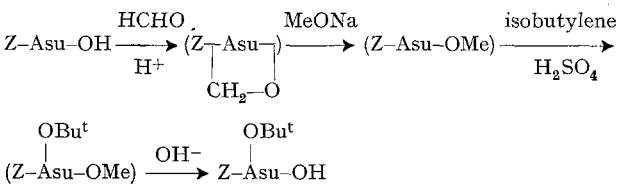
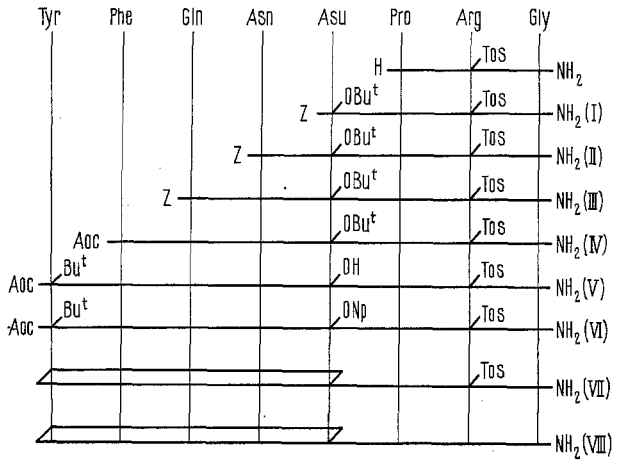
Benzyloxycarbonyl-L-α-aminosuberic acid ω-t-butyl ester (Z-Asu(OBu^t)-OH)[3,4], a key intermediate for the preparation of dicarba-analogs, was synthetized according to a procedure modified by MURAKAMI et al.[5] for the preparation of α-esters. The sodium methoxide treatment,



the second step in the reaction scheme, was carried out overnight at 10 °C in order to avoid possible racemization. Since all intermediates were obtained as oily compounds, completion of each reaction was ascertained by thin-layer chromatography, and the over-all yield of the key intermediate was satisfactory (66%). The partially protected octapeptide was synthetized by step-wise elongation of H-Pro-Arg(Tos)-Gly-NH_2[6] with the appropriate amino acid active esters[3,4] (Figure). Compound V was smoothly converted to the active ester VI by the trifluoroacetate method[7]. After removal of the remaining protecting groups from the tyrosyl residue, VI was treated with pyridine at 50 °C under high-dilution conditions (1 mmol/l), and a cyclic compound VII was obtained in about 60% yield. Removal of the tosyl group from VII was achieved by the HF-procedure[8] at 0 °C for 1 h in the presence of anisole. Remaining hydrogen fluoride in the crude product was removed by passing the aqueous solution through a short column of Amberlite IR-45 (OH^-). The HF-free product was purified on a column of Sephadex G-25 with 0.1 *N* AcOH as the solvent, and the homogeneous compound was obtained as lyophilizate in a yield of 51%. Paper chromatography showed this compound to give a single spot in 2 different solvent systems; Rf 0.36 (*n*-BuOH:AcOH:water = 4:1:1) and Rf 0.19 (*n*-BuOH:pyridine:water = 4:1:1); $[\alpha]_D^{16}$ – 69° (c 0.38, 0.1 *N* AcOH). Anal. calcd. for $C_{48}H_{67}N_{14}O_{12}\cdot CH_3COOH\cdot 2.5\,H_2O$: C 52.81; H 6.74; N 17.24. Found for a sample dried at 100 °C for 10 h in vacuo: C 52.87; H 6.71; N 17.18. Ratio of amino acids after acid hydrolysis: $Tyr_{0.99}$, $Phe_{1.08}$, $Glu_{1.08}$, $Asp_{1.00}$, $Asu_{1.06}$, $Pro_{0.96}$, $Arg_{1.00}$, $Gly_{1.01}$. Preliminary bioassay revealed that deamino-dicarba-Arg^8-vasopressin possesses approximately $^1/_{15}$ of the principal activities of natural-type deamino-Arg^8-vasopressin (see Table).



Synthesis of deamino-dicarba-Arg^8-vasopressin.

Zusammenfassung. Die Synthese und Resultate einer vorläufigen biologischen Prüfung von Desamino-dicarba-Arg^8-vasopressin, einem Neurohypophysenhormon-Analogon, in dem die Schwefelbrücke durch eine Äthylengruppe ersetzt ist, werden beschrieben.

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Inhibition of DNA Synthesis in Regenerating Rat Liver by Hydrocortisone

The inhibition of growth of lymphoid tissues by adrenal corticosteroids is well documented[1–3]. Natural corticosteroids or their analogs have also been shown to inhibit erythropoiesis[4] and the proliferation of fibroblasts[5], to induce growth failure in neonatal rats[6], to cause the abnormal development or death of embryonic tissues[7,8], and to cause the regression of tumors of lymphoid origin[9–11].

