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CONTENTS

ABSTRACTS OF SHORT PAPERS

Growth Hormone and Pituitary Tumors

1. *Ch. Hohbach and H. Ueberberg:* On the question of a specific secretory mechanism in the anterior pituitary.
2. *W. Saeger and D. Lüdecke:* Histologic and ultrastructural differential diagnosis of acidophilic adenomas and comparison with clinical data.
3. *M. Schleyer, K. H. Voigt, H. L. Fehm and H. Etzrodt:* Isolation and purification of porcine growth hormone.
4. *K. v. Werder, B. Schultz and S. Gallenberger:* Heterogeneity of pituitary and serum growth hormone in the rat.
5. *H. Etzrodt, M. Schleyer, H. L. Fehm, K. H. Voigt and E. F. Pfeiffer:* Radio-metric assay for animal growth hormone.
6. *S. Raptis, H. Hirth-Schmidt, K. E. Schröder and E. F. Pfeiffer:* The effect of beta-receptor blockade on exercise and arginine-induced growth hormone secretion in man.
7. *P. H. Althoff, J. Happ, V. Grabs, B. Schneider, J. Beyer and K. Schöffling:* The early rise of growth hormone in acromegalic patients following intravenous glucagon: sign for secondary hypothalamic acromegaly?
8. *C. Lucke and K. D. Morgner:* Sleep-induced growth hormone secretion: lack of fluctuations during the menstrual cycle.
9. *H. F. L. Meyer-Bahlburg:* Hypopituitary short stature as chronic handicap: psychological management.
10. *P. Heidemann, P. Stubbe and O. Buurman:* Effectiveness of a small uniform dose of human growth hormone during long-term treatment of hypopituitary dwarfism.
11. *O. Butenandt, R. Eder and F. Bidlingmaier:* Integrated growth hormone concentrations in boys with constitutionally delayed development.
12. *C. Schade, P. Meixner, P. H. Althoff, R. Simrock, M. Neubauer, J. Beyer and K. Schöffling:* Age-dependence of growth hormone response to hypoglycemia in Klinefelter patients with increased body height.
13. *D. Heesen, W. Hadam, K. Finke, R. Mies and W. Winkelmann:* Influence of TRH on HGH-secretion in patients with acromegaly or chronic renal insufficiency.
14. *W. Winkelmann, W. Hadam, D. Heesen, R. Mies, H. G. Solbach and W. Wiegelmann:* Pituitary function in patients with primary empty sella syndrome.

LH — RH

15. *W. Voelter, K. Zech and D. Gupta*: Synthesis of a peptide sequence Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ with biological activity of LH/FSH-RH.
16. *H. Kuhl, C. Rosniatowski and H.-D. Taubert*: The role of hypothalamic L-cystine arylamidase activity in the feed-back mechanisms of gonadotrophin release.
17. *H.-D. Taubert, J. S. E. Dericks-Tan and H. Kuhl*: Stimulation of hypothalamic L-cystine arylamidase activity by prostaglandins E₂ and F_{2α} in the rat.
18. *G. Leyendecker, L. Wildt and W. Nocke*: Radioimmunoassay of LH-releasing hormone.
19. *E. Keller, H. G. Dahlen, G. Schubring, W. Klemt, R. Richter, E. Friedrich, E. W. Joel, H. I. Wyss, A. E. Schindler, H. J. Staemmler and H. P. G. Schneider*: Human pituitary gonadotropin index (HPGI): statistical analysis of 413 LRH tests.
20. *J. Happ, J. Beyer, F. Szamak, M. Neubauer, K. Demisch and K. Schöffling*: LH, FSH, testosterone and estrogens in serum of healthy men after prolonged infusion of synthetic LH-RH.
21. *A. Römmler and J. Hammerstein*: Time-dependent alterations in pituitary responsiveness caused by LH-RH stimulations in man.
22. *J. Girard, J. J. Staub, J. B. Baumann, M. Stahl and P. W. Nars*: Assessment of hypothalamo-anterior pituitary secreting capacity with one single test.
23. *D. Lüdecke, P.-C. Czygan, R. Hebrmann and D. Schrader*: Combined function tests with LRF and TRF in correlation to pituitary processes confirmed by operation findings.
24. *H. K. Kley, W. Wiegelmann, H. G. Solbach and H. L. Krüskenper*: A combined test for evaluating the somatotropic, gonadotropic and adrenocorticotrophic function of the anterior pituitary gland.
25. *D. Schönberg, E. W. Joel, E. Jetter and J. Bierich*: Graded dose response of plasma HLH and HFSH to synthetic gonadotrophin releasing factor in boys and girls before and after puberty onset.
26. *R. Illig, M. Tolksdorf, G. Müerset and A. Prader*: Evaluation of the hypothalamic-pituitary-gonadal axis by LH-RH in children and adolescents with Klinefelter's and Turner's syndrome.
27. *S. Heller and H. Weber*: Influence on LH release by prostaglandin F_{2α} in the rat.
28. *J. Sandow and W. Heptner*: Positive feedback effect of small doses of LH-RH after chronic treatment.
29. *F. Elsaesser, F. Ellendorff, N. Parvizi and A. König*: Response of the pituitary and testes to LH-RH in the neonatal miniature pig.

