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# EXCRETION AND ANTICONVULSANT ACTIVITY OF STEROID HORMONES IN AN INFANT WITH INFANTILE SPASM AND HYPSARRHYTMIA TREATED WITH EXCESSIVE DOSES OF ACTH

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## SUMMARY

The excretion of steroids in urine and faeces was studied in an infant with infantile spasm with hypsarrhytmia. The analyses, which were carried out by gas-liquid chromatography and gas chromatography-mass spectrometry, were done before and after the infant was treated with excessive doses of ACTH. Prior to treatment the infant excreted less than 0·1 mg/24 h of individual steroids but after treatment the concentration in urine and faeces of several steroids increased 20–30 fold and the total excretion was about 12 mg/24 h both in urine and faeces. All the quantitatively predominant steroids excreted after ACTH treatment had a 3$\beta$-hydroxy-5-ene-structure: 3$\beta$,16$\alpha$-dihydroxy-5-androsten-17-one, 3$\beta$,17$\beta$-dihydroxy-5-androsten-16-one, 5-pregnene-3$\beta$,20$\alpha$-diol, 3$\beta$,16$\alpha$-dihydroxy-5-pregnen-20-one, 3$\xi$,20$\xi$-dihydroxy-5-pregnen-16-one and 5-pregnene-3$\beta$,17$\alpha$,20$\alpha$-triol. 5-Pregnene-3$\beta$,20$\alpha$-diol had an anticonvulsant activity when tested on newborn mice and the hypothesis is presented that the antiepileptic effects of ACTH in infants with hypsarrhytmia may be mediated *via* 3$\beta$-hydroxy-5-ene-steroids.

## INTRODUCTION

INFANTILE spasm with hypsarrhytmia is a severe form of epilepsy in infants with unknown etiology usually starting before the age of one year. The introduction of treatment with ACTH by Sorel and Dusaucy-Bauloye[1] has resulted in better control of seizures and possibly somewhat better prognosis for mental development in infants with this disease. The mechanism of action of the high doses of ACTH that are administered during several weeks is poorly understood. In grown-ups and older children seizures may follow treatment with steroids or ACTH for e.g. collagen disorders but the anticonvulsant effect in infants with infantile spasms and hypsarrhytmia is well documented[2]. Studies in newborn mice have shown an age dependent anticonvulsant effect of cortisol and deoxycorticosterone against high frequency electro-shock seizures[3]. It was considered possible that the presence of certain steroids in infants with infantile spasm who were given ACTH might be related to the successful treatment of the disease. Therefore, as part of a general investigation program on the effects of steroids on the development of the central nervous system, the present study of steroid excretion in a case of infantile spasm was undertaken. The finding of 3$\beta$-hydroxy-5-ene-steroids in large amounts after treatment with ACTH was striking and further studies will show if the steroid excretion pattern found in this case is correlated with the beneficial clinical response obtained.

## EXPERIMENTAL

*Case history.* The patient was first seen at the pediatric department, Karolinska Hospital, at the age of 5½ months when he was referred from a local medical officer for the observation of seizures starting one day before admittance. He was a product of a 40 week gestation to a then 32 yr-old mother whose pregnancy was uneventful. Delivery was normal with a birth weight of 3200 g.

Development was thought to be normal to the age of 5 months when the patient suddenly began to suffer episodes of fits characterized by extension of arms and flexion of legs of a few sec duration.

Physical examination on admittance did not reveal anything abnormal. CSF analysis showed no cells, protein concentration was 22 mg%, skull X-ray was normal as was the serum concentration of electrolytes. Electrophoresis of the CSF-protein one week after admittance showed a marked increase of alfa-1-globulin. EEG showed generalized epileptogenic activity of the type hypsarrhytmia. During the first weeks of hospitalization several typical nodding spasms were noted daily (4–15 episodes).

After an unsuccessful trial of pyridoxin, ACTH treatment was started during the fourth week with 180 units daily for a period of 22 days and then reduced to 120 units daily over 3 days, this dose was continued for 3 weeks and then gradually decreased. Phenobarbital, 45 mg daily, was given from the 5th week at hospital. A few days after the onset of ACTH treatment the nodding spasms disappeared and the EEG was normalized. Psychological evaluation at the age of 36 weeks during treatment with ACTH showed a psychomotor development corresponding to 28–32 weeks. At the age of 17 months the child could raise himself to the standing position and walk with support. Psychological evaluation at the age of 18 months showed that he functioned at a level of 13–15 months.

