ether. The GLC analysis of the ether extract showed a strong peak for cholesterol, but no peak corresponding to the C_{26} sterol could be detected. However, the compound could be clearly detected by GC/MS analysis (*9*) of the sample by means of selected ion detection technique and by monitoring the peaks at *m/e* 352 and 313 (Fig. 2). The estimated amount was about 40 ng/ml.

The C_{26} sterol (compound **2**) was first isolated by Idler *et al.* (*10*) from the scallop *Placopecten magellanicus*, and since then several 24-norcholesterol derivatives were identified in a number of marine sources, such as asteroid ($\Delta^{7,22}$) (*11*), clam ($\Delta^{5,22}$) (*12*), jellyfish (Δ^{22} and $\Delta^{5,22}$) (*13*), *Tunicier halocynthia roretzi* (Δ^{22}, $\Delta^{5,22}$, and $\Delta^{7,22}$) (*14*), and red algae (Δ^{22}, $\Delta^{5,22}$) (*15*). Two 27-norsterols, 22-*trans*-27-nor-24-methylcholesta-5,22-dien-3β-ol and its Δ^7-isomer, were isolated from the asteroid *Asterias amurensis* (*16*). But those are all from marine sources and are minor components of the sterol fraction.

This appears to be the first case of detection of a C_{26} sterol in mammalia. At present, we have no information on the source or the biosynthesis of this sterol. In view of the characteristic symptoms, the C_{26} sterol (produced by an abnormal metabolic pathway) may not be a suitable substrate of cholesterol $C_{20,22}$ lyase (cytochrome P 450), which is a key enzyme for steroid hormone biosynthesis. Alternatively, the function of cholesterol $C_{20,22}$ lyase might be disrupted for some reason, and the degradation of cholesterol side chain may be directed instead toward production of the C_{26} sterol.

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5. Retention times of GLC with the use of 1.5 percent OV-17 on Shimalite W (80 to 100 mesh), a column 150 cm by 4 mm (inside diameter), at 255°C, were as follows: 22-*trans*-27-norcholesta-5,22-dien-3β-ol (**1**), 6.5 minutes; 22-*cis*-27-norcholesta-5,22-dien-3β-ol, 6.4 minutes; 24-norcholesta-5,22-dien-3β-ol (**2**), 5.3 minutes, 24-norcholesta-3,23-dien-3β-ol (**3**), 7.6 minutes. GLC analysis of urinary steroids of a normal girl shows a similar profile as reported by Horning, *et al.* [E. C. Horning and M. G. Horning, *J. Chromatogr. Sci.* **9**, 129 (1971); A. L. German and E. C. Horning, *ibid.* **11**, 76 (1973)], indicating many small peaks of steroids in the region of retention time of 15 to 40 minutes on the analytical conditions of Fig. 1. The urine from the lipoid hyperplasia which is one of the congenital adrenal hyperplasia showed a major peak of cholesterol without C_{26} sterol in the GLC analysis.
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7. Mass spectrum of compound **2** showed *m/e* 442 (13 percent), 427(4), 352(23), 337(9), 313(22), 255(25), 129(45), and 97(100); that of compound **3** showed *m/e* 442 (14 percent), 427(12), 352(24), 343(48), 337(15), 313(34), 283(100), 253(20), and 129(68).
8. *Cis*- and *trans*-27-norcholesta-5,22-dien-3β-ols were separated by a glass capillary column with Poly-I 101, 30 m by 0.2 mm (inside diameter) at 245°C. The retention time for the *trans* isomer was 10.7 minutes, and for the *cis* isomer it was 10.3 minutes.
9. A Shimadzu-LKB-9000 GC-MS instrument with MID-PM was used for multi-ion monitoring; column, 1.5 percent OV-17 on Shimalite W(80–100 mesh), 150 cm by 4 mm (inside diameter) at 250°C.
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Non-Inactivation of an X-Chromosome Locus in Man

Abstract. *Cloned fibroblasts from women heterozygous for X-linked ichthyosis (steroid sulfatase deficiency) were examined to see whether or not this locus is subject to X-inactivation. Of 103 clones examined, all had normal levels of steroid sulfatase activity. Two of the women studied were also heterozygous for glucose-6-phosphate dehydrogenase deficiency. This allowed the demonstration that both X chromosomes were represented as the active X in various clones and that selection did not account for these findings. Thus, the steroid sulfatase locus, like the* Xg^a *locus to which it is linked, appears to escape X-inactivation in man.*

