moval of the cover) the spots appeared as expected, indicating the UV-light to be the responsible factor for this process.

The minimal time required for the beginning of appearance of pigment from 25 μg of material developed chromatographically was as follows: for MIT, DIT, T_2 and T_3 2–4 min, for T_4 – 10 min, for T – 30 min and 60 min for tyrosine. The shorter time required for the iodinated compounds suggests that the presence of iodine has some influence on the reaction.

With dopa and dopamine (applied in aqueous solution, 25 μg each) already during the chromatography pigments began to appear, which were intensified further by UV-irradiation. The much faster reaction observed with these compounds might be explained by the fact that dopa is a better precursor than tyrosine in both enzymatic and non-enzymatic production of melanin[5,10].

The minimal amounts of substances needed for the formation of a visible pigment after chromatography and 2 h irradiation were estimated. The sensitivity limit for MIT, T_3, T_4, Tyr and T was 2.5 μg while 1 μg was adequate for DIT and T_2.

The chromatographic properties of the UV-irradiated material were investigated by applying a plate with 25 μg of the above substances and immediate exposure to UV-irradiation for 22 h, prior to chromatography. The pigmented spots obtained at the origin did not migrate with the solvents used. However, upon subsequent reirradiation for 2 h, additional spots were obtained at the usual sites, except T_4 which occupied the place corresponding to T_2 or T_3, probably as the consequence of deiodination. The additional spots seem to be caused by small amounts of unaltered substances, present together with the pigment formed during the first irradiation. When various amounts of MIT and T_2 were treated as above, it was observed that the dose required for the development of additional spots was 5 times higher than the sensitivity limit. The formation of compounds with modified chromatographic mobilities from thyroxine and triiodothyronine upon exposure to UV has been explained as a possible polymer formation[11].

In our work, the chemical nature of the brown pigment has not been determined, but data available in the literature suggest that this pigment is a melanin-like polymer. Melanin can be formed not only enzymatically but also by autoxidation with the participation of free radical intermediates[6,7]. Such compounds were described to be formed from dopa[7,8] and also from thyroxine[1,12,13]. The effect of UV in the autoxidative process consists in a conversion of a 'nonautoxidizable compound into an autoxidizable one'[7,14].

Zusammenfassung. Nach Bestrahlung mit UV- und gewöhnlichem Licht entstehen in Dünnschichtchromatogrammen von Tyrosin, Thyronin und ihren Jodderivaten wie auch Dopa und Dopamin dunkle Flecken an den entsprechenden Stellen dieser Substanzen. UV-Licht ist für das Auftreten der Pigmente verantwortlich, welche melaninähnliche Polymere zu sein scheinen.

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Inhibition of Steroid Δ^4 Reductase by Heparin: Studies with Desoxycorticosterone, Progesterone, Androstenedione and Testosterone

Heparin has recently been shown to inhibit C-4 double bond reduction of cortisone, cortisol, corticosterone and aldosterone by rat liver homogenates[1,2]. Reduction of the C-20 carbonyl function and oxidation-reduction at C-11 of cortisone were not modified by heparin.

Hepatic enzyme systems for steroid reduction at the C-4 double bond are thought to interact with several widely scattered sites on the substrate molecule, i.e. the double bond, the 1 and 2 positions of ring A, carbon 11, and the C-17 side chain[3]. This being the case, information as to whether heparin interferes with the attachment of steroid to enzyme protein might be obtained by investigating steroids with various structural differences at C-11 and C-17. In the previous studies[1], heparin inhibited reduction of the C-4 double bond regardless of whether the C-11 position was occupied by a ketone or a hydroxyl group.

This report concerns the effect of heparin on ring A reduction of four C-11 desoxy, Δ^4-3 keto steroids by rat liver. Each of the steroids investigated has different structural characteristics at C-17.

Female Holtzman rats weighing between 200 and 250 g were used in this study. Details of preparing whole liver homogenates and of the incubation and extraction procedures have been published[1]. Steroid ring A reduction was determined by UV-absorption at 240 nm.

The Figure shows the manner in which increasing concentrations of heparin inhibited ring A reduction of androstenedione (4-androsten-3, 17-dione) progesterone, desoxycorticosterone and testosterone. It appears that heparin will inhibit reduction of the C-4 double bond of a variety of C-19 and C-21 steroids with various structural configurations at C-17. Rates of reduction for these 4 compounds were similar in the absence of heparin.