In a previous study[12] it was shown that pharmacological doses of hydrocortisone markedly inhibit RNA synthesis in the regenerating liver of hypophysectomized, but not of intact rats. The results of other studies suggest that adrenal corticosteroids inhibit mitosis in the regenerating liver of the mouse[13,14] and of the rat[15]. Furthermore, adrenocorticotrophic hormone was shown[16] to decrease the incorporation of tritiated thymidine into nuclei of the regenerating liver of the rat. In view of their importance as anticancer agents, the growth inhibiting action of the corticosteroids was studied further, using the regenerating liver system.

Male Sprague-Dawley rats (240–300 g) were maintained in plastic cages with purina chow and water ad libitum. Lighting was controlled from 06.00–18.00 h. Partial hepatectomy involved the surgical removal of approximately 70% of the liver[17]; the operations were routinely performed between 10.00–13.00 h. The rats were fasted in wire bottom cages following the operation. The labelled precursors, (^{3}H-methyl) thymidine or 6-^{14}C orotic acid, were injected i.p. 26 h after partial hepatectomy, when the rate of DNA synthesis is approximately

maximal[18], and 1 h before removal of the regenerating liver remnant. Hydrocortisone sodium succinate (Solucortef, Upjohn Co.) was also administered by i.p. injection.

To determine the specific activity of the DNA, the liver was homogenized in 2 volumes (w:v) of *tris* buffer (0.05 *M*, pH 7.5). Uniform sampling of the homogenate was ensured by diluting the latter six-fold with 0.125% sodium deoxycholate. The DNA was extracted from aliquots of the suspension by the procedure of FLECK and MUNRO[19]. The DNA extracted from a 2.5 ml aliquot of the deoxycholate-treated homogenate was dissolved in 1.0 ml of NCS solubilizer (Nuclear Chicago Corporation), then counted in 15 ml of scintillant (5.0 g of PPO/l of toluene). The efficiency for ^3H and ^{14}C was approximately 30% and 90%, respectively. The DNA extracted from a 5 ml aliquot was estimated by use of the diphenylamine reagent[20] using deoxyadenosine as a standard. The specific activity of the DNA is reported as cpm per μg deoxyadenosine.

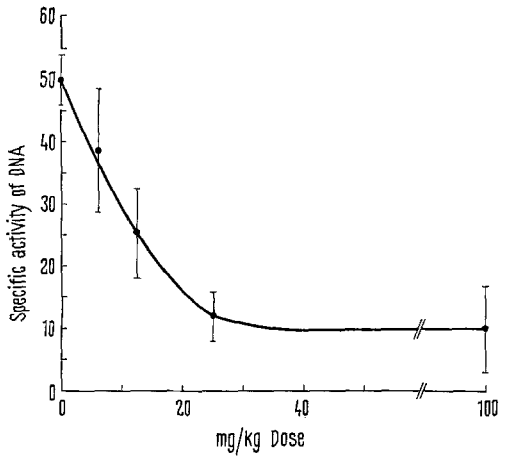

Fig. 1. Inhibition of incorporation of ^3H-thymidine into DNA in the 27 h regenerating liver by various doses of hydrocortisone sodium succinate. The indicated doses of the corticosteroid were administered at the time of partial hepatectomy and 12 h later. ^3H-thymidine (10 μc/200 g) was injected 1 h before removal of the liver. Control rats received 0.9% saline in place of the steroid. Each point represents the average of 2–3 rats, with the exception of the control value which represents the mean of 7 rats. The standard errors are shown.

Figure 1 shows a dose-response curve in which the specific activity of the liver DNA is plotted as a function of the size of the administered dose of hydrocortisone sodium succinate. With the exception of the controls each rat received the indicated dose at the time of partial hepatectomy with a second dose 12 h later; this schedule was found to inhibit RNA synthesis in hypophysectomized rats[12]. The ^3H-thymidine was administered 1 h before removal of the liver and 26 h after partial hepatectomy. It is clear from the results that low doses of hydrocortisone cause a marked inhibition (80%) of the incorporation of ^3H-thymidine into DNA. The small fraction of the cells which appear to escape the inhibitory action of the steroid may pas through the sensitive phase when the plasma hydrocortisone levels are low, since the clearance of the steroid is virtually complete within 3–4 h after administration[21].

