53. Improved methods for the isolation of cortisol metabolites from neonatal urine

DRAYER, N. M. and DERKS, H. J. G. M., Department of Paediatrics, State University, Gröningen, The Netherlands

Inadequate hydrolysis and extraction methods have greatly hampered previous investigations into the qualitative and quantitative aspects of cortisol metabolism in newborn infants. Therefore, a study was undertaken to find an analytical procedure which would allow complete hydrolysis of corticosteroid conjugates and quantitative extraction of the cortisol metabolites from neonatal urine. The analysed urine was left over from 48 h collections from newborn infants, older children and adults who received tracer amounts of tritiated cortisol for estimation of cortisol production rates. Both the original Amberlite XAD-2 method and three different modifications of this method were tested for their effectiveness in the isolation of the radioactive cortisol metabolites from these urines. One of these modifications showed an extraction efficiency of 93–95% of the total urinary radioactivity. The free and different types of conjugated cortisol metabolites were separated by DEAE-Sephadex anion exchange chromatography. It was shown that the conjugation pattern of cortisol metabolites in neonatal urine differs distinctly from those in the urine of older children and adults and that neonatal urine contained an as yet unidentified conjugate, which was hardly present in adult urine. Three different hydrolytic methods were tested on the neonatal steroid conjugates and it was found that the unknown conjugate in neonatal urine could be hydrolysed by solvolysis only.

54. The identification and quantification of 6α-hydroxylated cortisol metabolites in the urine of pregnant women

DERKS, H. J. G. M. and DRAYER, N. M., Department of Paediatrics, State University, Gröningen, The Netherlands

Recently, it was shown in our laboratory that the newborn infant excretes large amounts of 6α-hydroxylated corticosteroids and that these newly identified steroids play a quantitatively important role in neonatal cortisol metabolism. As part of a study to establish the role of 6α-hydroxylation in perinatal cortisol metabolism, 6α-hydroxylated corticosteroids were analysed both qualitatively and quantitatively in the urine of pregnant women (36–40 weeks of gestation). Urine samples were subjected to enzymatic hydrolysis and chromatographed on DEAE-Sephadex anion exchanger, subsequently. The free steroid fraction was collected and the steroids were extracted by Amberlite XAD-2 chromatography. The urinary steroids were separated by high performance liquid chromatography. The fractions containing the 6α-hydroxylated corticosteroids were collected and after evaporation of the eluent, methoxime-trimethylsilyl (MO–TMS) derivatives were prepared. The steroid-MO–TMS ethers were analysed by mass fragmentography. A urinary steroid was considered to be identified when HPLC and gas chromatographic retention times and mass fragmentographic properties were identical to those of reference compounds. The average amounts of the following steroids excreted by five pregnant women (μg/24 h) were: 6α-hydroxy-tetrahydrocortisone, 127.5; 6α-hydroxy-5α-THE, 19.0; 6α-hydroxy-tetrahydrocortisol, 266.9; 6α-hydroxy-5α-tetrahydrocortisol, 27.4; 6α-hydroxy-20α-cortolone, 126.0; 6α-hydroxy-20β-cortolone, 47.2.

55. Effects of steroids on the fructolysis index of buffalo (*Bubalus bubalis*) spermatozoa

SIDHU, K. S. and GURAYA, S. S., Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, 141004, India

A study has been made of the effects of steroids (androsterone, testosterone, dehydroepiandrosterone and androstenedione) on the fructolysis index of buffalo (*Bubalus bubalis*) spermatozoa. Testosterone, dehydroepiandrosterone and androstenedione inhibit the fructolysis index, percentage sperm motility and percentage live sperm and increase the percentage sperm abnormalities progressively with their increasing concentrations used. However, androsterone increases the fructolysis index and percentage sperm abnormalities. It also inhibits percentage motility but does not affect the percentage of live sperm. The possible mode of action of these steroids on metabolism of spermatozoa is through steroid dehydrogenases (HSDS) i.e., 3α, 3β, 17β, 5ene3β and 16α HSDs which have been localized cytochemically in spermatozoa. Thus but for androsterone, all other steroids can be used for prolonging the life span of spermatozoa in various dilutors.

56. Steroid metabolism in an arrhenoblastoma investigated by radio–gas chromatography and gas chromatography–mass spectrometry

LISBOA, B. P., HALKET, J. McK. and STEGNER, H.-E., Universitäts-Frauenklinik-Eppendorf, Martinistrasse 52, 2 Hamburg 20, Federal Republic of Germany

Radio–gas chromatography (RGC) and gas chromatography–mass spectrometry (GC–MS) were applied to study the *in vitro* metabolism of pregnenolone (**I**) in an arrhenoblastoma. After incubation of tissue slices at pH 7.5 with (**I**) or [4-^{14}C]-(**I**), extracts were purified on XAD-2 and subjected to chromatography on Sephadex LH-20 and (for RGC) thin-layer chromatography. Steroids were analysed by RGC as free, methoxime or trimethylsilyl ethers (TMS) on a QF-1 column. Steroids (as TMS) were analysed on a 25 m capillary column (OV-101) by GC–MS using repetitive scanning and the spectra were recorded *on line* for computerized evaluation. The following steroids were identified (tr = traces): androstenedione (tr), testosterone (tr), dehydroepiandrosterone (tr), androsterone (tr), etiocholanolone (tr), progesterone (0.6%), 5-pregnene-3β,20α-diol (11.6%), 20α-hydroxy-4-pregnen-3-one (0.72%) and 17α-hydroxy-pregnenolone (tr). This pattern differs from that found in the normal ovary.

57. Interaction of steroids with ascorbate via charge transfer complex formation

CHINOY, N. J., SEETHALAKSHMI, L. and RAO, M. V., Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India

The absorption maxima (E_{max}) of pure ascorbate (AA), some steroids and ATP were measured at different wavelengths individually, followed by a mixture of different concentrations of steroids with a constant level of ascorbate and vice versa. A change in E_{max} of either one or both the substances when mixed together indicates the formation of charge transfer complex (CTC). The CTC formation between AA and testosterone was greater than pregnenolone > cholesterol > progesterone > androstenedione. Testosterone did not form CTC with ATP except in the presence of low concentrations of AA. The results suggest the probable involvement of AA in the metabolism of steriods via its free radical and CTC formation. The results enable the postulation that tissue metabolism in steroid target organs is probably energized not only by high energy phosphate but also by the paramagnetic electron flow from monodehydroascorbic acid. This is the first report of its kind.