*Collection of faeces and urine.* Faeces was collected from diapers not contaminated with urine. Urine was collected in a plastic bag during 24 h periods. The samples were immediately frozen and stored at −20°C until analyzed. Collections were performed during the premedication period and 2 weeks after onset of ACTH therapy.

*Steroids.* 3β,16α-Dihydroxy-5-pregnen-20-one, 3β,16α-dihydroxy-5-androsten-17-one, 3β,17β-dihydroxy-5-androsten-16-one, and 5-androstene-3β,16α,17β-triol were supplied by Dr. R. W. Kelly. The following steroids were purchased from Ikapharm (Ramat-Gan, Israel): 5-pregnene-3β,20α-diol, 3β-hydroxy-5-androsten-17-one, 5-androstene-3β,17β-diol, 3β-hydroxy-5-pregnen-20-one, and 5-pregnene-3β,16α,20α-triol. Dr. L. Starka kindly donated 3β,7α-dihydroxy-5-androsten-17-one and 3β,7β-dihydroxy-5-androsten-17-one. Professor W. Klyne supplied 5-pregnene-3β,17α,20α-triol from the Medical Research Council Steroid Reference Collection. 3β,21-Dihydroxy-5-pregnen-20-one 21-acetate was purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). It was hydrolyzed with $K_2CO_3$ in methanol at room temperature over night. Sodium borohydride reduction of 3β,21-dihydroxy-5-pregnen-20-one yielded a mixture of 5-pregnene-3β,20α(and 20β),21-triol. Dr. C. H. L. Shackleton kindly donated 3β,17α-dihydroxy-5-pregnen-20-one and 3β,16β-dihydroxy-5-androsten-17-one.

*Extraction and purification of* $C_{19}$ *and* $C_{21}$ *steroids in faeces.* Faeces, 200 g, was extracted with chloroform/methanol (1:1, v/v) and with 0·2 M ammonium carbonate in 80% (w/v) ethanol. The extract was partitioned between hexane and

80% (v/v) ethanol and the material in the ethanol phase was separated into free steroid, glucuronide, monosulphate and disulphate fractions as described in a previous paper[4]. Aliquots of these fractions were analysed by thin-layer chromatography using the solvent system ethyl acetate/ethanol/15 M ammonium hydroxide (5:5:1, by vol.) and were found to contain material with the mobilities of corresponding free and mono- and disulphurylated reference steroids. The glucuronide fractions did not give any spots on the thin-layer plates with an anisaldehyde-sulphuric acid–acetic acid reagent.

The free steroid fraction was further purified by chromatography on Sephadex LH-20 using the solvent system chloroform/methanol, 1:1 (v/v), as described previously[4]. The steroid glucuronide fraction was hydrolyzed with Ketodase® [5]. Aliquots of the steroid mono- and disulphate fractions were treated in three different ways, as described earlier[4]: (1) Hydrolysis with Ketodase® followed by solvolysis of the remaining water phase after extraction of the liberated steroids from the incubation mixture with ethyl acetate. (2) Hydrolysis with enzymes from *Helix pomatia* followed by solvolysis. (3) Solvolysis without initial hydrolysis.

All steroid fractions obtained after these procedures were silylated and analyzed by gas chromatography-mass spectrometry.

*Purification and analysis of sterols in faeces.* The hexane phase obtained when partitioning the faecal extract between hexane and 70% ethanol (see above) was chromatographed on Sephadex LH-20 in the solvent system methylene chloride/benzene (2:1, v/v). This procedure gave separation of free and esterified sterols [4]. The free sterols were further purified by thin-layer chromatography in the solvent system benzene/ethyl acetate (7:1, v/v) and the sterol esters were saponified by 0·17 M KOH in methanol/dioxane (4:1, v/v) at 20°C for 18 h. The hydrolysate was neutralized by chromatography on a column of Amberlyst-15 in ammonium form in ethanol and was then chromatographed on Sephadex LH-20 in the solvent system methylene chloride/benzene (2:1, v/v) and further subfractionated by thin-layer chromatography. All sterol fractions obtained were silylated and analyzed by gas chromatography-mass spectrometry.