Steroid Metabolism (Part I)

30. *D. Beckmann and H. Breuer*: Metabolism of estrogens in liver and kidney of minipigs of different age and sex.
31. *J. H. H. Thijssen, J. Lourens and G. H. Donker*: Biogenesis of production of oestrogens in males with cirrhosis of the liver.
32. *P. Ball and E. Knuppen*: Metabolism of ethynodiol and mestranol in man and interaction between 2-hydroxy-ethynodiol and epinephrine.

33. *H. M. Bolt*: Kinetics of mestranol in humans in relation to its estrogenic activity.
34. *W. Wortmann, J. Schenker and J. C. Touchstone*: Aromatization of ^3H -androstenedione in the rhesus monkey liver in vivo.
35. *P. Barlas, K. Adelung, R. Röhner, A. Weimer, E. Keller and A. E. Schindler*: The effect of drugs on the steroid metabolism of the normal placenta.
36. *W. D. Lehmann*: The influence of prostaglandins and beta-mimetic substances on the aromatization rate in human placentas.
37. *L. Raith, A. Wirtz and H. J. Karl*: 5α -reduction of testosterone in mammary tumors.
38. *E. R. Lax and H. Schriefers*: $\Delta^{4-3\beta}$ -hydroxysteroid dehydrogenase activity in rat liver: intracellular distribution and sex dependency.
39. *H.-G. Hoff, R. Ghraf, E. R. Lax and H. Schriefers*: Regulation of the activities of the enzymes involved in the metabolism of steroid hormones in rat liver: the effect of 19-nortestosterone and the influence of cyproterone acetate on the action of testosterone and 5α -dihydrotestosterone.
40. *R. Ghraf, U. Vetter and H. Schriefers*: Organ specific sex differences in 17β -hydroxysteroid dehydrogenase activities in the rat.
41. *M. Krieg, R. Szalay and K. D. Voigt*: Binding and metabolism of testosterone and 5α -dihydrotestosterone in bulbocavernosus/levator ani muscle of male rats: in vivo and in vitro studies.
42. *H.-J. Horst, M. Dennis, J. Kaufmann and K. D. Voigt*: In vivo uptake and metabolism of $^3\text{H}-5\alpha$ -androstane- 3α , 17β -diol (3α -diol) and of $^3\text{H}-5\alpha$ -androstane- 3β , 17β -diol (3β -diol) by human benign prostatic hypertrophy.

