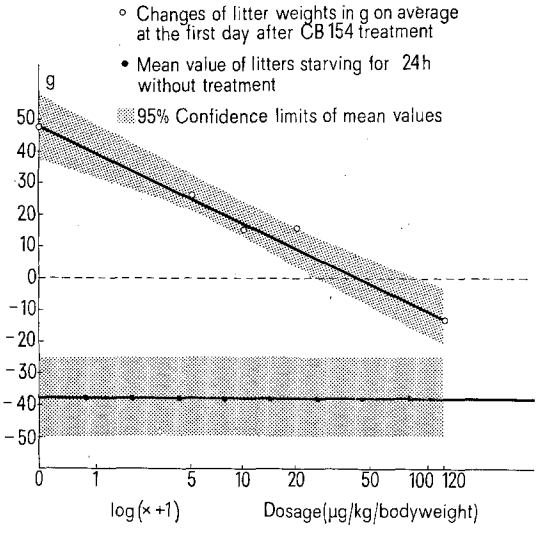
of life the pups were weighed daily at 08.00 and 17.00 h. The gain of weight of the pups within 24 h served as a direct measure for the amount of lactation of a bitch. A solution of CB 154 was administered i.m. to the bitches after weighing the pups in the evening of the 12th day post partum. The dosages used were 5, 10, 20 and 120 µg per kg bodyweight. For controls 1 group of bitches remained untreated. In another group the pups were removed at the same time for 24 h in order to show the effect of starvation.



Dose response curve for lactation inhibition by CB 154 i.m.

Results and discussion. The dose-response relation of CB 154 on the change of weight of pups is presented in the Figure and shows an inhibition of lactation in bitches. The statistical evaluation of the results was carried out by variance analysis. The regression of the curve is highly significant with $p < 0.001$. The ED_{50} (50% reduction of weight in comparison with untreated controls) of the inhibition of lactation in bitches was calculated to be 6 µg/kg bodyweight (95% confidence limit = 4–12 µg/kg).

These experiments show that CB 154 suppresses the lactation in the bitch and allow the assumption that prolactin is necessary to maintain lactation in bitches.

Zusammenfassung. Es wurde gezeigt, dass bei Hündinnen die Lactation durch den Prolactin-Sekretionshemmer 2-Br-α-Ergokryptin-methanesulfonat (CB 154, Sandoz) gehemmt wird. Gemessen über 24 Stunden beträgt die ED_{50} i.m. 6 µg/kg.

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Distribution of Potassium in Rat Adrenal Zones studied by Electron Probe X-Ray Microanalysis

Several factors are well known to be involved in the regulation of aldosterone production[1,2], still the mechanism of the regulation is far from being clarified. Experimental data from the literature[3] and from our previous work[4] yielded indirect arguments for the assumption that changes of the potassium and sodium content of the Zona glomerulosa might be the final stimuli for the regulation of aldosterone production.

The main obstacle to demonstrating potassium within tissues and cells lies in its excellent solubility in water and other solvents. At present no histochemical method providing reliable localization of potassium is available. Attempts have recently been made to use the electron probe X-ray microanalyzer to measure potassium content in tissue sections[5,6]. Kriz et al.[7–9] succeeded in determining relative potassium concentrations in different portions of the nephron.

We have made an effort to use the electron probe X-ray microanalyzer to study the distribution of potassium in the adrenal Zona glomerulosa and in the outermost cells of the Zona fasciculata.

26 male CFE rats weighing 150 g were decapitated. The adrenal glands were frozen to the specimen holders of a cryostat by means of isopentane chilled with liquid nitrogen, cut at 6 µm, and lyophilized. Quarz slides were coated with aluminium (ca. 100 Å), the frozen-dried cryostat sections were pressed on it, and coated again with aluminium.

