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STEROID SULPHATASE DEFICIENCY

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SUMMARY

Thirty-six pregnancies have been described in the literature with proven deficiency of steroid sulphatase activity in the placenta. The pregnancies were characterized by normal fetal growth and development despite urinary and plasma oestrogen levels usually associated with fetal death. Urinary oestriol levels rarely exceeded 5% of normal pregnancy values. In many of the pregnancies there was failure of normal parturitional mechanisms and delivery by Caesarean Section was common. The greatly reduced oestrogen production associated with the enzyme deficiency demonstrates (1) the major importance of sulphoconjugated steroids in oestrogen biosynthesis in the placenta and (2) the indispensable role of the sulphatase enzyme in this biosynthesis. Recent studies of steroid sulphatase activity in skin fibroblasts have revealed a generalized deficiency of the enzyme in individuals born of affected pregnancies. It has also been recently recognized that children born of affected pregnancies develop the skin condition, ichthyosis (of the X-linked type), in the first year of life. A causal relationship between steroid sulphatase deficiency and at least one form of X-linked ichthyosis is therefore likely. The progeny of all pregnancies with placental sulphatase deficiency, to date, have been male. Genetic considerations of the incidence of the disorder suggests an X-linked recessive inheritance.

INTRODUCTION

Since the initial discovery by Schachter and Marrian in 1938 [1] of oestrone sulphate in the urine of pregnant mares, numerous steroid sulphates have been isolated from various biological sources. Formation of the readily water soluble sulpho-conjugate was originally thought to be a detoxification mechanism, protecting the body from accumulating harmful levels of steroid hormones. It was believed that the steroid sulphates represented end-products of metabolism. While this role of sulpho-conjugation may be correct, the recognition of the existence of the sulphatase enzyme system and of secretion by the adrenal gland of certain sulphated steroids, particularly dehydroepiandrosterone sulphate (DHAS), suggested the possibility of other physiological functions.

The steroid sulphatase enzyme (sterol sulphate sulphohydrolase, EC 3.1.6.2) is located in the microsomal membrane of the cell and was first identified in ox and rat liver in 1956 [2, 3]. Subsequently, the enzyme was found to be widely distributed in mammalian tissues, including human. It has specificity for 3β-yl steroid sulphates and is capable of hydrolysing conjugates such as DHAS, pregnenolone sulphate, cholesterol sulphate, and oestrone sulphate, freeing the parent steroid for further metabolic transformation to more active hormones [4].

In man, DHAS secreted by the adrenal glands and cholesterol sulphate formed by the liver are present in blood in high concentrations. Current understanding suggests these two sulphated steroids function in the peripheral circulation as a precursor reservoir. Certain of the endocrine and other sulphatase-enriched tissues may utilize these substrates in hormone biosynthesis or other metabolism.

The importance of sulpho-conjugated steroids and the sulphatase enzyme in human metabolic processes is clearly demonstrated by the disorder of steroid sulphatase deficiency. This enzyme deficient state has only recently been recognized. It was first described in 1969 by France and Liggins[5] as a condition affecting placental oestrogen synthesis in pregnancy. A more recent finding by Shapiro *et al.*[6] has associated this inborn error of metabolism in a probably causal relationship with X-linked ichthyosis. The purpose of this paper is to review biochemical, clinical and genetic aspects of steroid sulphatase deficiency.

STEROID SULPHATASE DEFICIENCY IN PREGNANCY

Oestrogen biosynthesis and the sulphatase enzyme

The human feto-placental unit in its production of oestrogens represents most dramatically the utilization of steroid sulphates as intermediates in steroid hormone biosynthesis. In outline, it is believed that oestrogen synthesis in human pregnancy involves the following mechanisms [7, 8].

The fetal adrenal produces DHAS by *de novo* synthesis from acetate and to a minor degree from maternal cholesterol. The DHAS passes to the placenta where it is first deconjugated by the steroid sulphatase enzyme system and then converted to oestrone and oestradiol through a sequence of enzyme reactions (Fig. 1). Some of the oestrone and oestradiol is then shunted back to the fetus, where a part is converted to oestriol. The more important pathway to oestriol production, however, involves the precursor, 16α-hydroxydehydroepiandrosterone sulphate (16OH-DHAS). The fetal adrenal produces 16OH–DHAS but

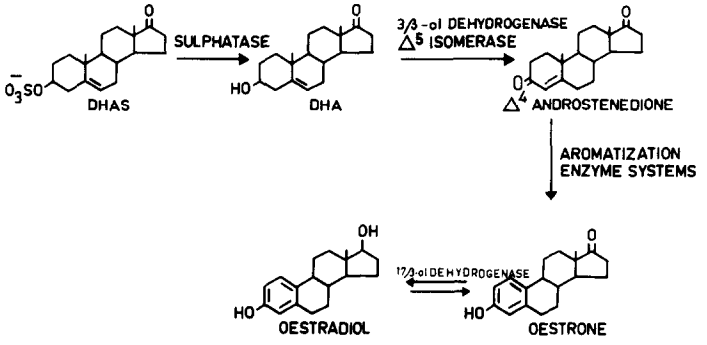

Fig. 1. Biosynthetic pathway of the conversion of DHAS to oestrogen in the human placenta.

the larger amount arises from 16α-hydroxylation of DHAS in the fetal liver. In the placenta, the 16OH-DHAS is converted to oestriol in a manner similar to DHAS conversion to oestradiol.