Intersexuality, Cryptorchidism

43. *K. Rager, D. Schönberg and D. Gupta*: Hormonal biotransformation in the testicular feminization syndrome.
44. *A. E. Schindler, A. Horvath and I. Sellin*: Steroid metabolism in the testes of a patient with testicular feminization.
45. *A. E. Schindler, E. Keller, E. Friedrich, E. R. Jaeger-Whitegiver, E. W. Joel, H. Pösl and E. Stolz*: Endocrine studies in patients with testicular feminization.
46. *H. Stolecke, R. A. Pfeiffer, H. A. Hienz and P. Strohmenger*: "Sex reversal" in a case of mixed gonadal dysgenesis with dicentric Y-chromosome (45, X/46, X, dic (Yq)).
47. *H. Cramer, R. Buchholz, E. Daume, H. Kalbfleisch and G. Sturm*: Mixed gonadal dysgenesis: male hermaphroditism with 46, XY/45, XO/46, X dic Y mosaicism.
48. *F. Majewski, J. R. Bierich, M. Barz, W. F. Haberlandt and M. Stoeckenius*: On the frequency of malformations in the different forms of Turner's syndrome.
49. *G. Mürset, A. Prader and W. Schmid*: Correlation between phenotype and karyotype in Turner's syndrome.
50. *U. Hirche*: Genitography: diagnostic procedure in intersex problems.
51. *A. Attanasio, K. Jendricke, J. R. Bierich, D. Gupta, G. Bulle, A. Flach, I. Reisert and E. Tonutti*: Cryptorchidism: clinical, hormonal and histological studies.

Renin-Angiotensin-Aldosterone System, Adiuretic Hormone, Catecholamines

52. E. Uhlich, P. Weber and U. Gröschel-Stewart: Angiotensin-stimulated vaso-pressin release in man; radioimmunologically determined plasma levels of vasopressin.
53. G. Benker, H. Kaulhausen, W. Oehm and H. Breuer: Renin activity and concentration of aldosterone in plasma during the menstrual cycle.
54. H. Kaulhausen, W. Mühlbauer, K. J. Beck and H. Breuer: The effect of 17 α -ethynodiol-17 β on plasma renin activity in ovariectomized women.
55. W. Oelkers, M. Schönesöfer, A. Blümel, H. Kutschke, H. Kreiser and R. Sterzel: Effects of progesterone and of four synthetic gestagens on the renin-angiotensin-aldosterone system in man.
56. H. J. Kramer and F. Krück: Natriuretic activity in plasma following extracellular volume expansion.
57. H. Thomas, D. Müller-Enoch and E. R. Lax: 3-hydroxy-4-methoxymandelic acid, a new metabolite of adrenaline and noradrenaline.

Adrenal Cortex

58. G. Müller, W. Adelsen, K. Leybold, W. Müller-Ruchholtz, K. Schemmel and L. S. Tschirch: Adrenal function in experimentally induced adrenalitis.
59. U. Schwedes, H. Wehner, U. Leuschner, K. Schöffling and K. H. Usadel: Development and function of isologous transplants of cell suspensions of fetal adrenal glands in the rat.
60. E. Mäusle, H. Scherrer and B. Kubatsch: Ultramorphometric studies regarding the effect of sex hormones on the adrenal cortex of the rat.
61. R. Mies, D. Heesen and W. Winkelmann: Disorders of Leydig cell function in primary adrenal insufficiency.
62. E. Nieschlag and H. K. Kley: Androgens in response to HCG in men with normal, suppressed and impaired adrenal function.
63. W. v. Petrykowski and P. Burmeister: Familial glucocorticoid insufficiency.
64. J. Braun, O. A. Müller and P. C. Scriba: Automated competitive protein binding analysis and radioimmunoassay of serum cortisol without prior organic solvent extraction.
65. W. Vielhauer, K.-H. Gless and P. Vecsei: Radioimmunoassay of 11-deoxycortisol in human plasma.
66. A. T. A. Fazekas, J. Homoki and W. M. Teller: Studies of the effect of ACTH on the cortisol content of different peripheral tissues and the adrenals in guinea pigs.
67. B. P. Lisboa, M. Strassner, L. Nocke-Finck, H. Breuer and J. M. Bayer: Studies on the metabolism of steroid hormones in a virilising tumour of the adrenal cortex.
68. D. Engelhardt, L. Raith and H. J. Karl: Female pseudohermaphroditism and hypertension due to 11 β -hydroxylase deficiency in two siblings.
69. F. Elsaesser, P. Stubbe and A. König: Plasma testosterone, androstenedione and progesterone levels in newborn infants, in children and as an index of congenital adrenal hyperplasia.
70. K. Holzmann, G. Wittenbecher and H. Mickan: Prenatal diagnosis of adrenogenital syndrome.
71. P. Edelmann and D. Gupta: Hormonal investigations in adolescent idiopathic scoliosis.

