Acta Dermatovener (Stockholm) 60: 503–507, 1980

A CONGENITAL ICHTHYOSIFORM SYNDROME WITH DEAFNESS AND ELEVATED SERUM STEROID DISULPHATE LEVELS

Aarne Oikarinen[1], Marja-Liisa Kääriäinen[2] and Aimo Ruokonen[3]

[1]*Departments of Dermatology,* [2]*Paediatrics and* [3]*Clinical Chemistry, University of Oulu, Oulu, Finland*

Abstract. This is a report on a child with a syndrome characterized by an extensive congenital ichthyosiform eruption, neurosensory deafness and abnormally elevated serum steroid disulphates neonatally. When analysed by gas–liquid chromatography (glc) and gas chromatography–mass spectrometry (gc-ms) the following serum steroid disulphates were very high 5 days after birth: 5-androstene-3β,17α-diol (56 μg/ml), 5-androstene-3β,17β-diol (25 μg/ml) and 5-pregnene-3β,20α-diol (26 μg/ml). The values are about one hundred times higher than the reference values at this age. At the same time serum steroid monosulphate concentrations were normal. The patient had normal steroid sulphatase activity in skin biopsies, indicating that enzyme deficiency was not the reason for the high steroid disulphate concentrations. When serum steroid disulphatases were next analysed at 16 months of age they were normal. No hepatomegaly was observed but the other laboratory data support the hypothesis that the serum steroid disulphate concentrations were due to neonatal hepatopathy. Later, no indications of chronic liver disease were observed. These indications have not been described earlier in ichthyosiform erythrodermia and it is possible that the patient represents a new type of this rare disease.

Key words: Ichthyosiform erythrodermia; Congenital deafness; Hepatopathy; Steroid sulphates

Steroid sulphatase activity has been shown to be diminished in X-linked ichthyosis (8). To study the correlations between this enzyme defect and blood steroid sulphates and their possible role in ichthyosis we analysed serum concentrations of pregnenolone sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β-diol,17β-diol sulphate by radioimmunoassay in various types of ichthyosis including one newborn with ichthyosiform erythrodermia (6). Steroid sulphate concentrations were usually normal—except in this child. His serum 5-androstene-3β,17β-diol sulphate concentrations were about hundred times higher than the mean of the control group. To establish the reason for this abnormal finding, serum steroid sulphates and the hydrolysing enzyme in the skin—steroid sulphatase, were further studied. These results and the other clinical and laboratory data are presented in this case report of a child with a rare congenital ichthyosiform eruption and deafness.

MATERIALS AND METHODS

Samples

Skin biopsies were taken under local 1% lidocaine (w/v)–0.001% adrenalin anaesthesia from both affected and healthy skin of the upper and lower extremities. For histological analysis the samples were routinely stained with haematoxylin and eosin. Skin biopsies (10–20 mg) were stored at −20°C until steroid sulphatase activity was determined.

Blood samples were drawn from the antecubital vein into glass tubes. Biochemical analyses were normally carried out routinely in the hospital laboratory. For steroid sulphatase determination, serum was frozen at −20°C until analysed. For amino acid analysis, urine was collected over 24 hours.

Determination of serum steroid sulphates

One ml of serum was extracted in 10 ml acetone–ethanol (1 : 1). This procedure denatured the proteins. The sample was filtered and the precipitate was resuspended in 10 ml chloroform–methanol (1 : 1). After filtering, the combined filtrates were evaporated *in vacuo* and the residue was dissolved in 3 and 1 ml portions of chloroform–methanol (1 : 1) containing sodium chloride (0.01 mole/l) and applied to a column of 20 g Sephadex LH-20 in the same solvent. Unconjugated steroids were eluted in the fraction at 50–95 ml and steroid monosulphates at 150–250 ml of the effluent. The solvent was changed to methanol and a disulphate fraction was collected at 250–350 ml of the effluent. The mono- and disulphate fractions were evaporated to dryness and solvolysed as described elsewhere (10).