One of the two X chromosomes in normal female somatic cells is functionally inactivated at an early stage of embryogenesis in all mammals (*1*). This inactivation process teleologically serves the function of achieving "dosage compensation" for X-chromosome loci between males and females (*2*). In eutherian mammals, either the maternally or paternally derived X chromosome is inactivated in a random fashion and the pattern of X-inactivation, once established, is genetically fixed for any given cell and its progeny. Much support for this theory, most clearly espoused by Lyon (*3*), has been obtained in many species, including man. Davidson *et al.* (*4*) used cloned fibroblasts from females, heterozygous for an X-linked biochemical marker [an electrophoretic variant of glucose-6-phosphate dehydrogenase (G6PD : E.C. 1.1.1.49)], to demonstrate

Table 1. Steroid sulfatase activity in cultured fibroblasts from normal individuals, patients with X-linked ichthyosis (including the sons of four obligate heterozygotes A, B, C, and D), heterozygotes for X-linked ichthyosis, and clones derived from these heterozygotes.

Fibroblasts	*N*	Cholesterol sulfatase (pmole/mg protein-hour)	
		Mean	Range
Control lines	18	4.38	1.10 to 9.20
Patients with X-linked ichthyosis	30	< 0.15	0 to 0.15
Heterozygote A		3.13	
Heterozygote B		1.59	
Heterozygote C		6.31	
Heterozygote D		3.47	
Heterozygote A clones	13	2.79	1.15 to 5.26
Heterozygote B clones	19	2.71	1.42 to 5.13
Heterozygote C clones	39	4.77	0.96 to 10.45
Heterozygote D clones	32	3.85	1.80 to 6.33
Son of heterozygote A (K III-8)		< 0.10	
Son of heterozygote B (F IV-2)		< 0.10	
Son of heterozygote C (C III-8)		< 0.10	
Son of heterozygote D (D III-3)		< 0.10	

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that two distinct types of clones could be derived in which loci of either the paternal or maternal X chromosome were expressed. Similar studies with hypoxanthine(guanine)phosphoribosyltransferase (*5, 6*), phosphoglycerate kinase (*7, 8*), iduronate sulfatase (*9*), α-galactosidase (*10*), phosphorylase B kinase (*11*), and dihydrotestosterone receptor protein (*12*) have been conducted and similar bimodality of enzyme expression in different clones demonstrated. Clinical and histochemical evidence for cellular mosaicism in females heterozygous for a number of other X-linked mutant alleles has also been presented (*13*). Cytologic expression of X-chromosome inactivation, coupled with biochemical studies of females heterozygous at two X-chromosome loci suggest that X-inactivation (Lyonization) encompasses a single and entire X chromosome in each normal female somatic cell (*7, 14*).

The concept of complete inactivation of all loci on a given X chromosome has, however, been challenged. A number of clinical observations suggest that the Xg^a blood group locus, which specifies an X-linked red blood cell surface antigen, might not demonstrate Lyonization (*15*). However, since Xg^a is only detectable on the surface of red blood cells, a somatic cell genetic approach to prove non-Lyonization has not been possible. The numerous clinical abnormalities and high intrauterine lethality associated with X-chromosome aneuploid states in man may reflect the requirement at some stage of female embryonic development for the expression of both sets of some X-linked alleles (*16*).

Studies in our laboratory have shown that deficiency of microsomal steroid sulfatase activity is the enzymatic basis of a relatively common genetic dermatologic condition, X-linked ichthyosis (*17*). Previous family studies demonstrated that the gene specifying this disorder is located approximately 10 centimorgans from the Xg^a locus (*18*). With the establishment of a biochemical marker for this condition (steroid sulfatase deficiency) which is detectable in cultured skin fibroblasts, it has become possible to ask whether the steroid sulfatase-ichthyosis locus demonstrates the X-inactivation phenomenon. To investigate this question, we cloned cultured fibroblasts from four female obligate heterozygotes for steroid sulfatase deficiency and examined the cells for steroid sulfatase activity by means of an assay described previously (*17*) (Table 1). Heterozygotes A and B are obligate carriers of the steroid sulfatase deficiency gene and were designated K family II-2 and F family III-10,

