«priming» der infantilen Holtzman-Ratten führt zu einer erhöhten LH-Sensibilität der pseudograviden Ovarien, so dass wir mit deutlichen Effekten rechnen durften.

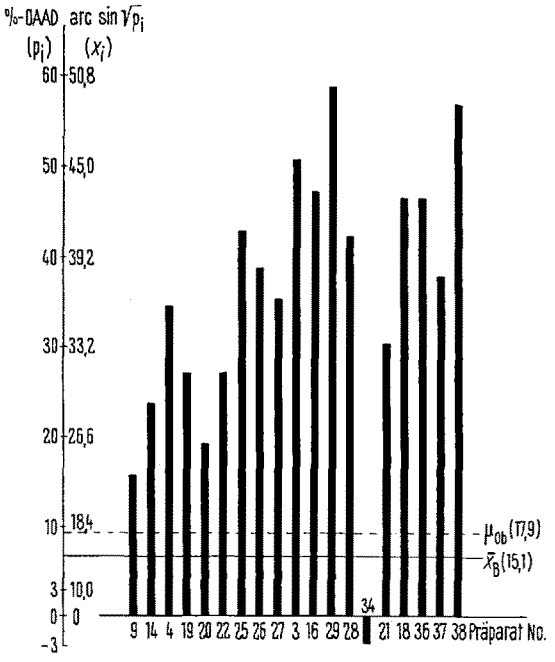

Fig. 2. Serum-LH-Werte (OAAD-%) nach Belastung vorbehandelter ovarektomierter Ratten mit humanen Hypothalamusextrakten verglichen mit dem mittleren Basiswert der Rechts-Links-Differenz nicht behandelter pseudogravider Tiere; in arcsin $\sqrt{p_i}$-Werten: $\bar{x}_B = 15{,}1$ ($s_B^2 = 5{,}5$; $n = 7$) mit oberer 99%-Vertrauensgrenze $\mu_{Ob.} = \bar{x} + ts/\sqrt{n} = 17{,}9$ entsprechend 9,4 OAAD-%.

Die Figur 2 stellt die Ergebnisse von 19 Belastungen mit menschlichen Hypothalamus-Extrakten dar. Die OAAD-Prozente wurden auf den mittleren Basiswert einer Vergleichsbestimmung des Ascorbinsäuregehaltes von rechtem und linkem Ovar pseudogravider Holtzman-Ratten[11] bezogen (vgl. Figur 2).

Alle oberhalb $\mu_{Ob.}$ ($\alpha = 0{,}01$) liegenden Werte, und das sind alle bis auf Fall 34, in dem keinerlei LH-Aktivität nachgewiesen werden konnte, belegen sehr eindeutig den Nachweis einer LH-Releasing-Aktivität in den Extrakten menschlicher Hypothalami. Die Aufgabe der Zukunft besteht darin, die Molekularstruktur speziell des LH-RF aufzuklären und einen Weg zu seiner Synthese zu finden, damit mögliche neue und bessere Wege zur Behandlung physiologischer und soziologischer Probleme der Reproduktion beschritten werden können[14].

Summary. There is evidence for LH-releasing-factor (LH-RF) in 18 crude acid extracts of human median eminence preparation following the procedure described by RAMIREZ and MCCANN.

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Hydrolysis of DHEA Sulphate after Oral Application

In recent investigations[1,2], the reabsorption of free and conjugated C_{19}-steroids from human intestines has been demonstrated in vivo. From such experiments it was concluded that double-labelled dehydroepiandrosterone (DHEA) sulphate is completely but slowly reabsorbed through the intestinal wall by virtue of its conversion to the lipophile steroid sulphatide. Hence, the oral application of DHEA sulphate should be as effective as the i.v. infusion, provided the conjugate reaches the small intestines without breakdown or alteration of the molecule. In order to obtain pertinent information, a fasting 23-year-old female subject was given orally 6.45 μg 4-^{14}C-DHEA with 493,000 dpm and 0.05 μg Na-7α-^{3}H-DHEA sulphate with 1,050,000 dpm dissolved in 20 ml water. Following the administration 91 ml of duodenal juice were collected over 45 min by means of a tube laying in the upper duodenum. The sample was processed by routine methods[1,3]. While free steroids were extracted with 3 times 2 volumes of chloroform, the combined extracts being extensively washed with alkali and water, total steroid conjugates were removed by extraction with ethyl acetate after addition of 50% (w/v) ammonium sulphate and acidification to pH 1. For cleavage of the conjugates the solvolysis in ether-perchloric acid[4] proved adequate. The fractions of free and liberated steroids were subjected to thin layer chromatography on silica gel G in chloroform-dioxane (94:6 v/v) (A) and the different labelled compounds rechromatographed on aluminum oxide in cyclohexane-butylacetate-*n*-butanol (50:45:5 v/v) (B). Subsequently, the individual steroids were converted into their corresponding 2,4-dinitrophenylhydrazones[5] and chromatographed in system (A). Radioactive zones on chromatograms were localized in a Berthold Dünnschicht-scanner LB-2720, while simultaneous counting of ^{3}H and

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^{14}C in aliquots of the various fractions was performed in a Packard Tricarb spectrometer 3004[6].