Thyroid Gland

72. *J. Herrmann, H. J. Rusche and H. L. Krüskenper:* Triiodothyronine and thyroxine kinetics in rabbits immunized with thyroid hormones.
73. *K. Horn, K. R. Blümel, D. Koeppen and P. C. Scriba:* Radioimmunoassay for T₃ in serum: necessity of prior extraction.
74. *R. D. Hesch, A. v. z. Mühlen and J. Köbberling:* T₃ levels and the TRH test in the early phase of treatment of thyrotoxicosis with antithyroid drugs.
75. *M. Hüfner and H. Munzinger:* Functional parameters of the pituitary-thyroid system in thyroidectomized patients under different conditions of substitution.
76. *R. Hehrmann, C. Schneider and I. H. Tewfik:* The efficiency of triiodothyronine therapy in athyroid patients.
77. *J. Herrmann, H. J. Rusche, W. Wildmeister, H. A. Horster and H. L. Krüskenper:* Triiodotyronine and thyroxine serum levels after administration of T₃/T₄ mixtures.
78. *K. W. Wenzel and H. Meinhold:* Serum T₄ levels measured by radioimmunoassay in a single 3 mg L-thyroxine test in comparison to the classical L-triiodothyronine test for thyroid suppressibility: evidence of lower toxicity during thyroxine suppression.
79. *A. Ishihara, U. Loos, G. Rothenbuchner and E. F. Pfeiffer:* Thyroxine assay in urine as an index for the diagnosis of thyroid function.
80. *K. Joseph, J. Mahlstedt and Th. Kranz:* Correlation of serum TBG concentrations with the results of conventional in-vitro tests.
81. *P. G. Livesey, F. Erhardt, K. Horn and P. C. Scriba:* Isolation and radioimmunoassay of thyroxine binding globulin.
82. *G. Rabenhorst, K. Lennert, W. Bindeballe, Hg. Lahrtz, K. Schemmel and G. Waschulzik:* Cytology, cytophotometrical measurement of Feulgen DNA and cytochemistry of cold nodules.
83. *J. Bommer, E. Ritz, B. Krempien, O. Mehls and B. Schulze:* Synthesis of chondroitin sulfate in experimental hypothyroidism.

Steroids: Transport Proteins and Determinations

84. *R. K. Wagner and W. Rüffert:* Assay of CBG and SHBG by agar gel electrophoresis.
85. *H. Kappus and H. M. Bolt:* Irreversible binding of ethynodiol metabolites to proteins and nucleic acids.
86. *D. Egert, W. Jonat and H. Maass:* Dependence of in-vitro progesterone metabolism on protein binding in the rat uterus.
87. *W. Geisthövel and K. D. Morgner:* Comparison of the binding capacity of the sexual hormone-binding globulin, free testosterone and stimulation of Leydig cells in patients with hypergonadotropic and hypogonadotropic hypogonadism.
88. *E. Nieschlag, H. K. Kley and K. H. Usadel:* Biological activity and metabolism of testosterone bound to circulating antibodies.
89. *E. Friedrich, E. R. Jaeger-Whitegiver, M. Bieder, H. Halverscheidt, B. Penke, P. Pallai, E. Keller and A. E. Schindler:* Standardization of specific radioimmunoassays for plasma progesterone, estradiol, estrone and androstenedione.

90. *B. Hoffmann and R. Hamburger*: Determination of progesterone in milk by radioimmunoassay and its application for the diagnosis of bovine fertility.
91. *P. Kemeter, H. Salzer, G. Breitenecker and F. Friedrich*: Progesterone, oestradiol- 17β and testosterone levels in the follicular fluid of tertiary follicles and Graafian follicles of human ovaries.
92. *E. R. Jaeger-Whitegiver, M. Bieder, U. Nickel, E. W. Joel, E. Keller, E. Friedrich and A. E. Schindler*: Plasma estrogens, progesterone and FSH/LH ratio in normal and abnormal cycles and in cycles under oral contraceptive treatment.