*Extraction and purification of steroids in urine.* Urine, 200 ml, was applied to an 80 g column of Amberlite XAD-2[6]. The column was washed with 1 l of distilled water. The steroids were eluted with 1 l of methanol and the methanol eluate was evaporated to dryness. The residue was dissolved in 100 ml of 70% (v/v) ethanol and passed through a 10 g column of Amberlyst-15 in the sodium form. The column was rinsed with 500 ml of ethanol and the eluates were evaporated to dryness under vacuum. The extract was then chromatographed on a Sephadex LH-20 column and was separated into the free steroid, glucuronide, monosulphate and disulphate fractions as described previously[4]. Aliquots of the different steroid fractions were analyzed by thin-layer chromatography and the material in the fractions had mobilities similar to corresponding reference steroid conjugates. The free steroid fractions did not give any spots on the thin-layer plates. The steroids in the different fractions were liberated as described above for the steroid fractions in faeces. Analysis was performed by gas chromatography-mass spectrometry of the silylated steroids.

*Gas chromatography-mass spectrometry.* Spectra were recorded on magnetic tape using the incremental mode of operation[7] and were then treated in an IBM

1800 computer[8]. A steroid was considered identified when the retention times of the silyl ether on SE-30 and QF-1 columns and the mass spectrum were identical with those of the reference compound.

*Pharmacological test of anticonvulsant activity of steroids.* The method employed was a high frequency electro-shock procedure with newborn mice as test objects. The method was described in detail by Ferngren and Paalzow in 1969[9]. A pulse stimulator induces square wave pulses, the frequency, pulse, width and total duration of which are present at optimal values for the age group of mice used. The voltage that just induces a tonic fore-limb seizure is determined individually in a pretest procedure, and the electro-shock is then repeated every ½ h up to 5 h and then after 24 h, since some drugs have a very long lasting action in newborn mice.

The duration of the induced seizure is measured and is used as a graded response since the duration of seizure has been shown to be shortened by well known anticonvulsants. A control group injected with the solvent is also included since seizure duration *per se* in some age groups is influenced by repetition of the electro-shock.

## RESULTS

*Steroids in faeces and urine.* Tables 1 and 2 show the steroids excreted in faeces and urine from the infant before and after administration of ACTH. Only six steroids in faeces and one in urine could be found during the premedication period. After treatment with ACTH the infant excreted nineteen different steroid hormone metabolites in faeces and twelve in urine. Most of the steroids had a 3$\beta$-hydroxy-5-ene- structure and were excreted as mono- or disulphates. In urine small amounts of predominantly saturated steroids were also recovered from the glucuronide fraction (see Table 2). During the medication period small amounts of 20$\alpha$,22$\xi$-dihydroxycholesterol were found in faeces.

After treatment with ACTH the infant excreted several 17$\alpha$,21-dihydroxylated, saturated $C_{21}$ steroids in faeces. These compounds which were presumably cortisol metabolites were quantitatively much less important than the 3$\beta$-hydroxy-5-ene-steroids; they were not studied further.

*Semiquantitative analysis.* In order to make an approximate estimation of the amounts of steroids present in faeces and urine from the infant before and after treatment with ACTH, aliquots of faeces and urine were worked up as described in the experimental section. The samples were silylated and analyzed by gas–liquid chromatography (Figs. 1–4).

In a previous gas–liquid chromatographic study[10] the "response" of the silyl ethers of six 3$\beta$-hydroxy-5-ene-$C_{19}$ and $C_{21}$ steroids was linear between 0·1–0·5 $\mu$g and varied about ± 10% between the different steroids. In the present study it was therefore considered sufficient for a semiquantitative estimation to use one 3$\beta$-hydroxy-5-ene-steroid to determine a standard graph. The peak areas were measured with a planimeter and compared to the peak areas produced by known amounts of 3$\beta$-hydroxy-5-pregnen-20-one silyl ether. The recoveries of [7$\alpha$-$^3$H]-3$\beta$-hydroxy-5-androsten-17-one added to faeces and urine were used to calculate losses in extractions, chromatographies and hydrolyses and solvolyses. Losses were about 40%. The results of the analyses are shown in Table 3. The figures have not been corrected for analytical losses.

