quappen von *Rana temporaria*[16]. Die Versuche, deren Ergebnisse an anderer Stelle veröffentlicht werden, zeigten eine Durchschnittshemmung der Wachstumsgeschwindigkeit der Tiere beim plasmatischen Mucoprotein von −61,7%, beim Serum von −55,3%; sie ist in beiden Fällen deutlich signifikant.

Die Ergebnisse beider Vorgänge bestätigen also dieselbe Tatsache, dass Mucoproteine animalischer Herkunft das Wachstum von biologischen Individuen hemmen können.

Summary. Anti-growth activity of animal mucoproteins on germinating seeds of *Lupinus albus* was tested; the inhibition with plasma mucoprotein was statistically significant. The inhibition with urine mucoprotein according to ANDERSON was the most effective.

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Extrahepatic 7α-Hydroxylation of Dehydroepiandrosterone

In a number of papers dealing with the *in vitro* catabolism of steroid hormones, the tissue localization of the metabolic activity has been studied predominantly in the liver and the kidneys. Several authors, however, have drawn their attention also to other organs: BROWN et al.[1] investigated the *in vitro* metabolism of cortisol in various rat organs and found the matabolic activity to be present not only in the adrenals and the liver, but also in the pituitary, the gastrointestinal tract, the kidneys, and the spleen. The extrahepatic catabolism of steroid hormones involving the action of dehydrogenases and Δ^4-hydrogenases is dealt with in several studies which are devoted almost exclusively to corticoids[2–4], occasionally also to progesterone[5]. In addition to endocrine organs, the extrahepatic catabolism was followed in the lungs, the kidneys, and the blood. The extrahepatic enzymatic hydroxylation of steroids *in vitro* has not yet been subjected to investigation.

Studies on the *in vitro* metabolism of 3β-hydroxy-Δ^5-androsten-17-one (dehydroepiandrosterone – DHA)[6–9] have as yet dealt with metabolic activity of the liver only. Our previous experiments concerned with the problem of the organ specificity of the liver in the enzymatic hydroxylation of dehydroepiandrosterone *in vitro* revealed some degree of the 7α-hydroxylating activity to be present also in several other organs. It is for this reason that we focused our attention on the extrahepatic transformation of DHA *in vitro*.

Female rats of the Wistar strain, weighing 100 to 170 g, were used for experimentation. 2 g of fresh tissue was homogenized in 25 ml of a Krebs-Ringer phosphate buffer solution. The homogenate with glucose added (0.05%) was subsequently transferred to a solution of 2 mg of dehydroepiandrosterone in 1 ml of triethylene glycol. The mixtures were incubated at 38°C in an oxygen atmosphere over a period of 1 h. 7α-Hydroxylation was also investigated in tris-buffer, pH 7.6, the yields being somewhat lower. Extraction of the incubation mixture with ethyl acetate, purification of the extract using chromatography on a thin layer of alumina, paper partition chromatography in the Bush B5 system, and evaluation of the chromatograms after detection with the antimony trichloride reagent were all performed as described elsewhere[10].

The lung, the liver, the kidney, the spleen, the heart, the blood, the muscle, and the adrenal, respectively, were tested for their hydroxylating activity. The results were repeatedly positive with all organs examined except for the adrenals, where a definite hydroxylating activity was found only in one case. When comparing the 7α-hydroxylating activities, considerable quantitative differences between individual organs, as well as varying results from individual experiments, were found, perhaps owing to a lack of completely adequate homogeneity of the biological material used in the present study. The results are shown in Table I.

To obtain more thorough information regarding the properties of the extrahepatic 7α-hydroxylating system, the results obtained for the lungs were compared with those for the liver. Because of the possibility of a species dependence of the activity of 7α-hydroxylase[11], 7α-hydroxylation of DHA was investigated also in the lungs and the livers of rabbits and calves, using identical methods. During the incubation, 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHA) was produced in all cases; yields from calf and rat tissues were closely parallel, whereas those from rabbit tissues were reduced to about one half.

When using thin-layer chromatography on alumina and paper partition chromatography, the chromatographic properties of the substance obtained by incubating 300 ml

Table I. Organ dependence of the *in vitro* 7α-hydroxylation of DHA in female rats. 2 g of tissue were incubated with 2 mg of DHA

Tissue	Range of yields 7α-OH-DHA/1 mg of DHA
	μg
Liver	4.0 – 30.0
Lung	2.0 – 34.0
Kidney	3.0 – 78.0
Spleen	5.0 –150.0
Heart	2.5 – 16.0
Blood	2.0 – 3.5
Muscle	4.5 – 13.5
Adrenal[a]	0.0 – 13.0

[a] 100 mg of adrenal tissue were incubated with 0.5 mg of DHA.