The DHAS secreted by the maternal adrenal can also be utilized by the placenta. Maternal DHAS is the principal precursor for oestrogen biosynthesis in early pregnancy but by mid-gestation precursors of fetal origin become of greater importance. In the last trimester of pregnancy, about 50% of the oestrone and oestradiol is produced from maternal DHAS but only 10% of the oestriol is derived from this source [9].

The production rate of oestriol increases almost 1000-fold during the course of gestation, making it quantitatively the most important oestrogen in the pregnant woman. Oestrone and oestradiol are also produced at rates several hundred fold greater than in the non-pregnant state. In late pregnancy, an average of 30 mg oestriol, 1.5 mg of oestrone and 0.7 mg of oestradiol is excreted in the urine per day [10].

Evidence of the major importance of sulpho-conjugated steroids as precursors in placental oestrogen biosynthesis and the associated indispensable role of the steroid sulphatase enzyme in this synthesis is provided by pregnancies with sulphatase deficiency, for in affected pregnancies, oestrogen production is only about 5% or less of normal pregnancy levels.

Clinical features of pregnancies with placental sulphatase deficiency

A steroid sulphatase deficient state may first be recognized in an individual before birth as the condition of pregnancy commonly termed placental sulphatase deficiency. Thirty-six such pregnancies, in which confirmative evidence has been established for the disorder, have been described in the literature [5, 11–24]. In addition, we have observed placental steroid sulphatase deficiency in four further pregnancies (France J., unpublished observations).

The disorder is distinguished clinically by a marked reduction in blood and urinary levels of oestrogens in the presence of normal fetal growth and development. Urinary oestriol excretion rarely exceeds 3 mg/24 h (10.4 μmol/24 h) at any period of gestation. Plasma concentrations of progesterone and urinary levels of pregnanediol are unaffected and are within the normal ranges [13, 21]. Plasma levels of placental lactogen and chorionic gonadotrophin are normal [16, 17].

Apart from the low oestrogen production, no clinical manifestations directly attributable to the enzyme deficiency have been observed up to term. However, prolongation of pregnancy beyond 40 weeks gestation can occur [12, 15, 16]. In a small percentage of affected pregnancies, spontaneous labour with normal vaginal delivery may occur [17, 22–24] but in the large majority of pregnancies, induction of labour is required and recourse to delivery by Caesarean Section may be necessary [5, 12–16, 18–24]. Several primigravid pregnancies, terminating in Caesarean Section, have been associated with an unripened, tightly closed cervix which failed to efface or dilate despite a normal uterine response to intravenous oxytocin [13, 14, 22].

Male babies of normal birthweight with a placenta of normal size and weight have been delivered in all reported pregnancies with placental sulphatase deficiency. Histological studies and electron microscopy of placental tissue have revealed no special features [17, 21]. No abnormalities attributable to the enzyme deficiency are evident in the baby at birth. Cord blood concentrations of the oestrogen precursors, DHAS and 16OH-DHAS, are in the range for normal pregnancy [13, 15].

Several tests have been employed as an aid to the antepartum diagnosis of steroid sulphatase deficiency. The failure of oestrogen levels to rise in response to a DHAS load administered into the maternal or fetal compartment has been an indicator used by a number of investigators [5, 11, 13, 15, 16, 18, 21, 22, 24]. The significance of the absence of response to DHAS can be enhanced by demonstrating the presence of a response in oestrogen levels to a DHA load [5, 13, 18, 21, 24]. Measurement of the levels of DHAS present in amniotic fluid of affected pregnancies [16, 17], has been also proposed as an antepartum test [17].

IN VITRO STUDIES OF PLACENTAL STEROID ENZYME ACTIVITIES

Steroid sulphatase

Conclusive proof that a particular pregnancy was associated with steroid sulphatase deficiency rests on *in vitro* metabolic studies demonstrating absence of activity of the enzyme in the placenta.