Table 1. Relative retention times (5α-cholestane = 1·00) on 1·5% SE-30 and 3% QF-1 columns of silyl ethers of steroids isolated from faeces from an infant with hypsarrhytmia before and after treatment with ACTH

| Compound | $t_R$ SE-30 | $t_R$ QF-1 | Found in fraction (Sephadex LH-20 chromatography) | Found before ACTH | Found after ACTH |
|---|---|---|---|---|---|
| 3β-Hydroxy-5-androsten-17-one | 0·51 | 1·40 | M | | + |
| 5-Androstene-3β,17α-diol | 0·52 | 0·50 | D | | + |
| 5-Androstene-3β,17β-diol | 0·60 | 0·58 | M | | + |
| 3ξ,16ξ-Dihydroxy-5ξ-androstan-17-one | 0·60 | — | D | | + |
| 3β-Hydroxy-5-pregnen-20-one | 0·79 | 1·80 | M | | + |
| 3β,16α-Dihydroxy-5-androsten-17-one | 0·79 | 1·46 | D | | + |
| 3β,16α-Dihydroxy-5α-androstan-17-one | 0·79 | | D | | + |
| 3ξ,17α-Dihydroxy-5ξ-pregnan-20-one | 0·87 | — | M | | + |
| 3β,16β-Dihydroxy-5-androsten-17-one | 0·89 | 1·70 | D | | + |
| 5-Pregnene-3β,20α-diol | 1·10 | 1·06 | M,D | + | + |
| 5-Androstene-3β,16α,17β-triol | 1·10 | 1·06 | M,D | + | |
| 5ξ-Pregnane-3ξ,16ξ,20ξ-triol | 1·11 | — | F | + | |
| 3β,16α-Dihydroxy-5-pregnen-20-one | 1·19 | 2·03 | M,D | + | + |
| 3ξ,21-Dihydroxy-5ξ-pregnan-20-one | 1·30 | — | M,D | | + |
| 5ξ-Pregnane-3ξ,17α,20ξ-triol | 1·30 | — | M | | + |
| 3ξ,20ξ-Dihydroxy-5-pregnen-16-one | 1·52 | — | M | | + |
| 5-Pregnene-3β,17α,20α-triol | 1·55 | 1·83 | M,D | + | + |
| 3β,21-Dihydroxy-5-pregnen-20-one | 1·64 | 2·08 | D | | + |
| 5-Pregnene-3β,16β,20β-triol | 1·86 | — | D | + | |
| 5-Pregnene-3β,20α,21-triol | 1·90 | 1·46 | D | | + |
| 5-Pregnene-3β,16α,20α,21-tetrol | 2·25 | 2·25 | M | | + |
| 20α,22ξ-Dihydroxycholesterol | 4·33 | — | M | | + |

F = free steroid fraction.
M = steroid monosulphate fraction.
D = steroid disulphate fraction.

The steroid excretion in faeces and urine from the infant prior to ACTH treatment was less than 0·1 mg per 24 h. After administration of ACTH about 12 mg of steroids was excreted in both faeces and urine. The quantitatively predominant steroids in both faeces and urine were 3ξ,20ξ-dihydroxy-5-pregnen-16-one and 5-pregnene-3β,17α,20α-triol. Three other steroids of quantitative importance were 3β,16α-dihydroxy-5-androsten-17-one, 5-pregnene-3β,20α-diol, and 3β,16α-dihydroxy-5-pregnen-20-one.

*Experimental pharmacology*. Two of the quantitatively predominant steroids that were identified in urine and faeces from the patient, when treated with ACTH, 5-pregnene-3β,20α-diol and 5-pregnene-3β,17α,20α-triol, were tested for anticonvulsant activity in the animal model described in the experimental section. The steroids were dissolved in peanut oil during slow heating and controls were injected by peanut oil. 5-Pregnene-3β,20α-diol diminished seizure duration in mice up to the age of 5 days in the moderate dose of 25 mg/kg but had no effect in 9-day-old mice (Fig. 5). It may be mentioned that some steroids like cortisone-acetate when injected 1–4 days after birth gave a prolongation of electro-shock seizure up to the age of 14 days (unpublished observations) but that no such effect was found for 5-pregnene-3β,20α-diol.

A similar anticonvulsant effect was found with 5-pregnene-3β,17α,20α-triol

Table 2. Relative retention times (5α-cholestane = 1·50) on 1·5% SE-30 and 3% QF-1 columns of silyl ethers of steroids isolated from urine from an infant with hypsarrhytmia before and after treatment with ACTH.

