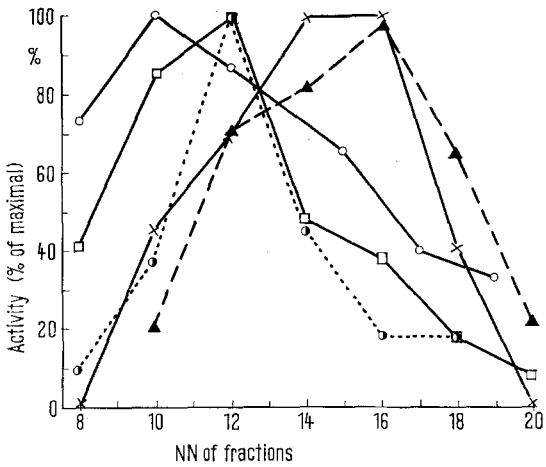
for automatic sample injection with simultaneous establishing of sucrose density gradient in borate buffer system (ref.[14], pp. 216 and 224). Electrophoresis was carried out at 200 V (12 mA) for 63 h. In 5 ml fraction rates of deamination of monoamines were determined using following final concentrations of the substrates: kynuramine.HBr 1 mM; pH 7.4 (ref.[15]), *p*-nitrophenylethylamine.HCl 0.83 mM and *m*-nitro-*p*-hydroxybenzylamine.HCl 0.53 mM, both at pH 7.0 (ref.[4]), tyramine.HCl 3.2 mM and 5-hydroxytryptamine. cratininesulphate 5 mM, both at pH 7.4 (ref.[16]).



Separation by means of density gradient electrophoresis of amine oxidases from rat liver mitochondria disintegrated by sonication in absence of detergents. Conditions of the experiment see text. ○—○, 5-hydroxytryptamine; □—□, tyramine; ◐---◐, *p*-nitrophenylethylamine; ×—×, kynuramine; △---△, m-nitro-*p*-hydroxybenzylamine. Activity in fractions in per cent of the highest activity.

A typical example of the results obtained (Figure 1) shows that the amine oxidases of rat liver mitochondria may be more or less distinctly separated. Similar results are observed in experiments with longer (up to 85 h) but not shorter (24–48 h) electrophoretic runs[17]. After the treatment with urea of sonicated rat liver mitochondria, separation of amine oxidases by density gradient electrophoresis was observed even within 43 h runs[18].

Выводы. Аминоксидазы озвученных митохондрий печени крысы могут быть разделены в отсутствие детергентов путем электрофореза в градиенте плотности.

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The Identification of Quantitation of Extremely Polar, Free Corticosteroids in Liquor amnii

During the course of work on the steroid content of liquor amnii, it has become apparent that a number of free polar corticosteroids are present in substantial amounts.

6β, 11β, 21α, 21 tetrahydroxypregn-4-ene-3, 20-dione (6β hydroxycortisol), 6β, 11β, 17α, 20α, 21 pentahydroxypregn-4-ene-3-one (20α dihydro 6β-OH-F) 6β, 11β, 17α, 20β, 21 pentahydroxypregn-4-ene-3-one (20β dihydro 6β-OH-F) as well as 11β, 17α, 21 trihydroxypregn-4-ene-3-20 dione (cortisol) and 11β, 17α, 21 trihydroxypregn-4-ene-3-11-20 trione (cortisone) have all been identified and a quantitative estimate made in a number of samples of liquor from full-term normal pregnancies.

The extraction of these extremely polar corticosteroids has been previously described[1–3]. The preliminary purification and identification procedures and the quantitative determination of 6β hydroxycortisol by gas-liquid chromatography (GLC) and the reproducibility of the assay has also been described previously[3]. The identification of other cortisol metabolites more polar than 6β hydroxycortisol has been concluded, following initial purification of the ethyl acetate extract by thin-layer chromatography in a distilled water system. The 20α and 20β derivatives of 6β hydroxycortisol were isolated as a mixture following subsequent thin-layer chromatography in a system comprising chloroform–methanol 84:16 by volume (Rf 0.30). They were then separated by chromatography on boric acid impregnated plates (3% boric acid in water) in a solvent comprising chloroform–methanol 84:16 by volume. In this system 17α, 20β-diols moved considerably faster than 17α, 20α-diols. The UV-absorbing zones (254 nm) were eluted and separately purified by thin-layer chromatography in a system comprising diethyl ether–ethanol 9:1 by volume, when a wide separation of the compounds occurred.

Alternatively it was found possible to separate the 2 epimers by preliminary paper chromatography in a benzene–ethyl acetate–methanol–water system (7:3:10:10 by volume) and following elution of the single spot subsequent paper chromatography in a system comprising benzene–tertiary butanol–water (70:43:86 by volume) with a 4 h overrun. 2 UV-absorbing zones were observed which corresponded to the markers of 20α and 20β dihydro 6β hydroxycortisol; the 20α epimer being more polar than the 20β.

Identification procedures on the eluted compounds were carried out as previously described[4, 5] and by comparative programmes of thin-layer, paper and gas liquid chromato-

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graphy. In all instances there was complete agreement between the unknown and the reference steroids.