MCGUIRE and TOMKINS[4], using a crude liver preparation, found about the same order of activity for reduction of the C-4 double bond with a wide variety of steroid

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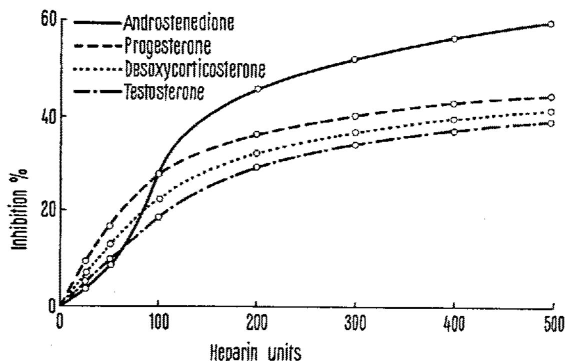
substrates. Upon purification, however, there was a disproportionate loss of correlation of the activities of the enzyme preparation with respect to various steroid substrates employed. Certain procedures inhibited the reductase activities but to different degrees for each steroid employed. These data were interpreted to mean that a group of steroid reductases are present in liver homogenates which are specific for each given steroid substrate.

In view of this specificity, it seems unlikely that heparin would have appreciable affinity for the steroid binding site of each reductase involved in this study and thus inhibit each in a somewhat similar manner. A more likely explanation for heparin inhibition is interference with a cofactor common to the reductases. NADPH is the specific cofactor for the Δ^4 reductases and heparin may compete with this coenzyme for attachment to the specific protein. We have previously shown[1] that excess NADPH will diminish the inhibitory effect of heparin on cortisone reduction. Reduction of the C-20 ketone[2] and C-11 ketone (unpublished data) groups of cortisone by rat liver homogenates is not inhibited by heparin and the NADPH requirements of these functions are probably less rigorous than for the Δ^4 reductases[5,6].



Heparin inhibition of steroid reduction by liver homogenates. %-inhibition refers to a comparison between steroid reduction in an experimental flask and in the corresponding control flask, both prepared with the same homogenate. Each point represents the mean of 5 experiments.

Zusammenfassung. Heparin hemmt die Reduktion der Doppelbindung in 4-Stellung des Rings A einer Reihe von C-11-desoxy, Δ^4-4-keto-Steroiden in der Rattenleber. Es scheint, dass Heparin mit NADPH um das Steroid-reduktase-Apoenzym konkurriert.

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Cation Inhibition of DNA Synthesis in Mammary Epithelial Cells in vitro

The onset of DNA synthesis and subsequent cell division are precisely controlled events in mammalian cells. Although the intracellular mechanisms important in regulating the DNA cycle are as yet undefined, various chemical and physical changes in the external environment of the cell may initiate DNA synthesis or prevent its onset. The ability of these environmental factors to vary the duration of the post-mitotic G_1 period, but not the duration of the S period suggests that mechanisms important in regulating cell proliferation are operative in G_1. Such factors as temperature[1], nutrient deprivation[2,3], hormones[4,5], tissue injury[6], and others have been shown to alter the duration of the G_1 period. This report describes the effect of various cations upon the initiation of DNA synthesis by mammary epithelial cells maintained in organ culture.

Both abdominal mammary glands of mid-pregnant (10–12 day) nulliparous C3H/HeN mice were removed aseptically, and explants were prepared and cultured in sterile Medium 199 (Microbiological Associates)[7]. Appropriate hormones were added to a final concentration of 5 μg/ml, and analytical grade crystalline salts were dissolved in Medium 199 to the desired final concentration. Each experiment was performed with tissue from a single animal, and replicate incubations were used for each determination. DNA synthesis was measured after exposing the explants to (Me-^{3}H)-thymidine (Schwarz, specific activity 8.0 C/mM) at a concentration of 0.5 μC/ml for 4 or 12 h labeling periods. The tissue was then weighed, de-fatted in acetone, and assayed for tritium-labeled DNA as previously described[5], except that dissolution of the explants in hydroxide of hyamine was accelerated by heating at 70 °C for 10 min. Explants taken for autoradiography were fixed in Bouin's solution and sectioned at 5 μ. The slides were dipped in Kodak photographic emulsion, developed 3 weeks later, and stained with Delafield's hematoxylin. Casein synthesis was measured by exposing explants to medium containing ^{32}P (30 μC/ml) for 4 h pulse-labeling periods. After the explants were weighed and homogenized, radioactive casein was isolated from the 105,000 g supernatant by precipitation with rennin and calcium ions in the presence of bovine casein carrier, as previously described[7].

As shown in the Figure, mouse mammary explants cultured in the presence of insulin show a marked augmentation in rate of DNA synthesis. Previous studies[4] indicated that insulin acts upon these epithelial cells in the G_1

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