| Compound | $t_R$ SE-30 | $t_R$ QF-1 | Found in fraction (Sephadex LH-20 chromatography) | Found before ACTH | Found after ACTH |
|---|---|---|---|---|---|
| 3α-Hydroxy-5β-androstane-11,17-trione | 0·52 | — | G | | + |
| 5-Androstene-3β,17α-diol | 0·52 | 0·50 | D | | + |
| 3β,7β-Dihydroxy-5-androsten-17-one | 0·71 | — | M | | + |
| 3β,16α-Dihydroxy-5-androsten-17-one | 0·79 | 1·46 | M | | + |
| 3ξ,17α-Dihydroxy-5ξ-pregnan-20-one | 0·87 | — | G | | + |
| 3β,17β-Dihydroxy-5-androsten-16-one | 0·89 | 1·90 | M | | + |
| 5-Pregnene-3β,20α-diol | 1·10 | 1·06 | D | | + |
| 5-Androstene-3β,16α,17β-triol | 1·10 | 1·06 | M | | + |
| 5ξ-Pregnane-3ξ,16ξ,20ξ-triol | 1·16 | — | G | | + |
| 3β,16α-Dihydroxy-5-pregnen-20-one | 1·19 | 2·03 | M | | + |
| 5ξ-Pregnane-3ξ,17α,20ξ-triol | 1·30 | — | G | | + |
| 3ξ,20ξ-Dihydroxy-5-pregnen-16-one | 1·52 | — | M | | + |
| 5-Pregnene-3β,17α,20α-triol | 1·55* | 1·83 | G,M,D | + | + |

G = steroid glucuronide fraction.
M = steroid monosulphate fraction.
D = steroid disulphate fraction.

Table 3. Approximate amounts of steroids in the mono- and disulphate fractions of faeces and urine from an infant with hypsarrhytmia treated with excessive doses of ACTH. The values are expressed as mg of free steroid excreted per 24 h

| Steroid | Faeces | | Urine | |
|---|---|---|---|---|
| | Steroid monosulphate fraction | Steroid disulphate fraction | Steroid monosulphate fraction | Steroid disulphate fraction |
| 5-Androstene-3β,17β-diol | 0·2 | 0·1 | | 0·3 |
| 3β,16α-Dihydroxy-5-androsten-17-one | | 0·1 | 2·5 | |
| 3β-Hydroxy-5-pregnen-20-one | 1·1 | | | |
| 3ξ,17ξ-Dihydroxy-5ξ-pregnan-20-one | 0·2 | | | |
| 3β,16β-Dihydroxy-5-androsten-17-one | | 0·5 | | |
| 3β,17β-Dihydroxy-5-androsten-16-one | | | 1·5 | |
| 5-Pregnene-3β,20α-diol | 1·7 | 1·3 | | 0·3 |
| 5-Androstene-3β,16α,17β-triol | | | 1·1 | |
| 3β,16α-Dihydroxy-5-pregnen-20-one | 0·2 | 0·5 | 1·4 | |
| 5ξ-Pregnane-3ξ,17α,20ξ-triol | 0·7 | | | |
| 3ξ,21-Dihydroxy-5ξ-pregnan-20-one | | 0·1 | | |
| 3ξ,20ξ-Dihydroxy-5-pregnen-16-one | 2·3 | | 3·1 | |
| 5-Pregnene-3β,17α,20α-triol | | 3·0 | | 2·2 |
| Total | 6·4 | 5·6 | 9·6 | 2·8 |

in a single dose of 25 mg/kg in all age-groups studied (Fig. 6). The duration of action was at least 5 h in 1-day-old mice. The effect was less in 3 and 9 day-old mice but the effect was significant 1–2 h after the injection.

*Sterols in faeces.* The nonesterified and fatty acid esterified sterols in meconium were the same as those found in a previous study of meconium from new-