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of homogenate from 25 g of calf lungs with 250 mg of DHA were identical with the behaviour of the referent synthetic 7α-OH-DHA. To identify this substance, the following reactions were performed: (a) Dehydration by treatment with 4% HCl in 90% methanol over a period of 24 h yielded $\Delta^{5,7}$-androstadiene-3β-ol-17-one; (b) Reduction with $NaBH_4$ in a water-ethanolic medium yielded Δ^{5}-androstene-3β,7α,17β-triol; (c) Acetylation using acetanhydride in a pyridine medium yielded 3,7-diacetate.

Table II. Influence of several inhibitors on the *in vitro* 7α-hydroxylation of DHA in the livers and the lungs of female rats. 2 g of tissue were incubated with 2 mg of DHA

Inhibitor	7α-OH-DHA/1 mg of DHA	
	Liver	Lung
	μg	μg
∅ (control)	6.0	34.0
N_2 atmosphere	2.9	4.0
95°C/10 min	0.0	0.0
KCN ($10^{-2}M$)	10.4	27.0
$HgCl_2$ ($10^{-3}M$)	1.5	0.0
α,α'-Dipyridyl ($5.10^{-4}M$)	2.5	–
$Na_4P_2O_7$ ($10^{-3}M$)	–	0.0

The identities of the resulting products were proved by their chromogenic properties and chromatographic mobilities in at least two systems.

Analogically, 40 μg of the substance obtained by incubating DHA with the homogenate from rat lungs was identified.

The *in vitro* 7α-hydroxylation of DHA in lung and liver homogenates fails to show any significant dependence on the sex of the rats. The influence of the action of several inhibitors during incubation and the influence of preheating the homogenate is obvious from Table II. The presence of TPNH slightly increased yields from both the lungs and the livers.

The presence of 7α-OH-DHA in tissue incubates is not occasioned by an autooxidation of DHA, as confirmed by the negative results of blind experiments without the use of tissues.

Zusammenfassung. In verschiedenen Rattenorganen (Lunge, Niere, Milz, Blut, Muskelgewebe) konnte ein System nachgewiesen werden, das imstande ist, *in vitro* Dehydroepiandrosteron an der 7α-Stellung zu hydroxylieren. Dies Ergebnis weist auf die allgemein mögliche extrahepatale und extraadrenale Steroidhydroxylation hin.

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Determination of the Structure of the Peptide Moiety of the Antibiotic Albomycin

The albomycin[1] molecule consists of two moieties[2]: the peptide part containing 3 L-serine residues, 3 N^{δ}-hydroxyornithine residues, and iron bound in complex; and the pyrimidine part containing a sulphur atom. Both moieties are linked through the oxygen atom of the hydroxy group of one of the three serine residues. By acid hydrolysis of the pyrimidine moiety 3-methyluracil is formed, and by alkaline hydrolysis 4-(N'-methyl)cytosine arises. Partial acid hydrolysis of albomycin releases three peptides containing 3-methyl-uracil linked with one, two, and probably three serine residues[2]. From the partial acid hydrolysate of deferrialbomycin (preparation with carefully removed iron) a great amount of ninhydrin positive degradation products (formed from unstable N^{δ}-hydroxyornithine) was obtained from which, however, it was not possible to construct the amino acid sequence in the peptide part of the antibiotic. Neither albomycin nor deferrialbomycin were cleaved in experiments of enzymatic hydrolysis. Although N^{δ}-hydroxyornithine is transformed into stable ornithine by hydrogenation, deferrialbomycin is hydrogenated in the pyrimidine part of the molecule only since N^{δ}-hydroxyornithine is acetylated in the antibiotic and forms three hydroxamic acid groups.

The most adequate procedure for stabilizing N^{δ}-hydroxyornithine in the cyclopeptide of deferrialbomycin is, according to our experience, its transformation to glutamic acid by oxidation with performic acid as described previously[3,4]. The sequence of its amino acids can then be solved by partial acid hydrolysis. Six peptides, P 32, P 33, P 71, P 72, P 73, and P 82, were obtained thereby, which were isolated by paper chromatography and electrophoresis. The pattern of these peptides in Figure 1 shows the unambiguously determined sequence of the oxidized cyclopeptide moiety of the antibiotic. X denotes the degradation product formed by oxidation of the pyrimidine part. The previously mentioned peptides

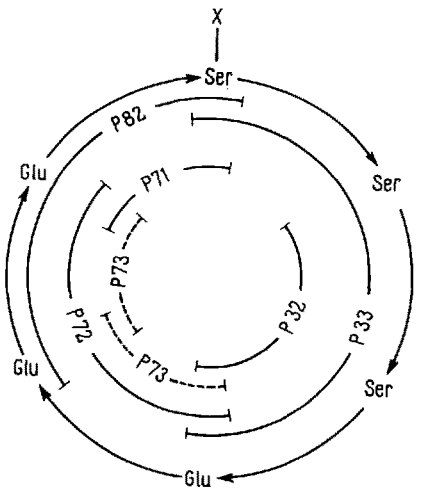

Fig. 1

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