Carbohydrate and Lipid Metabolism

93. *G. Klöppel and H.-J. Schäfer*: Localization and identification of calcium in B-cells of mice. Morphologic-functional studies on insulin secretion.
94. *R. Landgraf, M. Klingenburg, M. Landgraf-Leurs, R. Hörl and I. Marschner*: Kinetics of insulin and glucagon release from the isolated pancreas under extreme nutritional conditions.
95. *V. Maier, M. Lambach, H. Laube and E. F. Pfeiffer*: On the mechanism of insulin secretion: in-vitro stimulation by butandioles.
96. *U. Krause, J. Beyer, K. H. Usadel and F. Stelzner*: Different insulin-producing tumors in a patient with polyadenomatosis of the islet cells.
97. *K. H. Usadel, U. Schwedes, U. Leuschner and K. Schöffling*: Development of isologous transplants of cell suspensions of the fetal pancreas in the rat.
98. *H. Bojar, K. Balzer, M. Stannek, W. Reipen and W. Staib*: Hormone sensitivity of isolated liver parenchymal cells.
99. *K.-G. Petersen, W. Doll, S. Steinhilber and L. Kerp*: Specific binding of insulin in the liver of steroid-diabetic mice.
100. *H. U. Tietze and R. D. Schmid*: Impaired regulation of cortisol and of epinephrine release in children with ketotic hypoglycemia.
101. *L. Tharandt, R. I. Diekers, N. Breuer and W. Staib*: Effects of testosterone on glucagon and lactate-induced gluconeogenesis studied with the model of isolated perfused (without hemoglobin) mice livers.
102. *C.-J. Estler and P. Mitznegg*: Effect of clomiphene on lipolysis.
103. *G. G. Hofmann, G. Schneider and L. Krick*: Lipolytic effect of L-triiodothyronine and L-thyroxine on insulin-stimulated fat cells of obese and normal-weight subjects.
104. *D. Heinz, G. J. Kremer, G. Hoffmann, M. Schirazi and G. W. Oertel*: metabolism of sulfoconjugated 7α - 3 H-DHEA in hyperlipoproteinemia.

Prolactin, HCS

105. *M. Schmidt-Gollwitzer and B. B. Saxena*: A homologous radioimmunoassay of human prolactin.
106. *W. Wuttke and M. Fenske*: Effects of hypothalamic noradrenaline depletion on serum prolactin and LH in male rats.
107. *A. v. z. Mühlen, G. Schwinn, J. Köbberling, R. D. Hesch, F. Adlkofer and H. Schleusener*: Prolactin response to TRH and chlorpromazine in normal subjects and in patients with pituitary diseases.
108. *K. v. Werder, C. R. Pickardt, B. Glöckner, M. Gottsmann, H. K. Rjosk and P. C. Scriba*: Prolactin secretion in patients with pituitary tumors.

109. *H. G. Bohnet, H. G. Dahlen and H. P. G. Schneider*: Hyperprolactinemia and pulsatile LH fluctuation.
110. *P. Delwoye, H.-D. Taubert, O. Jürgensen, M. L'Hermite, J. Delogne and C. Robyn*: Influence of circulating prolactin increased by a psychotropic drug on gonadotrophin and progesterone secretion.
111. *D. Schams, U. Andreae, V. Reinhardt and H. Karg*: The influence of TRH on release of prolactin and lactation in the bovine.
112. *W. Geiger and R. Kriegel*: Proteohormones with growth promoting activity in maternal and fetal serum and amniotic fluid during pregnancy.
113. *R. Goebel, H. K. Rjosk and K. v. Werder*: Concentration of prolactin and estrogens during suppression of puerperal lactation in humans.
114. *H. K. Rjosk, K. v. Werder and R. Goebel*: Serum prolactin levels in galactorrhea.
115. *W. Geiger, H. Würz and M. Höfinghoff*: Investigations on the appearance of human chorionic somatomammotropin with high molecular weight in serum.
116. *K. Kreikenbaum, F. Elsaesser and N. Hähn*: Serum levels of HCS and progesterone during normal and pathological pregnancies.