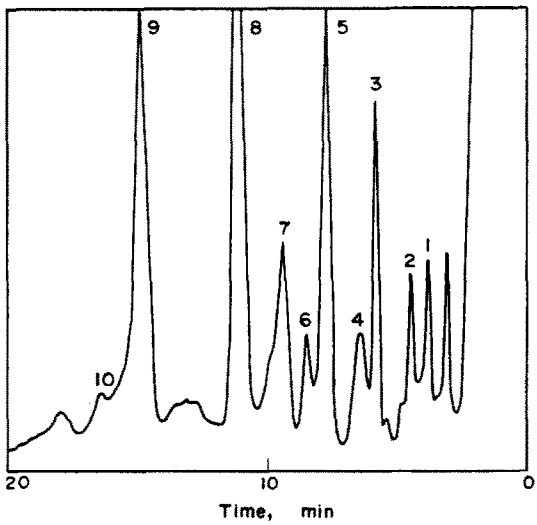


Fig. 1. Gas chromatographic analysis (SE-30 column) of silyl ethers of steroids in the monosulphate fraction of faeces from an infant with hypsarrhytmia treated with excessive doses of ACTH. The steroids were liberated by solvolysis. The numbered compounds were identified as the silyl ethers of 3$\beta$-hydroxy-5-androsten-17-one (1), 5-androstene-3$\beta$,17$\beta$-diol (2), 3$\beta$-hydroxy-5-pregnen-20-one (3), 3$\xi$,17$\alpha$-dihydroxy-5$\xi$-pregnan-20-one (4), 5-pregnene-3$\beta$,20$\alpha$-diol (5), 3$\beta$,16$\alpha$-dihydroxy-5-pregnen-20-one (6), 3$\xi$,21-dihydroxy-5$\xi$-pregnan-20-one and 5$\xi$-pregnane-3$\xi$,17$\alpha$,20$\xi$-triol (7), 3$\xi$,20$\xi$-dihydroxy-5-pregnen-16-one (8), 5-cholestene-3$\beta$-ol (9), and 5-pregnene-3$\beta$,16$\alpha$,20$\alpha$,21-tetrol (10).

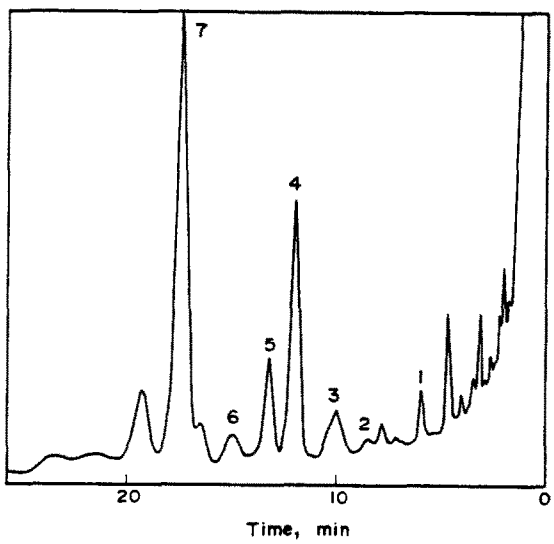


Fig. 2. Gas chromatographic analysis (SE-30 column) of silyl ethers of steroids in the disulphate fraction of faeces from an infant with hypsarrhytmia treated with excessive doses of ACTH. The steroids were liberated by solvolysis after hydrolysis with enzymes from *Helix pomatia*. The numbered compounds were identified as the silyl ethers of 5-androstene-3$\beta$,17$\beta$-diol (1), 3$\beta$,16$\alpha$-dihydroxy-5-androsten-17-one (2), 3$\beta$,16$\beta$-dihydroxy-5-androsten-17-one (3), 5-pregnene-3$\beta$,20$\alpha$-diol (4), 3$\beta$,16$\alpha$-dihydroxy-5-pregnen-20-one (5), 3$\xi$,21-dihydroxy-5$\xi$-pregnan-20-one (6), and 5-pregnene-3$\beta$,17$\alpha$,20$\alpha$-triol (7).

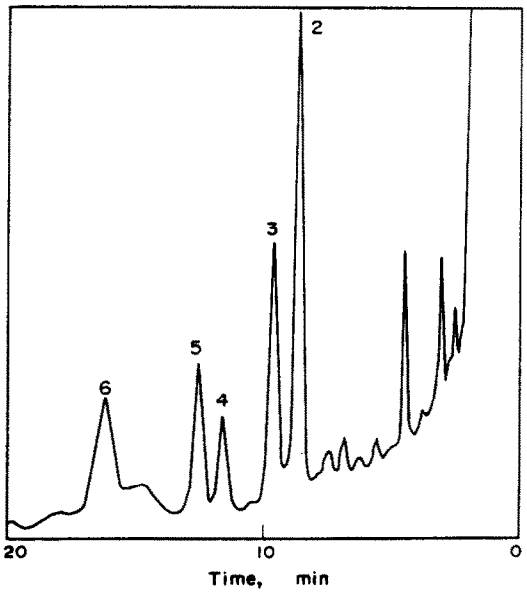


Fig. 3. Gas chromatographic analysis (SE-30 column) of silyl ethers of steroids in the monosulphate fraction of urine from an infant with hypsarrhytmia treated with excessive doses of ACTH. The steroids were liberated by solvolysis after hydrolysis with Ketodase®. The numbered compounds were identified as the silyl ethers of 3β,7β-dihydroxy-5-androsten-17-one (1), 3β,16α-dihydroxy-5-androsten-17-one (2), 3β,17β-dihydroxy-5-androsten-16-one (3), 5-androstene-3β,16α,17β-triol (4), 3β,16α-dihydroxy-5-pregnen-20-one (5), and 3ξ,20ξ-dihydroxy-5-pregnen-20-one (6).