This procedure liberated the steroids from their sulphate groups. The liberated steroids were purified on 200 mg silicic acid columns using a solvent system of ethyl acetate–toluol (7 : 93). The sample was applied to the column in 1 and 0.5 ml portions of the solvent. The first 3 ml

---

Trivial and systematic nomenclature of the steroids: dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one; pregnenolone, 3β-hydroxy-5-pregnen-20-one; stigmasterol, (24S)-24-ethyl-5, 22-cholestadien-3β-ol.

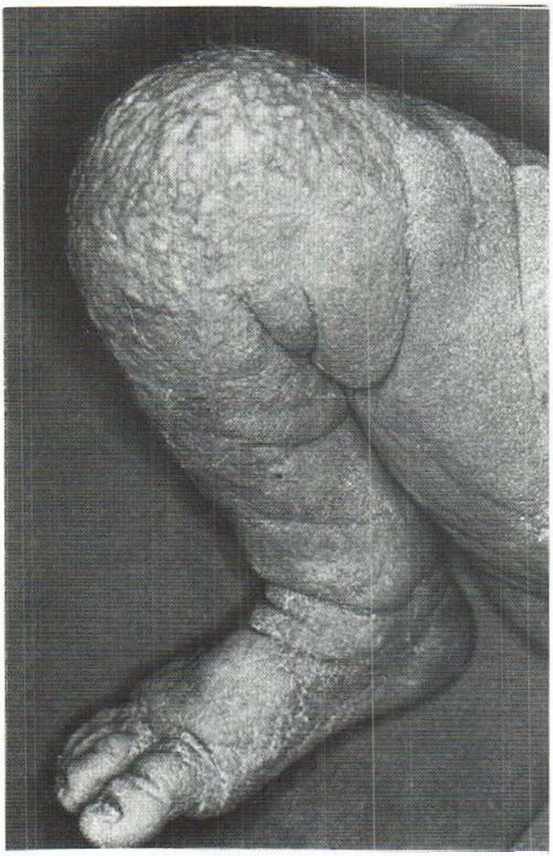

Fig. 1. Skin status at birth.

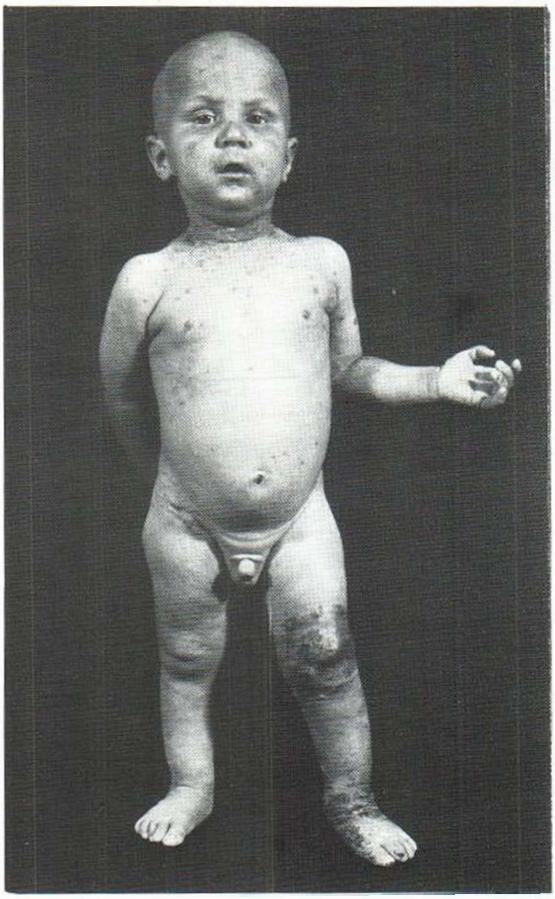

Fig. 2. Skin status at 16 months of age.

was discarded. The solvent was changed to ethyl acetate and steroids were eluted with 4 ml of the effluent. Gas-liquid chromatography (glc) was performed on 3% QF-1 and 2.2% SE-30 columns. Before analysis, the steroids were converted to trimethylsilyl (TMS) ether derivatives as described elsewhere (10). Stigmasterol was added as an internal standard before glc. Steroid quantities and peak areas in glc chromatograms were in linear correlation. Final identification of the steroid was carried out in a gas chromatograph (Carlo Erba 2 300)–mass spectrometer computer system (Jeol LMS D-100 Mass Data System) using the same steroid TMS derivatives.