Incubation methods, employing either isotopically labelled DHAS or oestrone sulphate as substrate, for whole tissue homogenates [5, 13, 15, 18, 22–24], tissue slices [12], 10,000 *g* supernatants [14, 16, 17, 20], or microsomal preparations [13] have been used by various investigators to determine sulphatase activity in placentas suspected of having the enzyme deficiency. Placental perfusion techniques have also been employed [11, 18]. All procedures have demonstrated negligible sulphatase activity in affected tissue compared with high levels of activity in normal placental tissue. Pregnenolone sulphate also has been shown to be unmetabolized by sulphatase deficient placentas [13, 14, 18, 20].

Other enzymes involved in steroid metabolism

The conversion of unconjugated DHA (or 16OH-DHA) to oestrogen involves the enzyme systems, 3β-hydroxysteroid dehydrogenase, Δ^{4-5} isomerase, 17β-hydroxysteroid dehydrogenase, and the aromatase complex (Fig. 1). These enzymes are functional in placental tissue deficient in sulphatase activity [5], though controversy exists as to whether or not they show some impairment of activity [14, 16]. By far the bulk of evidence, however, suggests that the metabolic transformations of unconjugated oestrogen intermediates occur in the sulphatase deficient placenta at rates similar to those found in placentas of normal pregnancies [11, 13, 15, 18, 22, 24].

Placental progesterone production, judged by plasma progesterone [19, 21] and urinary pregnanediol levels [5, 12, 13, 21], appears quantitatively to be uninfluenced by absence of sulphatase action, indicating normal activity of the enzymes involved.

Arylsulphatases

The arylsulphatases are a group of enzymes which catalyse the hydrolysis of a variety of aromatic sulphate esters. In human tissue, two soluble arylsulphatases, arylsulphatase A and arulsulphatase B, occur in the lysosomes of the cell, while a third, arylsulphatase C, exists as an insoluble microsomal enzyme.

Recent work by Shapiro *et al.*[20] with specific assay techniques, showed that in a placenta lacking steroid sulphatase the tissue also was deficient in arylsulphatase C but exhibited normal activities for the soluble lyososomal enzymes arylsulphatases A and B. Studies in our own laboratory have confirmed Shapiro's findings (France J. T., unpublished observations). The occurrence of normal levels of arylsulphatases A and B in serum and leucocytes of children born of pregnancies with placental sulphatase deficiency [25] is consistent with these observations.

STEROID SULPHATASE DEFICIENCY—A GENERALIZED ENZYME DEFECT

In 1977, Shapiro *et al.*[20] demonstrated for the first time that steroid sulphatase deficiency involved other somatic tissues besides the placenta. Using cultured skin fibroblasts from an infant born of an affected pregnancy, they measured steroid sulphatase activity, to the substrate DHAS, and found undetectable levels (< 10 pmol hydrolysed/h/mg protein). In comparison, levels of the enzyme in normal skin fibroblasts ranged from 636 to 2566 activity units. The work of Shapiro *et al.*[20] has been confirmed in a later study also demonstrating negligible levels of skin fibroblast sulphatase in several more individuals born of pregnancies with proven placental sulphatase deficiency [6] (see Fig. 2). Thus, the important question which remained unresolved from the time of the first reported pregnancy with the enzyme disorder has been answered. Individuals born of such affected pregnancies do have a generalized deficiency in steroid sulphatase and the enzyme defect persists throughout life.

STEROID SULPHATASE DEFICIENCY AND X-LINKED ICHTHYOSIS

A new clinical manifestation of steroid sulphatase deficiency has been recognized recently. A collabora-

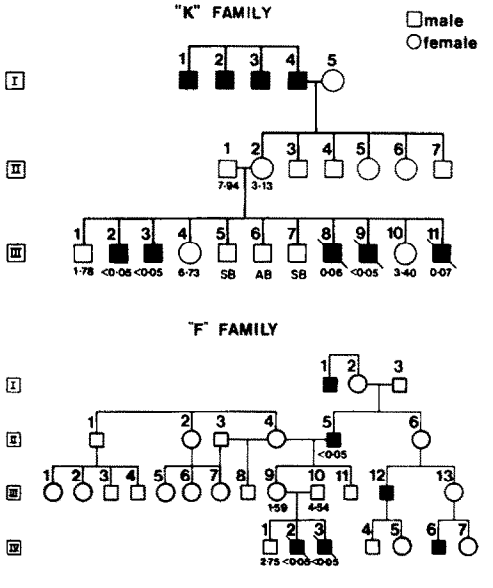

Fig. 2. Pedigrees of two families with histories of ichthyosis and pregnancies with placental sulphatase deficiency. Individuals with clinical ichthyosis are indicated by filled-in symbols. A slash denotes a case in which the placenta was steroid-sulphatase deficient. Levels of cholesterol-sulphatase activity in skin fibroblasts for each individual tested are shown below the symbol. Normal levels of steroid sulphatase activity in this assay, 4.64 ± 1.99 pmol desulphated/mg protein/h (n = 11). (From Shapiro *et al. Lancet* i (1976) 70–72. Reproduced by courtesy of the Editor of *Lancet*.)

tive study by Shapiro *et al.*[6] of two families with multiple occurrences of pregnancies with placental sulphatase deficiency revealed an association of the enzyme defect with the skin disorder X-linked ichthyosis. Jobsis *et al.*[26] have also reported, in abstract form, an observation of the same relationship.