TRH — TSH

117. *W. Voelter, K. Zech and D. Gupta*: Structure of TRH in water solutions.
118. *R. Hehrmann, J. Mulden and S. Westphalen*: The TRH infusion test.
119. *H. Wagner, H. Vosberg, K. Böckel, M. Hrubesch, G. Grote and W. H. Hauss*: Influence of age on response of TSH to thyrotropin releasing hormone in normal subjects.
120. *E. G. Weber*: Ultrastructural changes of the thyroid of *Notophthalmus viridescens* after hypophysectomy and TSH and prolactin stimulation.
121. *H. Schleusener, G. Fangerau, F. Adlikofer, K. W. Wenzel and H. Meinhold*: Incidence of latent hypothyroidism after subtotal thyroidectomy.
122. *J. Beyer, F. Kollmann, J. Happ, H. Menzel, V. Grabs and F. Ball*: TSH secretion in children with different diseases of the thyroid gland.
123. *K. v. Puttkamer, M. Ranke, D. Schönberg and D. Gupta*: Thyrotropin releasing hormone induced TSH response in children with primary congenital hypothyroidism under treatment.
124. *W. Bindeballe, R. Gutekunst, Hg. Labrtz, G. Rabenhorst, K. Schemmel and L. Weisbecker*: Examination of thyroid function in patients suffering from Graves' disease after long-term therapy with antithyroid drugs in combination with thyroid hormones.
125. *S. Zabransky, A. v. z. Mühlen and J. Köbberling*: Influence of dextro-triiodothyronine on thyrotropin secretion in man.
126. *F. Adlikofer, H. Schleusener, P. Kotulla, J. Faulhaber, U. Loos, G. Rothenbuchner and J. Birk*: Heterogeneity of TSH activity.
127. *J. Golstein, L. Vanhaelst, M. Bonnyns, G. Rothenbuchner, J. Birk and E. F. Pfeiffer*: TSH determination in rat serum by a homologous radioimmunoassay.
128. *A. v. z. Mühlen, M. Lammers, J. Köbberling and R. D. Hesch*: TSH, corticosterone and Ts in rats under various endogenous and environmental conditions.

Gonadotropins

129. *K. Schmidt-Gollwitzer, B. B. Saxena, M. Schmidt-Gollwitzer and J. Nevinny-Stickel:* Radioimmunological determination of FSH, LH and HCG with antibodies specific for β -subunits.
130. *D. Graesslin, Chr. Weise, W. Braendle and F. Leidenberger:* The different molecular forms of human gonadotropins.
131. *N. Parvizi, F. Ellendorff and D. Smidt:* LH secretion during the estrous cycle and the effects of pentobarbital in the miniature pig.
132. *F. Ellendorff, F. Elsaesser, A. Hartjen, N. Parvizi and D. Smidt:* Plasma LH and testosterone levels in response to copulation in the male miniature pig.
133. *K.-D. Döhler and W. Wuttke:* Gonadotropins, prolactin and progesterone in immature rats.
134. *O. Jürgensen, R. Karschnia, A. Baloussa, I. Fritz, E. May, C. Robyn and H.-D. Taubert:* Testing the reactivity of the hypothalamic-pituitary axis with the clomiphene stimulation test in women with secondary amenorrhea.
135. *P. Doerr and K. M. Pirke:* Response of plasma oestradiol, oestrone and testosterone to the administration of HCG, fluoxymesterone and dexamethasone in normal adult males.
136. *M. Neubauer, K. Demisch, F. Bidlingmaier and K. Schöffling:* Effect of HCG on plasma concentrations of estrone, estradiol and testosterone in male hypogonadism.
137. *F. Friedrich, P. Kemeter and H. Salzer:* Ovulation inhibition with human chorionic gonadotrophin.
138. *V. Pahnke, F. Leidenberger and J. Künzig:* Correlation between rat testicular HCG (LH)-binding capacity, Leydig cell number and testicular secretory function throughout pubescence.
139. *W. Braendle, P. Brucks, D. Graesslin and G. Bettendorf:* Receptor analysis of gonadotrophin-treated ovaries.
140. *F. Leidenberger, V. Pahnke, W. Braendle and D. Graesslin:* Attempts on the solubilization of LH-receptors from Leydig cell membranes.
141. *L. Wildt, G. Leyendecker and W. Nocke:* Binding of FSH- J^{125} to rat ovarian homogenates.