Determination of steriod sulphatase activity in skin biopsies

Skin biopsies were minced and 6 mg of the sample was incubated in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 20 000 cpm [7α-^{3}H]-dehydroepiandrosterone sulphate (specific activity 1–5 Ci/mmol). Incubation was performed at 37°C for 4 h using air as the gas phase. After incubation the samples were extracted three times with 0.5 ml of ethyl ether. The ether phases containing the liberated dehydroepiandrosterone were combined, dried, and counted in a scinatillation counter (LKB-Wallac 81000).

CASE REPORT

This 16-month-old boy was the product of a normal gestation and delivery. Both parents and one brother and one sister are healthy. There are no special skin diseases in relatives. At birth the boy was noted to have erythematous skin all over his body (Fig. 1). Scaly skin also covered the scalp, palms and soles. Other conditions at birth were normal. At 5 days after delivery hyperbilirubinemia was noted (350 nmol/l) and the blood was changed. At 6 weeks of age SGOT was 135 and SGPT was 55 (normal ranges at this age, SGOT<70 lu/l and SGPT<50 lu/l). Later these values became normal. During the first months his skin was problematic. The scaly erythematous layer thickened and hardened and formed verrucoid-like lesions. In some places the skin was macerated and infected. Because of the infected skin his weight did no increase. In order to rule out malabsorption, a jejunum biopsy was done; it proved normal. The verrucoid-like skin lesions were treated with verrruxin and his skin status became gradually better, his weight increased and the overall status gradually improved. At 6 months of age the boy was noted

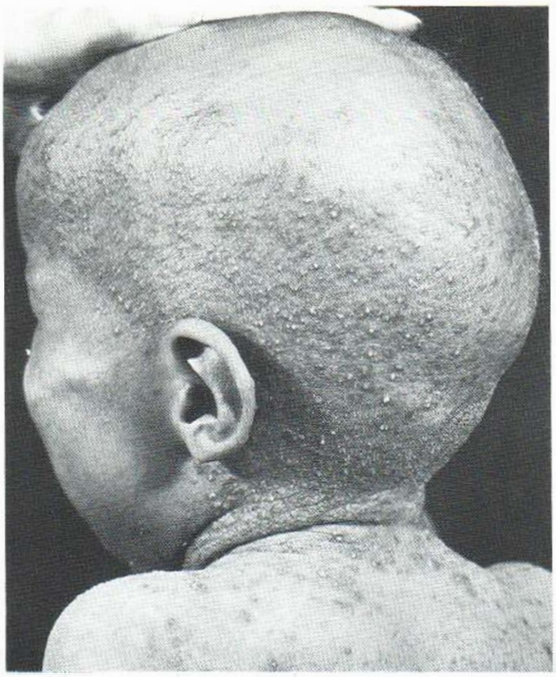

Fig. 3. Scalp alopecia at 16 months of age.

to be deaf and at 16 months of age he reacted in a bone conductive hearing test only to 250 Hz at 100 dB. Other psychomotor development progressed well; the boy walked at 14 months and is very lively. The mother has noticed that he is slightly intolerant of heat and gets fever in hot wether.

The skin status at 16 months of age is illustrated in Figs. 2, 3. Typical features are verrucoid granulomatous lesions in the ankle, knee, neck and head. Alopecia of the scalp and eyebrows and nail dystrophy were also noted. The rest of the skin is dry and slightly thickened.

Histopathological findings

Skin biopsy at the age of 2 weeks (Fig. 4): There was marked hyperkeratosis and slight parakeratosis. The stratum granulosum was normal or slightly thickened and there was vacuolization in the stratum granulosum. The epidermis was of normal thickness. Histological samples at the age of 16 months revealed mild hyperkeratosis and slight verrucosis in normal-looking skin (not shown). In verrucoid lesions there was marked hyperkeratosis, the granulosa layer was minimal and in the upper dermis there were inflammatory changes (not shown).

Urine analysis: Amino acids of urine were determined by amino acid analyzer and were found to be within normal limits.