Quantitation was carried out by GLC. Aliquots of both the 20α and its 20β epimer of 6β hydroxycortisol were oxidized to 17 oxosteroids[3]. The trimethyl silyl ether (TMSi) derivatives were then prepared using the methods described for 6β hydroxycortisol[3] with either pyridine or ethylene dichloride as solvent. All derivatives were chromatographed on 2 columns, a hybrid 0.9% cyanoethyl and dimethyl silicone (XE60) + 0.1% neopentyl glycol succinate (NGS) and an 1% NGS column. In each instance the isolated steroids had identical retention times with that obtained for the reference steroid 6β,11β-hydroxyandrost-4-ene-3-17 dione treated in the same way. It may therefore be assumed that both the compounds differ only in their side-chain orientations. As the 11-hydroxyl grouping can only be reacted for TMSi-ether derivative formation when pyridine is used, it must be assumed that both compounds have a hydroxyl grouping in this position.

Aliquots of the 20α and 20β dihydro derivatives of 6β hydroxycortisol were again reacted with TMSi-ether using pyridine as solvent and with the side-chain intact[6].

Determination of free corticosteroids in liquor (μg/l) collected after full-term normal delivery

6β,20α-OH-F μg/l	6β,20β-OH-F μg/l	6β-OH-F μg/l	Cortisol μg/l	Cortisone μg/l
29.0	24.8	38.8	18.2	N.D.[a]
36.0	28.0	32.0	26.6	17.3
32.0	29.0	24.9	13.1	11.0
28.6	25.1	–	–	–
30.0	27.0	–	–	–

[a] N.D., not detectable.

GLC of the resultant products achieved a wide separation of the 20α and 20β epimers although the retention times were long and wide peaks were obtained. Peak areas were compared with those of the standard. The results obtained from 5 full-term samples of liquor are shown in the Table. In 3 samples of liquor the steroidal content of 5 unconjugated polar compounds have been estimated in each, while in the other liquor samples particular attention has been paid to the 20α and 20β epimers of 6β hydroxycortisol.

It is obvious from the Table that the amounts of these polar compounds in the liquor is often higher than the amounts of cortisol and cortisone. This would agree with the results previously reported by ULSTROM et al.[7] during their work on neonatal urine, and with the known ability of the placenta to oxygenate steroids at the C-6 position[8–10].

Résumé. Identification et mesure quantitative des stéroïdes libres dans le liquide amniotique. Leur concentration est supérieure à celle du cortisol et du cortisone.

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Nondialyzable Toxic Factor in Uraemic Blood Effectively Removed by the Activated Charcoal

FEHER et al.[1] reported in this Journal that the uraemic blood contains a non-dialysable toxic factor, causing the rat's death under characteristic symptomatology. We present here the results of some experiments that further support the findings of the previous authors and prove moreover that this factor can be removed directly from the blood by the activated charcoal.

Material and methods. 2 ml of a preparate from 20 ml serum of 13 patients in a state of advanced renal failure (urea concentration 360–830 mg/100 ml) were given i.p. to 13 pairs of 30–35-day-old Sprague-Dawley rats, weighing 75–85 g. The preparation is obtained as follows: 20 ml of serum are added drop by drop under continuous agitation in a mixture of 20 ml of ethyl alcohol 95% and 0.4 ml of acetic acid. The contents is well mixed and placed in a boiling water bath for 10 min. 50 ml of ethyl alcohol 95% are added and the content is mixed again and centrifuged for 30 min at 4000 rpm. The supernatant is transfered to a suitable container and is condensed in vacuum at 70–75 °C to a volume of 5–7 ml. 50 mg of $NaHCO_3$ and 25 ml of ethyl alcohol 70% are added. The mixture is condensed once more by the same procedure to a volume of approximately 2 ml. The preparate for the first rat of each pair (A) was obtained from 20 ml of serum dialysed 12 h using the cellophane membrane of the Travenol coil artificial kidney against running ion-free water, while for the second rat (B, control) from 20 ml of the same serum, which had been previously dialysed, brought in contact for 10 min with 100 mg of activated charcoal (Charcoal Merck, No. 9624).

Results and discussion. Some minutes after the injection, certain reactions were observed on the rats A, especially restlessness, rigor, salivation, thirst, apathy, reduced reactivity to external stimuli, incoordinated movements, profound hypothermia (below 34 °C), clonic and tonic spasms. These reactions became evident in different intensity at all 13 rats A. None of the reactions mentioned were observed in the rats B, except for a simple reduction of their spontaneous activity now and then (Table). All the rats A died within 8–70 h. Immediately before death, the rat fell in coma and assumed opisthotonus with hyperextensibility of the posterior extremities. In contrast to this, only 3 of the 13 controls died within 6–26 h. The difference in mortality rate between experimental and control groups is highly significant ($P<0.001$).

The results agree with those of others[1] who originally worked on this subject and were able to observe also the following findings: (1) The larger the preparate, the quicker and more intense the appearance of reactions;

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