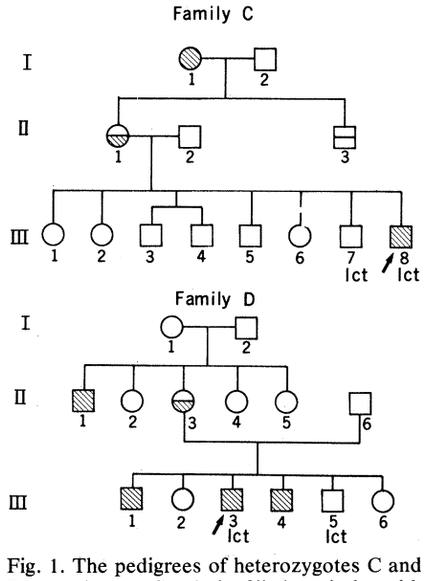

Fig. 1. The pedigrees of heterozygotes C and D are shown. Symbols filled entirely with cross-hatching indicate homozygosity or hemizygosity for G6PD deficiency, while half-shaded symbols denote females heterozygous for G6PD deficiency. The abbreviation *Ict* indicates individuals affected with X-linked ichthyosis. A single horizontal line through a symbol indicates an individual that was unavailable for examination. Arrows indicate the probands. The doubly heterozygous women included in the present study are individuals II-1 in the C family (heterozygote C) and II-3 in the D family (heterozygote D).

respectively, in our earlier reports (*17*). Heterozygotes C and D were ascertained by means of family studies of X-linked ichthyosis in Sardinia. Each of these women has two sons with proven X-linked ichthyosis. Their positions in their respective pedigrees are C II-1 and D II-3 (Fig. 1).

Fibroblast cultures were established from skin biopsies and grown in Eagle's minimal essential medium supplemented with 30 percent fetal calf serum. At very early passage, cells were trypsinized and plated at a dilution which gave only one to three colonies per 60-mm dish. These colonies were isolated with cloning cylinders and grown to a density suitable for assay of steroid sulfatase, G6PD, lactate dehydrogenase (LDH), and protein (*19*). If the steroid sulfatase locus undergoes X-inactivation like other X-chromosome loci, we would have expected to observe two populations of clones with respect to steroid sulfatase activity. One group of clones would have had normal steroid sulfatase activity and the other group of clones would have had absent sulfatase activity comparable to probands with X-linked ichthyosis (for example, their sons). As is indicated in Table 1, 103 clones from these four women were examined and none were deficient in steroid sulfatase activity.

Although these findings might be interpreted as strong evidence for non-inactivation of this locus, there are at least two possible alternative explanations. The first is that the steroid sulfatase locus might not, in fact, be X-linked and so would not be expected to Lyonize. The preponderance of family evidence presented by us and others and the linkage data with Xg^a would make this very

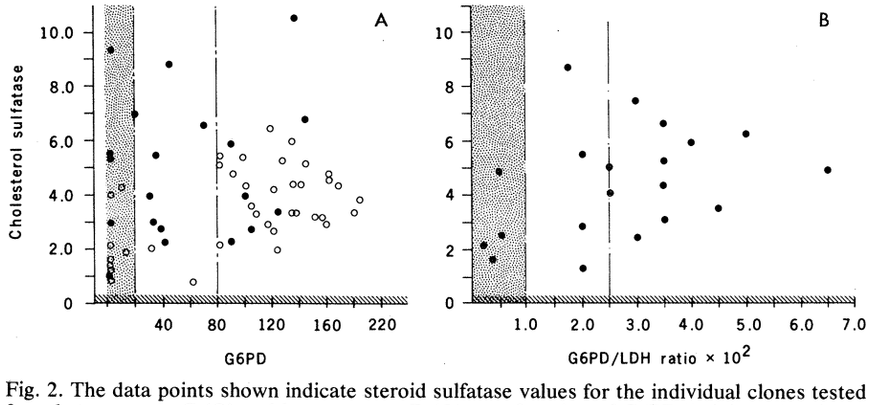

Fig. 2. The data points shown indicate steroid sulfatase values for the individual clones tested from heterozygote C (●) and heterozygote D (○) as a function of G6PD activity. (A) Clones were grown until they filled a single 75-cm² flask, and the cells were harvested for estimation of cholesterol sulfatase (picomoles per milligram of protein per hour) and G6PD (micromoles per milligram of protein per minute) activities. The stippled area demonstrates G6PD-deficient levels and the cross-hatched area denotes cholesterol sulfatase-deficient values. Multiple studies of three G6PD-deficient males consistently gave G6PD levels below 20 units. In contrast, values in normal controls were always greater than 80 units. Studies of more than 30 patients with X-linked ichthyosis have shown that all have cholesterol sulfatase activities of less than 0.15 pmole per milligram per hour (*17*). A second cloning experiment gave rise to 20 independent clones from heterozygote C (B). These were grown to confluence in 15-mm microtiter wells and assayed for G6PD, LDH, and cholesterol sulfatase activities. This procedure ensured that we studied vigorously growing cells early in their replicative life-spans. The G6PD activity in this instance was expressed as a ratio of G6PD to LDH, another cytoplasmic enzyme. Once again, the G6PD-deficient and steroid sulfatase-deficient boundaries were set by studying suitable control cultures and are indicated by the stippled and cross-hatched zones, respectively.