Steroid Receptors and Effects

142. *K. Klinga and B. Runnebaum:* Determination of estradiol- 17β binding proteins in human myometrium at term of pregnancy.
143. *K. Klinga, C. Hassenbach, J. Hohneck, H. Heep and B. Runnebaum:* Relationship between estradiol- 17β receptor and free estradiol and between corticosteroid binding globulin and progesterone in human myometrium during the menstrual cycle.
144. *H. J. Kyrein and B. Hoffmann:* Effects of in-vivo pretreatment with anabolic preparations with estrogenic activity on uterine estrogen receptors of calves.
145. *J. Poortman, J. H. Prenen, J. H. H. Thijssen and F. Schwarz:* Interaction of Δ^6 -androstene- 3β , 17β -diol with oestrogen and androgen receptors in human uterine tissue and mammary tumour tissue.

146. *A. Hughes* and *P. W. Jungblut*: The effect of estradiol and mesterolone on the concentrations of cytoplasmic estrogen and androgen receptors.
147. *P. I. Szendro*, *M. Little* and *P. W. Jungblut*: The biosynthetic sequence of the various forms of cytoplasmic estradiol receptor.
148. *P. W. Jungblut* and *G. Bayerköhler*: Extraction of "nuclear" estradiol receptors from purified nuclei.
149. *P. Benes* and *G. W. Oertel*: Effects of DHEA and its sulfoconjugates upon c-AMP in human erythrocytes.
150. *H.-G. Dahnke* and *K.-O. Mosebach*: Effect of testosterone on ^3H -labelling of the RNA and proteins in the prostate and seminal vesicles of immature rats after administration of ^3H -uridine.
151. *A. Edelmann*, *C. Burgmann*, *R. v. Berswordt-Wallrabe* and *K.-O. Mosebach*: Influence of growth hormone and prolactin (bovine) on the uptake of testosterone and progesterone into the prostate and seminal vesicles of immature rats.
152. *F. Lehmann*, *I. Just-Nastansky*, *B. Behrendt*, *P.-J. Czygan* and *G. Bettendorf*: Influence of exogenous steroids on corpus luteum function.

Steroid Metabolism (Part II)

153. *J. C. Plasse* and *B. P. Lisboa*: Quantitative study of the metabolism of Δ^4 -3-oxo-C₁₉-steroids in the rat prostate.
154. *U. Hoffmann* and *B. P. Lisboa*: Metabolism of Δ^4 -3-oxo-C₁₉-steroids in the rat uterus.
155. *O. Freire*, *M. Breckwoldt* and *B. P. Lisboa*: In-vitro study on the metabolism of oestrone and testosterone by the rhesus monkey kidney.
156. *B. P. Lisboa*, *M. Strassner*, *C. Wulff* and *U. Hoffmann*: 5 β -reductase in the human fetal brain.
157. *E. Bergheim* and *G. W. Oertel*: Influence of G-6-PDH on the metabolism of DHEA in the guinea pig.
158. *G. Hoffmann*, *G. Hoffmann-Treffz*, *B. Morsches*, *H. Holzmann* and *G. W. Oertel*: Metabolism of DHEA in the erythrocytes of psoriatics.
159. *J. R. Strecker* and *Ch. Lauritzen*: Load-test for feto-placental function with DHA-S and determination of plasma estrogens by radioimmunoassay.
160. *H. J. Künzig*, *W. Geiger* and *P. Gwuzdz*: Effect of DHEA-S on the plasma level of estrone, estradiol-17 β and estriol in the last trimenon of pregnancy.

ACTH

161. *R. Lang*, *I. Hilwig*, *H. L. Fehm*, *K. H. Voigt* and *E. F. Pfeiffer*: Monolayer culture of rat anterior pituitary cells as a model for studies of ACTH.
162. *H. L. Fehm*, *K. H. Voigt*, *R. Lang*, *M. Schleyer* and *E. F. Pfeiffer*: Spontaneous release of immunoreactive ACTH from isolated rat pituitary cells.
163. *A. Espinoza*, *I. Andresen* and *M. Kroll*: Biological ACTH determination in human plasma in the pg-range.
164. *O. A. Müller*, *X. Baur*, *I. Marschner*, *P. Schwandt*, *P. C. Scriba* and *P. Weisweiler*: ACTH bioassay with isolated rat adrenal cells: ACTH activity of the porcine lipotropic peptide B.
165. *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 adenohypophyseal cells and hormonally active adenohypophyseal 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. Gozariu, 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.
170. *H. R. Henrichs, H. H. Heissmeyer, R. Karg, K.-G. Petersen, A. Petrin and L. Kerp*: Dose response relation between synthetic salmon calcitonin and gastric acid output with and without stimulation by pentagastrin.
171. *P. O. Schwille, N. M. Samberger and D. Scholz*: Pentagastrin-induced changes in plasma calcium fractions and pancreatic glucagon in humans.
172. *P. O. Schwille, U. Koch and N. M. Samberger*: Serum calcium fractions in subtotal 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.
175. *A. Schulz and G. Delling*: Long-term effect of calcitonin on delayed bone mineralization and bone remodelling in experimental hypoparathyroidism.