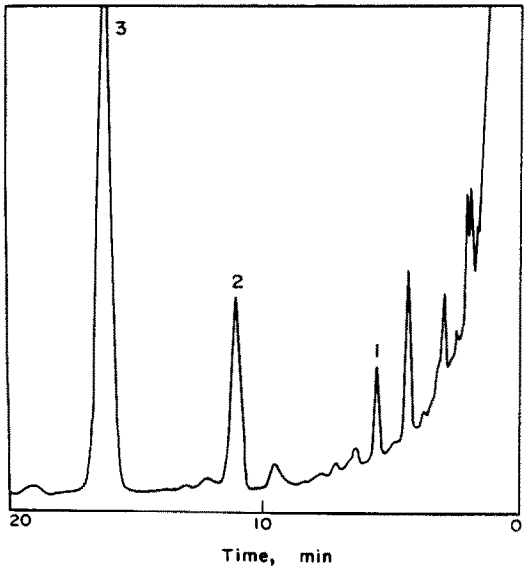


Fig. 4. Gas chromatographic analysis (SE-30 column) of silyl ethers of steroids in the disulphate fraction of urine from an infant with hypsarrhytmia treated with excessive doses of ACTH. The steroids were liberated by solvolysis after hydrolysis with enzymes from *Helix pomatia*. The numbered compounds were identified as the silyl ethers of 5-androstene-3β,17β-diol (1), 5-pregnene-3β,20α-diol (2), and 5-pregnene-3β,17α,20α-triol (3).

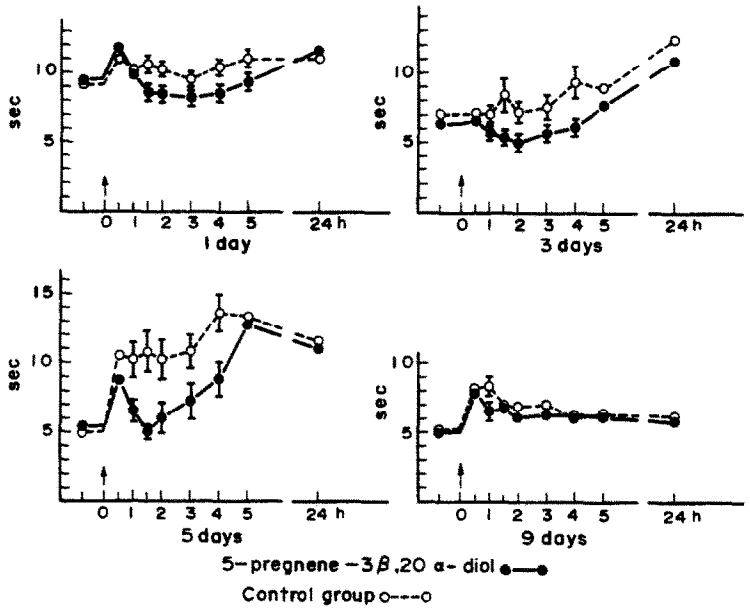


Fig. 5. Mean duration of tonic fore-limb seizures in 1–9 day-old mice after intraperitoneal administration of 5-pregnene-3$\beta$,20$\alpha$-diol (25 mg/kg). Controls were injected with peanut oil. Vertical bars indicate s.e.m. and have been plotted where a significant difference (p 0·05, student's-test) between steroid-injected and control animals was found. The anticonvulsant effect against high frequency electro-shock seizures was followed the first 5 h and at 24 h after injection.