unlikely. Furthermore, we have demonstrated X-linkage of human steroid sulfatase by human-rodent somatic cell hybridization techniques (*20*). The second alternative is that there has been some strong selective pressure operative either in vivo or in vitro which favors the growth of cells with normal steroid sulfatase activity and inhibits the growth of sulfatase-deficient cells. In appropriate growth and mixing experiments, we have not observed such selection against steroid sulfatase-deficient cells in vitro. To rigorously exclude this possibility however, we have made use of the fact that subjects C and D are also heterozygous for G6PD deficiency of the Mediterranean type (*21*). As indicated in Fig. 2, when clones from these doubly heterozygous subjects were examined for G6PD activity as well as steroid sulfatase activity, clones with normal and deficient G6PD levels could be identified, but all clones had normal levels of steroid sulfatase activity. Thus, regardless of whether the X chromosome bearing the normal or the mutant G6PD allele was inactivated, steroid sulfatase was always expressed.

The data presented here provide evidence in a somatic cell system for non-Lyonization of an X-chromosome locus. It is of particular interest that this locus, which affects steroid sulfatase expression, is in relatively close proximity to another non-Lyonized locus, Xg^a. It should now be possible to utilize X-autosome translocations in somatic cell hybridization studies to localize cytologically the noninactivated region of the X chromosome which contains these two loci.

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Separation of D and L Amino Acids by Liquid Chromatography: Use of Chiral Eluants

Abstract. *An aqueous eluant containing a chiral copper-proline complex effects the separation of underivatized amino acid enantiomers on an ion-exchange column. The stereoselectivity is ascribed to differences in stability of the diastereomeric amino acid–copper complexes formed in solution. A simple change in the chirality of the eluant reverses the order of the enantiomer elution. For detection and quantification of picomole amounts of amino acids, the eluant is monitored for fluorescence after reaction with* o*-phthalaldehyde, a reagent insensitive to proline but highly sensitive for amino acids containing a primary amino group.*

Chromatographic resolution of optical isomers (*1*) requires the introduction of an asymmetric environment either *intra*molecularly, by conversion to diastereomers, or *inter*molecularly, by the use of chiral stationary or mobile phases. In gas chromatography, excellent resolution of derivatized amino acids has been achieved with diastereomers (*2*), as well as with chiral stationary phases (*3*). Similarly in liquid chromatography, both these approaches have led to good separations of, for example, diastereomeric dipeptides (*4*) and helicenes on optically active supports (*5*).

In contrast, the effect of chiral *eluants* has not been extensively investigated (*6*). We now report a simple procedure for the separation of a number of α-amino acid enantiomers without the need for prior derivatization.

The method is based on the addition of a metal cation–amino acid complex to the eluant of a cation-exchange column. In the specific application reported, Cu^{2+}-proline complexes are dissolved (the molar ratio of Cu^{2+} to proline being 1/2) in a sodium acetate buffer. After the column is equilibrated (*7*), an amino acid sample is injected and is resolved into its enantiomers (Fig. 1). The chromatographic system in which the experiments were carried out had been developed (*8*) for the rapid, highly sensitive ion-exchange analysis of amino acids with the use of 5-μm bead resins. Separation was monitored by fluorometry (*9*) after the postcolumn reaction of the eluant with *o*-phthalaldehyde (*10*). To prevent precipitation of copper compounds by the *o*-phthalaldehyde solution, EDTA was added to the reagent (2.5 g/liter). *o*-Phthalaldehyde does interact with primary but not with secondary amines, so that proline does not interfere (nor does Cu^{2+}). Because of these circumstances the resolutions reported could be observed. The method is sensitive to picomole amounts.

Cysteic acid retention time was taken to represent the void volume of the column and the detection system (Table 1). The order of elution of the enantiomers (Fig. 1a and Table 1) with the chiral eluant is the reverse of that found by Rogozhin *et al.* (*11*) and Lefebvre *et al.* (*12*), who bonded a chiral proline-copper complex to the stationary support. This

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