Serum steroid sulphates

Fig. 5 shows glc analysis of steroid in the serum disulphate fractions of the ichthyosiform erythrodermia patient 5 days after birth and the corresponding chromatogram of a healthy control baby. In the patient sample the following disulphated compounds were identified and quantified by gc-ms: 5-androstene-3β,17α-diol (56 μg/ml), 5-androstene-3β,17β-diol (25 μg/ml), 5-pregnene-3β,20α-diol (26 μg/ml) Monosulphates were normal. At 16 months age the serum steroid disulphate concentrations were normal.

Table 1. *Steroid sulphatase activity in 6 mg skin biopsy samples from the patient with ichthyosiform erythrodermia, from a normal subject, and from a patient with X-linked ichthyosis*

Skin biopsies were incubated for 4 hours in Krebs-Ringer bicarbonate buffer using 20000 cpm [7α-^{3}H]-dehydroepiandrosterone sulphate as a substrate. The sulphatase activity is expressed in cpm values of the unconjugated dehydroepiandrosterone hydrolysed from its sulphate conjugate by the enzyme

Skin biopsy	Unconjugated dehydroepiandrosterone (cpm)
Ichthyosiform erythrodermia	1 896
Normal skin	1 850
X-linked ichthyosis	135
Reagent blank	172

Steroid sulphatase in skin biopsies

In Table 1, steroid sulphatase activities are given in 6 mg skin biopsy samples from the patient, from a normal control subject, and from a patient with X-linked ichthyosis. During 4 h incubation in Krebs-Ringer bicarbonate buffer with 20000 cpm of [7α-^{3}H]-dehydroepiandrosterone sulphate about the same amount of the substrate became hydrolysed in normal and erythrodermic skin biopsies. It is concluded that in the patient studied, steroid sulphatase activity is normal. A skin biopsy from a patient with X-linked ichthyosis was included as a negative control because it has been shown earlier that these patients have steroid sulphatase deficiency in their cultured skin fibroblasts (8). In this case too, when the sulphatase activity was determined in skin biopsies, no enzyme activity could be noted in X-linked ichthyosis.

DISCUSSION

Ichthyosis associated with deafness is a rare clinical condition. Up to now fewer than 20 cases of this kind of syndrome have been reported. Deafness has also been shown to be associated with Rud's and Refsum's syndromes, but in these, deafness is not a constant feature. In these latter syndromes there are also other neurological distrubances.

Rycroft et al. (7) have described two patients with ichthyosiform erythrodermia, deafness and vascularizing keratitis and normal intelligence. Baden