In our collaborative study [6], the steroid sulphatase activity was measured in cultured skin fibroblasts from individuals born of pregnancies with the enzyme deficiency as well as from all other available members of their families. Family histories and dermatological examination revealed an incidence of ichthyosis segregated as an X-linked trait in each of the two families investigated. All offspring of affected pregnancies had both absence of steroid sulphatase activity in their fibroblasts and clinically apparent ichthyosis. The fibroblasts of all individuals with ichthyosis were deficient in steroid sulphatase activity. The birth of several of the subjects with ichthyosis predated the first report of placental sulphatase deficiency. The results of steroid sulphatase determinations with cholesterol sulphate as substrate are summarized in Fig. 2. Similar findings were obtained with DHAS as substrate.

The skin of individuals with steroid sulphatase deficiency appears normal at birth. However, in the first year of life and usually by 3 months of age, ichthyosis becomes recognizable and thereafter persists throughout life. The greatest involvement is most often on the trunk and extensor surfaces of the extremities.

It is highly probable that a causal relationship exists between steroid sulphatase deficiency and at least one form of X-linked ichthyosis. The biochemical basis of this relationship is, however, not clear. It may be of relevance that ichthyosis can be a side-effect of treatment with drugs that inhibit cholesterol biosynthesis [27, 28]. It could be possible, therefore, that cholesterol sulphate is an important source of cholesterol for utilization in normal skin metabolic processes.

GENETIC ASPECTS

Male infants were born in all 36 pregnancies with proven steroid sulphatase deficiency described in the literature and in our four additional confirmed but unpublished cases. It is almost certain, therefore, that steroid sulphatase deficiency is a sex-linked inherited trait.

Although autosomal dominant transmission with sex limited expression cannot be wholly excluded, reference to the two family pedigrees in Fig. 2 suggests that genetic transmission of the enzyme defect is X-linked recessive. This form of inheritance is reinforced by the subsequent history of the family who provided the pregnancy with the sulphatase disorder reported by us in 1973 [13]; the next pregnancy was associated with a normal production of oestriol, indicating normal sulphatase activity although the infant again was a male. It is of further significance that this family also had a history of X-linked ichthyosis. The child born of the pregnancy with sulphatase deficiency subsequently developed ichthyosis. His brother, born of the unaffected pregnancy, has a normal skin.

If a causal relationship can be conclusively established between steroid sulphatase deficiency and X-linked ichthyosis, the genetics of the enzyme disorder will be proven unequivocally to be X-linked recessive. Assuming that this relationship is so, the incidence of individuals and pregnancies with the enzyme disorder should be the same as that of X-linked ichthyosis, namely 1 in 13,000 [29].

CONCLUSION

Steroid sulphatase deficiency is a newly recognized inborn error of human metabolism. Originally identified as an enzyme disorder of the placenta, it is now known that the defect generally extends to at least one other somatic tissue. It is probable that inheritance of the enzyme defect is as an X-linked recessive. Approximately 1 in 6000 males may be deficient in steroid sulphatase, making this genetically determined enzyme disorder one of the commonest affecting steroid metabolism.

The disorder involves both the sulphatase enzyme responsible for hydrolysis of 3β-yl-steroid sulphates and the probably different enzyme, arylsulphatase C. These two enzymes are microsomal in origin. The lysosomal arylsulphatases A and B are unaffected. The disorder can thus be discerned as a distinct entity, different from the rare and lethal autosomal recessive, multiple sulphatase-deficient state affecting all the sulphatase enzymes [30] and different from the autosomal recessive, arylsulphatase A deficient state associated with metachromatic leukodystrophy [31, 32].

In fetal life, growth and development occur normally in an affected individual but the enzyme deficiency may indirectly pose a possible lethal threat through failure of parturitional mechanisms. Safely delivered, the child with steroid sulphatase deficiency develops ichthyosis of the X-linked type within the first year of life. Growth and physical and mental development otherwise appear to progress normally.

Pregnancies associated with steroid sulphatase deficiency are characterized by markedly depressed oestrogen production. Thus, the importance of sulphoconjugated precursors and of the placental steroid sulphatase enzyme in oestrogen biosynthesis in human pregnancy is clearly defined.

The newly recognized relationship of steroid sulphatase deficiency with X-linked ichthyosis suggests a major role of steroid sulphates and the sulphatase enzyme in normal skin metabolism.

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