The potassium K_α radiation was analyzed in a JXA-5 microanalyzer. The diameter of the electron beam was

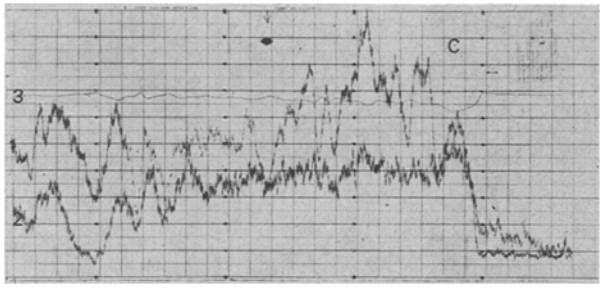

Fig. 1. Strip chart record of the potassium K_α radiation registered along a straight line perpendicular to the adrenal surface in a 6 µm section (line 1). Arrow indicates the borderline between Zona glomerulosa (right) and Zona fasciculata (left). C, capsule. Further right aluminium-coated quarz slide only. Line 2 is the graph for sodium, not considered here. Line 3, specimen current.

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ca. 1 μm, accelerating voltage: 30 kV, specimen current ca. 2×10^{-8} Å.

The intensity of the potassium K_α radiation was registered along lines perpendicular to the surface of the adrenals, from the surface to the first few cells of the Zona fasciculata. The incident-light microscope of the microanalyzer, and the refracted electron picture served for orientation within the section. In some areas of the specimen potassium distribution pictures were studied.

The quantitative analysis of our graphs was crossed by several technical factors, we were confined therefore to the comparison of data obtained in the same section and with the same adjustment of the instrument.

Sensitive detection of the potassium K_α radiation arizing from the tissue specimen was possible as compared to the background detected in the quarz slide near the specimen. The counts detected in areas with moderate potassium content was 8–10 times background.

In the Zona glomerulosa and the marginal layer of the Zona fasciculata the intensity of the potassium K_α radiation fluctuated but some maximum values occurred repeatedly (Figure 1). Variation of the potassium signal intensity might result from the presence in the electron beam of cell organelles with potassium concentrations different from that of the cytoplasmic matrix, from the cell being only partially in the section and from local thickness variations of the specimen. The recurring maxima were considered as the potassium level of a cytoplasmic area filling the whole thickness of the section.

Potassium level of the Zona glomerulosa cells usually exceeded that of the capsule and of the marginal fasciculata cells. In a deeper layer of the Zona glomerulosa higher potassium concentrations were regularly found than elsewhere in the Zona glomerulosa (Figure 1). These potassium-rich sites did not form a continuous layer but were confined to separate areas (Figure 2).

Analysis of potassium distribution pictures confirmed that the glomerulosa cells were in general somewhat richer in potassium than the marginal fasciculata cells (Figure 3). Condensations were seen near the borderline between the 2 zones. A number of them were identified at higher power as cells.

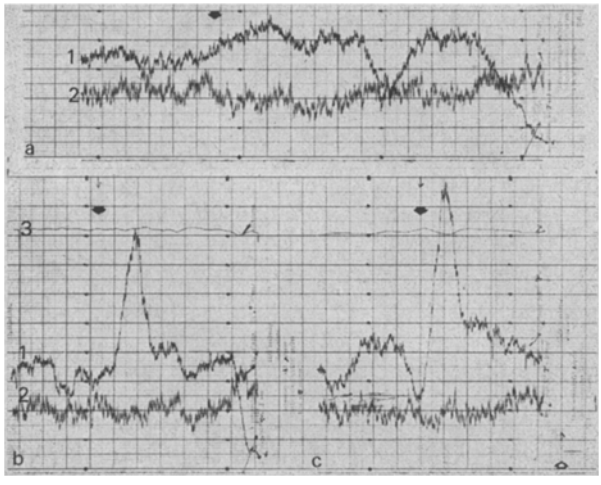

Fig. 2. Strip chart records of potassium K_α radiation along 3 parallel lines in the same section (lines 1). Chart speed for graph a) was twice as much as for graphs b) and c). Black arrows indicate borderline between Zona glomerulosa (right) and Zona fasciculata (left). Maximum seen in the same depth of the Zona glomerulosa in graphs b) and c) is lacking in graph a). Lines 2 and 3 not to be considered.