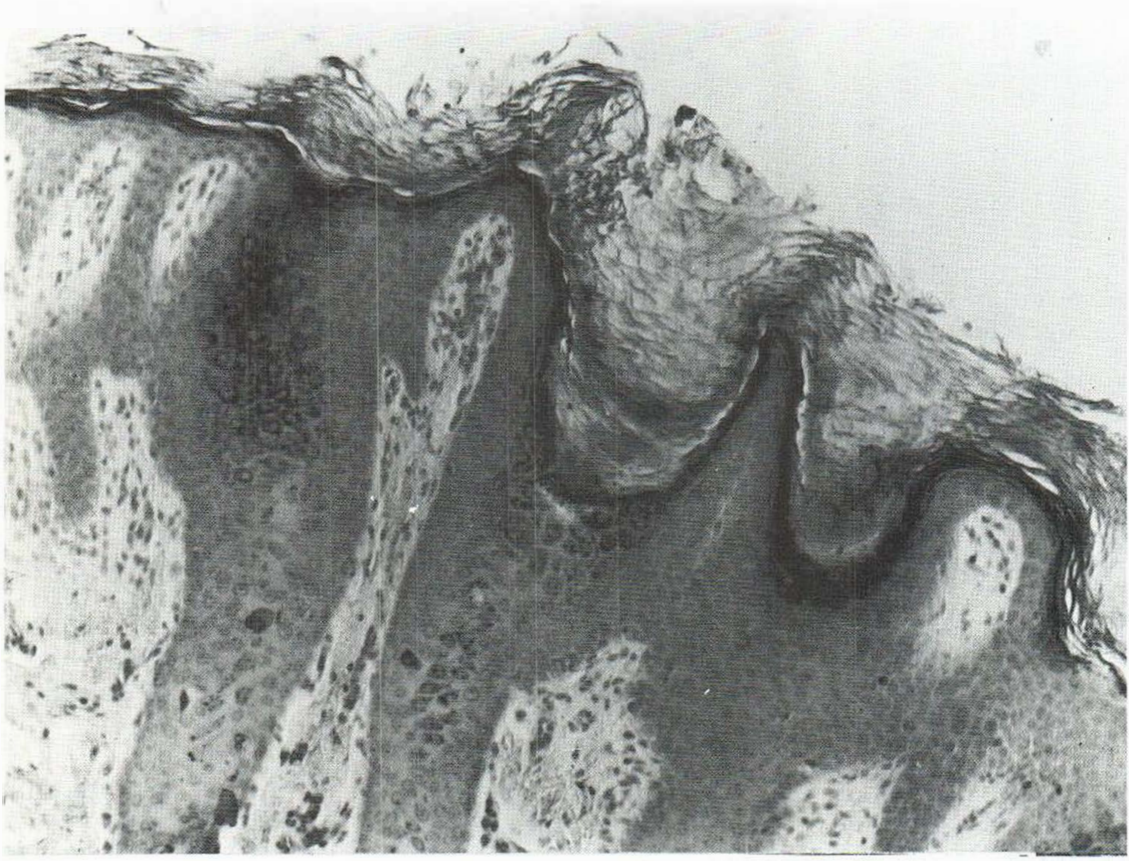

Fig. 4. Histological picture of thigh skin at the age of 2 weeks: ×160. Note hyperkeratosis, and vacuolization in the stratum granulosum.

et al. (1) have reported two patients with similar ichthyosiform erythrodermia, congenital deafness and vascularizing keratitis. The intelligence was also normal in these cases. Recently, Cram et al. (2) have reported two children with extensive ichthyosiform erythrodermia, deafness, hypotrichosis, and partial anhidrosis. The children were also of normal intelligence. Thus our case fits rather well with those features described by Cram. The skin was erythematous and ichthyotic. There was a grave disorder of keratinization: alopecia, nail dystrophy, and follicular hyperkeratosis. There was also congenital deafness and anamnestically-evident anhidrosis. The intelligence is normal in our case and the inheritance pattern is evidently autosomal recessive as in Cram's cases (2). In Cram's cases there was also a tight heel cord and vascularization in the eye. These features were not found in our case. However, it is possible that vascularization could develop later.

Alopecia and ichthyosiform erythrodermia can also be associated in the Netherton-syndrome (5), but our case showed normal amino acids in the urine and so does not fit with this syndrome.

In the preliminary study, disturbances in steroid sulphate metabolism were observed in this case. Because it has been shown that steroid sulphatase deficiency is present in X-linked ichthyosis we were interested to establish the reason for the high serum steroid sulphate concentrations in this infant.

Using radioimmunoassay it was earlier observed that the concentration of sulphated 5-androstene-3β,17β-diol was very high in the patient serum but the type of conjugation in the steroid nucleus was not known (6). This information is important in drawing conclusions as to the origin of the com-

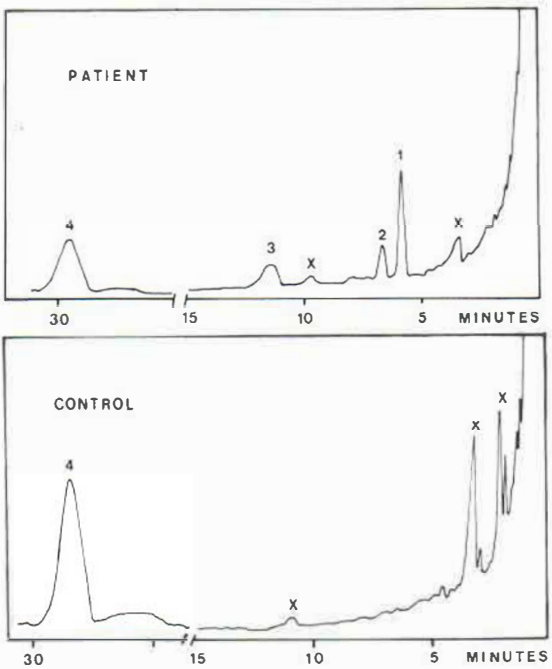

Fig. 5. Gas chromatographic analysis of the trimethylsilyl (TMS) derivatives of steroids in the serum disulphate fraction of the patient 5 days after birth, and in one control neonate 1=5-androstene-3β,17α-diol, 2=5-androstene-3β,17β-diol, 3=5-pregnene-3β,20α-diol, 4=stigmasterol (internal standard), ×=non-steroidal compound.