The fraction of free steroids, obtained from 91 ml of duodenal juice contained 18,304 dpm ^{14}C and 3,789 dpm ^{3}H. After separation into individual steroids a total of 17,526 dpm ^{14}C and 3,691 dpm ^{3}H could be detected in the 3 different compounds, of which DHEA represented 95.5% and 65.2% respectively. In addition to DHEA 2 unknown 17-ketosteroids with practically no ^{14}C-activity were isolated from the fraction of free steroids. In the very non-polar compound (Rf in system A = 0.86, in system B = 0.53 (androst-4-ene-3,17-dione: 0.80 and 0.47) 14.2% of ^{3}H-activity were found, as compared with 20.6% in the compound more polar than DHEA (Rf in system A = 0.19, in system B = 0.14 (DHEA: 0.48 and 0.32)). The fraction of steroid sulphates with 429,000 dpm ^{3}H in contrast consisted exclusively (98.1%) of DHEA sulphate.

From the recovery of merely 3.54% of administered free 4-^{14}C-DHEA the hydrolysis of 7α-^{3}H-DHEA sulphate was calculated to have reached 9.89%. The rapid disappearance of free 4-^{14}C-DHEA and liberated 7α-^{3}H-DHEA, probably due to immediate reabsorption from the duodenum (or the stomach?) is in agreement with previous findings[7]. Conversely, the reabsorption of DHEA sulphate proceeded very slowly, as indicated by a 40.93% recovery of ^{3}H-activity, thus leaving ample time for acid hydrolysis of DHEA sulphate in the stomach. Furthermore, in view of the collection of 91 ml duodenal juice during 45 min corresponding to 2–3 l/24 h (2–4 l/24 h in normal adults) the sampling may be considered sufficiently complete in order to exclude major losses of steroids through the intestinal passage. Since it can be assumed that the remaining portion of DHEA sulphate is exposed to hydrochloric acid in gastric juice for an additional period of time, the 10% hydrolysis, observed over 45 min, may actually represent a minimum. The isolation of 2 unknown 17-ketosteroids from the fraction of ^{3}H-labelled free steroids reflects the well-known formation of artefacts during acid hydrolysis of DHEA sulphate[8]. Whereas the non-polar compound exhibited chromatographic properties similar to those of androsta-3,5-diene-17-one, the identity of the more polar material could not as yet be established. No 17-ketosteroid resembling 3β-chloro-androst-5-ene-17-one could be detected.

Zusammenfassung. Nach oraler Gabe von 4-^{14}C-DHEA und Na-7α-^{3}H-DHEA-sulfat liessen sich mittels einer Duodenalsonde 91 ml Duodenalsaft innerhalb von 45 min gewinnen, aus denen man u.a. ^{3}H-markierte, freie 17-Ketosteroide isolierte. Anhand der Ausbeute an 4-^{14}C-DHEA konnte eine zumindest 10% betragende Hydrolyse verabreichten DHEA-sulfats nachgewiesen werden.

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Adrenocorticotropic Effect in Humans of a Synthetic β^{1-24} Corticotrophine Derivative

Synthetic β^{1-24} corticotrophine, consisting of the first 24 amino acids of β^{1-39} corticotrophine, possesses the full biological activity[1]. The adrenocorticotropic effect of β^{1-24} corticotrophine can be enhanced by replacement of certain amino acids[2]. A derivative of β^{1-25} corticotrophine, synthesized by BOISSONAS et al. is more active in humans blocked by dexamethasone than β^{1-24} corticotrophine[3,4].

We investigated the steroidogenic potency of a new peptide related to β^{1-24} corticotrophine, which has been synthesized by TESSER and SCHWYZER[5,6]. In D-serine^{1}-17,18 diornithine corticotrophine 1-24 tetracosactid, the arginine residues 17 and 18 are replaced by ornithine. Furthermore L-serine^{1} is replaced by D-serine^{1}. It has been shown that this derivative has a more potent action in animals than β^{1-24} corticotrophine[7]. The increase of the secretion of corticosterone into the adrenal vein of hypophysectomized rats is 10 times higher with a 4-fold prolonged effect.

Methods. β^{1-24} corticotrophine tetracosactide, subsequently named 30920-Ba, and D-serine^{1}-17,18 diornithine β^{1-24} corticotrophine tetracosactide, subsequently named 40401-Ba, were injected into healthy volunteers or patients without evidence of endocrinopathy, which were not blocked by dexamethasone. In crossover experiments all of them received the ACTH preparations within 1 week at the same dose level of 1 mg or 2 mg i.m. All experiments were started at 07.30. The urinary 17-hydroxycorticoids (HOC) were analysed by the method of LIDDLE et al.[8]. The plasma 11-hydroxycorticoids were measured with a modified method of MATTINGLY, the whole procedure being carried out at constant temperature of 25 °C[9].

Results. The effect of 2 mg 30920-Ba and 2 mg 40401-Ba on the urinary excretion of 17-hydroxycorticoids is shown in Figure 1. Measuring the 24 h excretion in 12 persons, there is a rise from 5.5 $\pm$ 0.75 mg to 8.0 $\pm$ 1.4 mg after 30920-Ba. Following the administration of 40401-Ba, the 17-hydroxycorticoids in urine increase from 6.4 $\pm$ 0.7 mg to 18.9 $\pm$ 3.7 mg. The difference of the values before and

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