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## ADVANCE ABSTRACTS OF PAPERS

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1557. *E. Bergheim and G. W. Oertel*: Influence of G-6-PDH on the metabolism of DHEA in the guinea pig.
1558. *G. Hoffmann, G. Hoffmann-Treffz, B. Morsches, H. Holzmann and G. W. Oertel*: Metabolism of DHEA in the erythrocytes of psoriatics.
1559. *J. R. Strecker and Ch. Lauritzen*: Load-test for fetoplacental function with DHA-S and determination of plasma estrogens by radioimmunoassay.
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1622. *H. L. Fehm, K. H. Voigt, R. Lang, M. Schleyer and E. F. Pfeiffer*: Spontaneous release of immunoreactive ACTH from isolated rat pituitary cells.
1633. *A. Espinoza, I. Andresen and M. Kroll*: Biological ACTH determination in human plasma in the pg-range.
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1655. *K. H. Voigt, H. L. Fehm, R. Lang and E. F. Pfeiffer*: Degradation of ACTH by isolated adrenal cells: influence of plasma.

166. *A. Espinoza and M. Neuss*: Secretion dynamics and hormone synthesis of isolated human adenohipophyseal cells and hormonally active adenohipophyseal tumour cells.
167. *K. Demisch, M. Neubauer, J. Happ, J. Beyer and K. Schöffling*: Influence of tetracosactrin on plasma levels of testosterone, LH and FSH.

*Calcium Metabolism*

168. *K. Forster, L. Gozarin, J. D. Faulhaber, H. Minne and R. Ziegler*: Influence of parathyroid hormone and calcitonin on the lipolysis of human adipose tissue.
169. *E. Altenähr and E. Kampf*: Suppression of parathyroid gland activity by growth hormone in-vivo.
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172. *P. O. Schwille, U. Koch and N. M. Samberger*: Serum calcium fractions in subtotally thyroidectomized patients.
173. *M. A. Dambacher, Th. Lauffenburger, J. Guncaga and H. G. Haas*: Calcitonin in Paget's disease of bone: a study of the dose response to the human and salmon synthetic peptide.
174. *H. Schmidt-Gayk, H. Seitz, E. Ritz and E. Böhme*: Primary hyperparathyroidism: influence of renal function on urinary cyclic AMP.
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II. Medizinische Klinik der Universität München

AUTOMATED COMPETITIVE PROTEIN BINDING ANALYSIS  
AND RADIOIMMUNOASSAY OF SERUM CORTISOL  
WITHOUT PRIOR ORGANIC SOLVENT EXTRACTION\*

J. Braun, O. A. Müller and P. C. Scriba

*Problem:* So far competitive protein binding analysis (CPBA) and radioimmunoassay (RIA) of cortisol (F) necessitated extraction with organic solvents. The extraction procedure represents an obstacle for automatization. We have devised a new method, by which the three main steps of a CPBA or RNA for F-displacement from its serum protein bindings, the reaction with a binding reagent (CBG or antibody) and the bound/free separation — take place consecutively on the same dextran gel column. This principle favours automatization by simultaneous column-chromatography.

*Method:* A mixture of 100  $\mu$ l standard, respectively unknown, 100  $\mu$ l  $^3$ H-F and 100  $\mu$ l 0.1 N HCl are transferred by a micropump to an acidified (0.1 N HCl, pH = 1.0) dextran gel column (2 g Sephadex G-10). While F remains quantitatively bound to the gel, the proteins are eluted with 0.2 M Tris-HCl-buffer pH = 8.0. The steroid containing columns are subsequently equilibrated with CBG-serum or antibody, respectively, followed by the elution of the bound hormone with buffer. The free F is eluted with excess serum, followed by 0.1 N HCl. The column is now ready for the next assay. The time for the whole chromatography is 52 minutes. An automatization was derived from the automatization of the  $T_3$ -uptake-test and  $T_4$ -assay (1), allowing the measurement of 25 samples at a time. A sensitivity (CPBA) was chosen which permitted to read concentrations ranging from less than 1 to 20  $\mu$ g $^0$ / $o$  from the same standard curve.

*Results (CPBA):* The within assay precision (serum) was  $7.5 \pm 0.68 \mu$ g $^0$ / $o$  (SD, N = 21). The between assay precision (serum) was  $14.2 \pm 1.7 \mu$ g $^0$ / $o$ , with a variation coefficient of 12.1% (N = 46). The recovery of standards added to serum was  $96 \pm 5\%$ , all serum dilutions were parallel to the standard curve. Specificity was comparable to the F-CPBA using solvent extraction, however progesterone, 11-desoxy-cortisol, 7 $\alpha$ -OH-progesterone and testosterone show less interference, since they are adsorbed more strongly to the gel and exhibit less binding to CBG. Normal values: The 9 a. m. levels ranged from 4.5 to 20  $\mu$ g $^0$ / $o$  (mean: 11.1  $\mu$ g $^0$ / $o$ , N = 68); the mean F-level after an overnight dexamethasone suppression (2 mg p. o.) was  $2.6 \pm 0.8 \mu$ g $^0$ / $o$  (SD, N = 14). After a 4-hour infusion of 25U ACTH the serum-F rose to  $32.8 \pm 7.1 \mu$ g $^0$ / $o$  (N = 19). 30 minutes after a single i. v. injection of 25U ACTH the same subjects showed a similar rise up to  $29.6 \pm 8.2 \mu$ g $^0$ / $o$ . — The use of an antiserum instead of CBG-serum increases sensitivity and specificity depending on the quality of the antibody. The RIA-results will be compared with the results obtained by CPBA.

1. Horn, K. et al.: Z. anal. Chem. 259 (1972) 222.

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