ABSTRACTS OF MAIN PAPERS

<i>L. Krulich</i> : Hypothalamic regulation of the secretion of growth hormone	p. 179—180
<i>D. Schönberg</i> : Growth hormone in plasma; levels under physiological conditions and after provocative stimuli	p. 181—182
<i>E. R. Froesch, B. Morell, J. Zapf, A. E. Zingg, C. Meuli, U. Schlumpf, R. Heimann, E. Eigenmann and R. E. Humbel</i> : NSILA-S: Insulin-like properties, receptor binding activities and relationship to somatomedin p. 183—184	
<i>W. Lenz</i> : Prenatal sex differentiation: genetic and hormonal control p. 185—186	
<i>M. Ammermann and R. A. Pfeiffer</i> : Chromosomal aberrations and intersexuality	p. 187—188
<i>H. Nowakowski</i> : Hermaphroditismus verus	p. 189
<i>A. Prader</i> : Pseudohermaphroditismus masculinus	p. 190—191
<i>W. Blunck</i> : Female Pseudohermaphroditism	p. 192—193
<i>A. A. Erhardt</i> : Psychosexuality; psychological problems in intersexuality	p. 194
<i>J. L. Van den Brande and M. V. L. Du Caju</i> : Plasma somatomedin activity in children	p. 195—196
<i>A. Flach</i> : Plastic operations in cases of intersexuality	p. 197

I.¹ und II.² Medizinische Klinik der Universität München

ACTH BIOASSAY WITH ISOLATED RAT ADRENAL CELLS:
ACTH ACTIVITY OF THE PORCINE LIPOTROPIC PEPTIDE B*

O. A. Müller², X. Baur², I. Marschner², P. Schwandt¹,
P. C. Scriba² and P. Weisweiler¹

The recently developed ACTH bioassay using isolated adrenal cells (1) represents a progress as to sensitivity and assay capacity. ACTH contaminations have to be estimated in lipotropin preparations during the search for a separate lipotropic pituitary hormone.

Methods: Rat adrenal cells were isolated as described (1). After 0.1 ml of standards or unknowns were added to 0.9 ml cell suspension ($8-15 \times 10^4$ cells/ml), the mixture was incubated for 2 hours at 37° C, followed by fluorometric determination of corticosterone. The assay sensitivity depends on careful preparation of the cells with the aim of little cell debris and a low basal corticosterone level. The addition of Trasylol® to the ACTH standards enhanced sensitivity, enabling us to measure 1—2 pg ACTH per vial. Reproducibility was good in contrast to other bioassays. — The preparation of the lipotropic fractions tested has been published (2).

Results: Despite adequate sensitivity of the assay, almost no ACTH activity was found in the serum of normal controls, probably due to the presence of ACTH binding factors (3). In addition the recovery of exogenous ACTH (both of synthetic 1—23 or 1—39 and of homologous porcine 1—39 peptide) was only 20—50%. Further, the high endogenous ACTH activity estimated in Nelson's syndrome by this method was up to 10 times lower than measured in the Lipscomb-Nelson assay. — In contrast to the findings with serum, complete recovery of exogenous ACTH from a medium containing pituitary extracts was observed. The ACTH content of the different lipotropic porcine pituitary fractions depended on the degree of purification: in raw extracts (fraction G) we found 0.1—0.4% ACTH, calculated on a weight basis. In the most purified and lipolytically most active fraction (peptide B) we found only ACTH activities of approximately 10 pg per 100 µg fraction. This demonstrates that ACTH cannot be the lipolytic principle of peptide B.

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