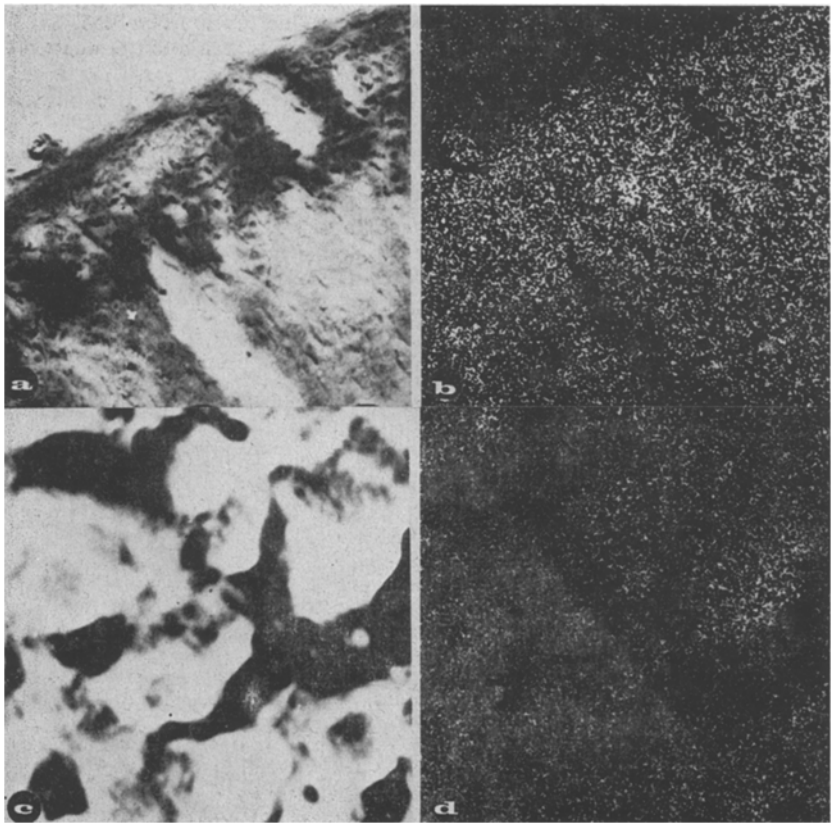

Fig. 3. Potassium distribution pictures (b and d) and corresponding refracted electron pictures (a and c) of the adrenal cortex. With lower power (a and b, × 270) a generally higher potassium content of the glomerulosa and further accumulations in its deeper part can be observed. With higher power (c and d, × 2,200) potassium seems to be well localized in the deeper glomerulosa cells with higher concentration in some of them than in others.

Our results are in agreement with the data of GLICK et al.[10,11], who concluded on the basis of flame photometry of adrenal slices of monkey and guinea-pig, that the borderline between glomerulosa and fasciculata contains more potassium than other parts of the adrenal gland. Their method, however, was insufficient in separating clearly these two zones from the capsule and each other, and was unsuited to reveal differences between individual cells. It turned out from our experiments that, in rat, potassium is not uniformly distributed in the Zona glomerulosa but is cumulated in certain cells in its deeper part. The question arises whether these cells have some key role in the regulation of aldosterone secretion.

Studies are in progress aiming at the quantitative determination of the potassium content of Zona glomerulosa cells in different experimental circumstances influencing aldosterone secretion[12].

Zusammenfassung. An gefriergetrockneten Kryostatschnitten der Rattennebennierenrinde wurden anatomisch drei verschiedene Zonen im Hinblick auf die Kaliumablagerung untersucht und festgestellt, dass sich Kalium vor allem in der tiefen Region der Zona glomerulosa ablagert.

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Thioglycolate de sodium et hormones hypothalamo-neurohypophysaires

Des travaux antérieurs ont montré qu'un extrait total d'hypophyse d'anguille révèle à la fois une activité de type ocytocique[1-3] et vasopressique[1]. La chromatographie sur papier[1] ou en couche mince[4-6] appliquée à ces substances montre qu'elles ne se distinguent pas de l'isotocine (ICT) et de l'arginine-vasotocine (AVT) hautement purifiées ou de synthèse[1]. Des observations analogues peuvent être faites à partir d'un extrait total provenant du noyau préoptique de ce poisson[2-6].