pound, since it is known that steroid monosulphates are mainly products of endocrine function in the adrenals and gonads, while steroid disulphates are mainly synthesized by the liver (4). It was shown in this study that 5-androstene-3β,17β-diol was conjugated at positions 3 and 17. In addition to this compound, the other steroid disulphates 5-androstene-3β, 17α-diol and 5-pregnene-3β,20α-diol, were present in very high concentrations in the same serum sample obtained 5 days after birth. In the skin biopsy, steroid sulphatase activity was normal and hence this could not be the reason for the high steroid disulphate concentrations. At 16 months of age the steroid disulphates were already within normal limits. It has been shown earlier that in hepatopathy, blood concentrations of steroid disulphates increase (9). In this case too, serum transaminase activities were higher than the upper limits of the normal ranges for some weeks after birth, and normalized later. Together, these findings suggest that in newborn life the infant had disturbances in liver function, though later no signs of a chronic liver disease were observed. This patient seems to have some similarity to the patients reported by Desmons et al. (3) although in our case no hepatomegaly was noticed. However, it seems more probable that in this patient liver affections were not so serious as in the same types of patient reported by Desmons et al. (3). Our case is therefore clinically closer to cases described by Cram et al. (2). The steroid disulphate concentrations reported here have not been published before in this rare disease and it is possible that the patient represents a new type of ichthyosiform erythrodermia.

REFERENCES

1. Baden, H. P. & Alper, J. C.: Ichthyosiforme dermatosis, keratitis and deafness. Arch Dermatol *113:* 1701, 1977.
2. Cram, D. L., Resneck, J. S. & Jackson, B. J.: A congenital ichthyosiforme syndrome with deafness and keratitis. Arch Dermatol *115:* 467, 1979.
3. Desmons, M. F., Bar, J. & Chevillard, Y.: Erythrodermie ichtyosiforme congénitale sèche, surdi-mutité, hépatomégalie, de transmission recessive autosomique. Bull Soc Fr Dermatol Syphiligr *78:* 585, 1971.
4. Laatikainen, T., Peltonen, J. & Nylander, P.: Determination of estriol, estriol sulfate, progesterone and neutral steroid mono- and disulfates in umbilical cord blood plasma. Steroids *21:* 347, 1973.
5. Michalowski, R., Urban, J. & Kucharska, D.: Netherton-Syndrome mit Alopezie und Prolinurie. Hautarzt *29:* 205, 1978.
6. Ruokonen, A., Oikarinen, A., Palatsi, R. & Huhtaniemi, I.: Serum steroid sulphates in ichthyosis. Accepted by Br J Dermatol.
7. Rycroft, R. J. G., Moynahan, E. J. & Wells, R. S.: Atypical ichthyosiform erythroderma, deafness and keratitis. Br J Dermatol *94:* 211, 1976.
8. Shapiro, L. J., Weiss, R., Webster, D. & France, D. J.: X-linked ichthyosis due to steroid-sulphatase deficiency. Lancet *i:* 70, 1978.
9. Sjövall, J. & Vihko, R.: Identification and quantitation of solvolysable steroids in human plasma by gas chromatography–mass spectrometry. Excerpta Medica International Congress Series *132:* 210, 1966.
10. Vihko, R.: Gas chromatographic–mass spectrometric studies on solvolyzable steroids in human peripheral plasma. Acta Endocrinol Suppl. *109:* 1, 1966.

Received February 11, 1980

Aarne Oikarinen, M.D.
Department of Dermatology
University of Oulu
SF-90220 Oulu 22
Finland