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;

[1] I. FEHER, I. DESI and E. SZOLD, Experientia *14*, 292 (1958).

Rat	Tachypnea Tachycardia	Reactions Rigor	Salivation	Thirst	Apathy	Hypothermia	Hyperextension of posterior extremities	Clonic and tonic spasms	Time of death (h)
1 A	+	—	—	—	+	—	—	+	7
B	—	—	—	—	+	—	—	—	6
2 A	—	—	—	—	—	—	—	+	40
B	—	—	—	—	—	—	—	—	–
3 A	—	—	—	—	—	—	—	+	40
B	—	—	—	—	—	—	—	—	–
4 A	+	+	+	+	+	+	+	+	12
B	+	—	+	—	+	—	—	—	26
5 A	+	+	—	+	+	+	+	+	12
B	—	—	—	—	—	—	—	—	–
6 A	+	+	—	—	+	+	+	—	20
B	—	—	—	—	—	—	—	—	24
7 A	+	+	—	+	+	+	+	+	40
B	—	—	—	—	—	—	—	—	–
8 A	+	+	+	—	+	+	+	+	18
B	—	—	—	—	—	—	—	—	–
9 A⁻	+	+	—	+	+	+	+	+	70
B	—	—	—	—	—	—	—	—	–
10 A	+	+	—	—	+	+	+	+	20
B	—	—	—	—	—	—	—	—	–
11 A⁺	+	+	—	+	+	+	+	+	16
B	—	—	—	—	—	—	—	—	–
12 A	+	+	—	—	+	+	+	+	8
B	—	—	—	—	—	—	—	—	–
13 A	+	+	+	+	+	+	+	+	2
B	—	—	—	—	—	—	—		–

(A) Travenol coil artificial kidney; (B) activated with charcoal. Reactions observed on 13 pairs of rats.

the rat dies sooner. A parallel relation exists between the injected amount and the drop of the rat's rectal temperature which sometimes reaches levels of 10 °C less than the initial. (2) Preparates obtained from the serum of normal individuals or non-uraemic patients failed to produce any reaction. (3) There is no difference in K, P, Na, Cl, protein and non-protein nitrogen concentration between normal and uraemic serum preparates. (4) The toxic factor is a peptide existing in blood as a macromolecular complex, bound to plasma proteins or other macromolecules, and (5) the charcoal treated alcohol preparates from uraemic serum remains highly toxic to rats.

Our results suggest the presence in uraemic blood of a toxic factor non-dialysable by the membrane of the artificial kidney. The preparate, entirely free of the known electrolytes and uraemic substances, provoked in all rats A some chacteristic reactions resembling certain symptoms noted in patients with severe renal failure. The absence of these reactions in the rats B is indicative that the charcoal directly inactivates the toxicity of uraemic plasma in almost all of the patients. The fact that the charcoal-treated alcoholic preparate remains toxic is not at all in disagreement; it is well known that many of the strongly absorbed substances on activated charcoal can be completely recovered by exposure to ethyl alcohol[2].

The symptoms of chronic renal failure, as well as its effect on the different systems, are attributed to the accumulation of the whole of the uraemic substances. The toxic effect of each substance is not yet determined. Most of these are easily dialysed by the membrane of the artificial kidney. The persistence of certain manifestations such as anaemia, polyneuritis, itching, skin pigmentation, on gainful survived patients by maintenance haemodialysis are probably due to the continued presence of non-dialysable toxic factor (s). Therefore it is justified to hope that our charcoal artificial kidney, recently applied in experimental and clinical studies for the treatment of endogenous and exogenous intoxications, would lead eventually to a more satisfactory treatment of patients in terminal uraemia[3–8].

Résumé. Les auteurs ont vérifié les résultats des travaux précédents concernant l'existence dans le sang urémique d'un facteur (s) toxique macromoléculaire nondialysable. De plus, ils ont demontré que ce facteur peut être effectivement absorbé du plasma par le charbon actif. La signification et l'importance de ces nouveaux faits sur l'insuffisance rénale et son traitement sont discutées.

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