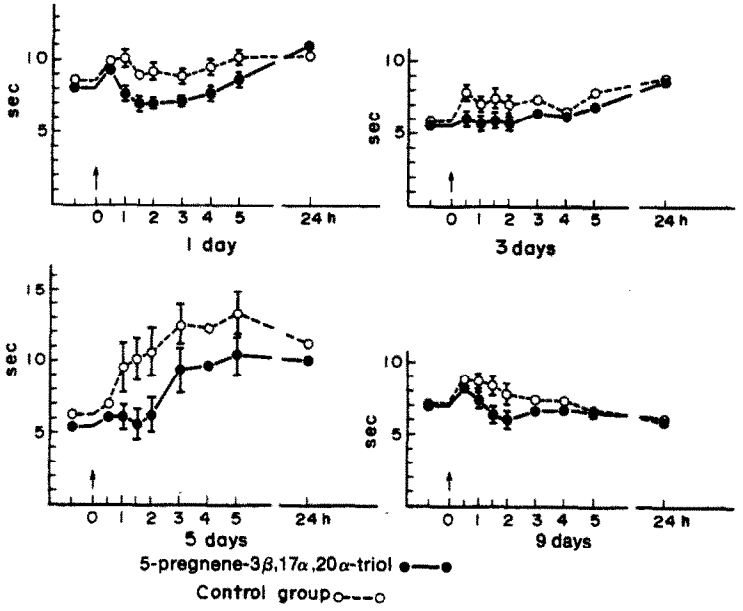


Fig. 6. Mean duration of tonic fore-limb seizures in 1–9 day-old mice after intraperitoneal administration of 5-pregnene-3$\beta$,17$\alpha$,20$\alpha$-diol (25 mg/kg). Controls were injected with peanut oil. Vertical bars indicate s.e.m. and have been plotted where a significant difference (p 0·05. student's t-test) between steroid-injected and control animals was found. The anticonvulsant effect against high frequency electro-shock seizures was followed the first 5 h and at 24 h after injection.

born babies[11], i.e. lanosterol, 8-ene- and 9-ene-dihydrolanosterol, and 4,4- and 4,14-dimethyl substituted 7-ene- and 8-ene-5α-cholestane derivatives. Cholesterol, lathosterol, 7-ene- and 8-ene-methostenol as well as the plant sterols campesterol, β-sitosterol, and cycloartenol were also identified.

## DISCUSSION

During the first weeks of life newborn infants excrete large amounts of 3β-hydroxy-5-ene-steroids in urine and faeces (see Refs. 12, 13). These compounds are probably synthesized in the fetal adrenal cortex. When the fetal cortex gradually atrophies during the first year of life[14] a parallel decrease in the excretion of 3β-hydroxy-5-ene-steroids occurs[10, 13]. An anencephalic fetus whose adrenal cortex only consisted of a permanent zone did not excrete any 3β-hydroxy-5-ene-steroids either in urine or in meconium[4].

The infant studied in the present investigation excreted less than 0·1 mg/24 h of individual 3β-hydroxy-5-ene-steroids in urine and faeces prior to treatment with ACTH. Little information is available on the excretion of 3β-hydroxy-5-ene-steroids in urine from normal infants of corresponding age. In a semiquantitative study of the faecal excretion of steroids in infants[10], infants six months of age were found to excrete low amounts of 3β-hydroxy-5-ene-steroids and none of the steroids were excreted in amounts higher than 0·1 mg/24 h. However, more information of both urinary and faecal excretion of 3β-hydroxy-5-ene-steroids in healthy infants is needed before definite conclusions can be drawn concerning the levels of urinary and faecal steroids in infants with various disorders.

Administration of large doses of ACTH to the infant studied resulted in the excretion of large amounts of 3β-hydroxy-5-ene-steroids in urine and faeces (about 24 mg/24 h). This indicates that at the age of 6 months the infant had a fetal adrenal cortex which was still responsive towards stimulation with ACTH. The collection of urine and faeces from the infant was started two weeks after the onset of ACTH treatment and one week after the onset of phenobarbital administration. In some investigated cases where infants were treated with phenobarbital for other reasons the total amount of steroids excreted in urine and faeces was not increased, neither did the relative amount of 3β-hydroxy-5-ene-steroids increase (unpublished observations). Therefore it seems justifiable to regard the changes in steroid excretion in the patient in the present study as the result of ACTH administration and not as the result of phenobarbital administration.

The administration of ACTH to the infant with hypsarrhytmia was remarkably efficient in abolishing epileptogenic seizures. It was also found that two of the quantitatively important steroids excreted by the infant after treatment with ACTH, 5-pregnene-3β,20α-diol and 5-pregnene-3β,17α,20α-triol, had anticonvulsant activity when tested on newborn mice. This indicates that the antiepileptic effects of ACTH in infants with hypsarrhytmia may be mediated *via* 3β-hydroxy-5-ene-steroids or some of their metabolites.

The clinical outcome in infants with the typical EEG picture of hypsarrhytmia was significantly better regarding mental development after steroid or ACTH treatment as shown in a recent follow up study[15] but still only 26% developed normally. It may be that one important factor in infants who recover is their ability to respond properly to ACTH administration by producing large amounts of 3β-hydroxy-5-ene-steroids. More studies are needed to settle this question and

to evaluate the possible therapeutic value of 3$\beta$-hydroxy-5-ene-steroids in cases of hypsarrhytmia.

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