Compte-tenu de ces résultats expérimentaux, il apparaît comme très vraissemblable de penser que l'anguille, sur le plan de la nature des ses hormones hypothalamo-neurohypophysaires, ne se distingue pas des autres poissons Téléostéens. Toutefois, chez l'anguille, l'hydrolyse des peptides hormonaux et la détermination de leur composition en acides aminés n'ont pas été réalisées.

De ce fait, nous avons tenté d'apporter quelques précisions complémentaires en faveur de la nature – ICT et AVT – de ces substances, en étudiant leur comportement en présence d'un thiol qui, par réduction, doit couper le pont disulfure assurant normalement la fermeture de la partie cyclique de la molécule[7-9].

Matériel et méthodes. Les expériences sont réalisées sur des anguilles d'eau douce, de poids compris entre 350 et 630 g. Après prélèvement du noyau préoptique et de l'hypophyse, les fractions tissulaires sont extraites à l'acide acétique et centrifugées. Les surnageants sont alors chromatographiés en couche mince de poudre de cellulose d'après la technique habituelle[4-6]. Les éluats correspondants à l'AVT et à l'ICT sont employés pour les tests. Parallèlement aux hormones d'extraction, l'action du thioglycolate de Sodium (TNa) est étudiée sur les peptides de synthèse: AVT, ICT et ocytocine.

Dans chaque cas nous préparons une solution-mère d'hormone. Au temps zéro nous y ajoutons la solution de TNa et nous enregistrons la contraction d'un fragment de corne utérine de ratte[10]. La manipulation est alors répétée à intervalles de temps moyens de 5 à 10 min. Pour chaque peptide 4 concentrations de TNa sont étudiées (40, 30, 20 et 10 mM), ainsi que l'influence éventuelle de la présence (0,10 g/l) ou de l'absence de magnésium dans la solution physiologique[11] baignant la corne utérine.

Résultats et discussion. Action du TNa sur l'AVT de synthèse (AVT_s) et d'extraction (AVT_e). Si l'expérience est faite en l'absence de magnésium, on constate qu'en présence de TNa 40 mM l'activité de ces peptides n'est plus décelable après 10 min de contact. Au fur et à mesure que l'on diminue la concentration en TNa, le temps nécessaire à l'inactivation augmente et passe à 20, 30 et 50 min pour le TNa 30, 20 et 10 mM. En présence de magnésium les résultats obtenus sont parfaitement superposables, et l'on aboutit dans tous les cas à une inactivation complète des activités hormonales.

Action du TNa sur l'ICT de synthèse (ICT_s) et d'extraction (ICT_e). En l'absence de Magnésium l'activité hormonale est abolie totalement au bout de 20, 30, 40 et 65 min pour des concentrations respectives en TNa de 40, 30, 20 et 10 mM, qu'il s'agisse du peptide de synthèse ou extrait à partir du tractus hypothalamo-neurohypophysaire de l'anguille. En présence de magnésium aucune différence n'est notée.

Action du TNa sur l'ocytocine de synthèse. Les expériences montrent que ce peptide est inactivé de la même façon que l'ICT_s ou l'ICT_e, quelles que soient les concentrations en TNa, que la corne utérine soit placée ou non en présence de magnésium. La simulitude de comportement entre l'ICT et l'ocytocine se retrouve donc non seulement sur le plan de leurs propriétés de migration (Rf superposable), mais également sur le plan de l'inactivation due au TNa. Les problèmes qui se trouvent posés dans cette étude concernant donc l'action du TNa et la différence de comportement constatée entre l'AVT d'une part, l'ICT et l'ocytocine d'autre part.

Comment expliquer l'action du TNa? Si l'on estime que l'inactivation hormonale suit une loi linéaire, on peut

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