblasts and keratinocytes now makes it possible to perform prenatal diagnosis in families with sex-linked ichthyosis. One can now distinguish normal from affected males, and this information could be made available to individuals seeking genetic counseling.

Ichthyosis vulgaris, although similar in appearance to sex-linked ichthyosis, is clearly different based on its mode of inheritance, dominant, and certain clinical features. The demonstration that fibroblasts from ichthyosis vulgaris have sulfatase activity, now clearly separates the two diseases at the molecular level.

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