Shown in Figure 2 is the specific activity of the DNA as a function of the time after partial hepatectomy at which 50 mg/kg of hydrocortisone sodium succinate was administered. Although 2 doses effectively inhibited RNA synthesis when given at zero and 12 h (Figure 1), a single dose at 12 h gave a slight stimulation. Maximum inhibition (approximately 80%) with a single dose was observed when the steroid was injected 19–23 h after partial hepatectomy. This corresponds to the period when the rate of DNA synthesis rises exponentially, then plateaus, in the regenerating liver of young adult rats[18].

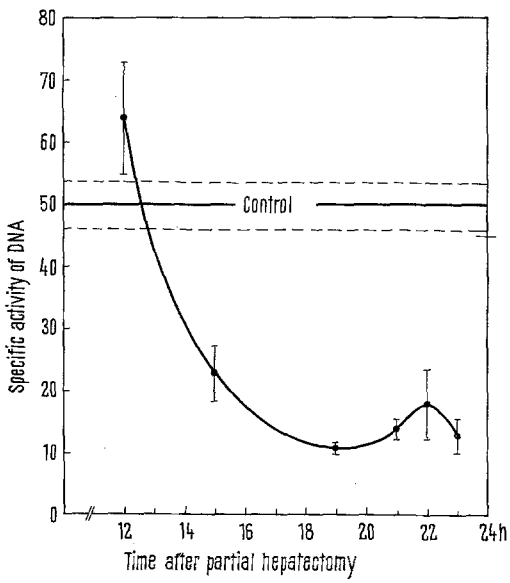

Fig. 2. Inhibition by one dose (50 mg/kg) of hydrocortisone sodium succinate of the incorporation of ^3H-thymidine into DNA of regenerating liver as a function of the time of administration of the corticosteroid. The rats were injected with ^3H-thymidine (10 μc/300 g) 26 h after partial hepatectomy and 1 h before removal of the liver. With the exception of the control value which is based on 18 rats, each point is the mean of 3–9 rats. The standard errors are shown.

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The inhibition of DNA synthesis by hydrocortisone is also observed when ^{14}C-orotic acid is used as the labelled substrate. The data in the Table show that the percent inhibition of the incorporation of label from ^{14}C-orotic acid, which must enter DNA via the de novo pathway, is essentially identical to that observed when ^{3}H-thymidine, which enters via the salvage pathway, is used. In these experiments a single dose of hydrocortisone (50 mg/kg) was administered 19 h post-operative. Either 10 μc of ^{3}H-thymidine or 5 μc of ^{14}C-orotic acid was administered 26 h after partial hepatectomy and 1 h before removal of the liver. Separate experiments confirmed that the incorporation of label from ^{14}C-orotic acid into DNA was linear for 1–2 h, and that the incorporation of label into the acid-soluble fraction 0.5 h after the administration of either precursor, was 5–10% higher in the hydrocortisone-treated rats as compared to the controls. The values for both the control and treated rats have been corrected for the presence of a small amount of RNA contamination in the DNA preparations, which contains label from the ^{14}C-orotic acid. The small error (about 12%) was estimated by extracting the DNA from the 12 h regenerating liver of rats treated as above; label from ^{14}C-orotic acid would appear only in the RNA at this time since DNA synthesis is negligible. The conclusion that the changes in the labelling pattern of DNA are a true indication of the degree of inhibition of DNA synthesis was also supported by the results of mitotic counts. Mitoses were absent in eosin-stained 10 μ sections from the 30 h regenerating liver of rats which received a single dose of 50 mg/kg or 100 mg/kg of hydrocortisone 19 h after partial hepatectomy; approximately 3.5% of the cells were in metaphase in similar preparations from control rats.

These results indicate that the regenerating liver is most sensitive to the steroid if the latter is administered during the period of rapid DNA synthesis in the parenchymal cells, which occurs approximately 17–19 h post-operation in young adult rats[18]. It is this latter finding which may be pertinent to the problem of optimizing schedules for the use of corticosteroids as cancer chemotherapeutic agents, since DNA synthesis shows a diurnal variation in some tumors[22]. Whether some tumors grown in a host animal exhibit this marked sensitivity to corticosteroids when they are in the vicinity of the S-phase must await further study[23].

Percent inhibition of DNA synthesis

Precursor	Specific activity ± S.E.[a] Control	Treated	% inhibition
^{3}H-thymidine (10 μc)	50 ± 3.7 (18)	11 ± 0.89 (9)	78
^{14}C-orotic acid (5 μc)	13 ± 1.9 (7)	3 ± 0.75 (7)	77

[a] Number of rats shown in parenthesis.

Résumé. Dans le foie du rat en voie de régénération, la biosynthèse de DNA est inhibée au maximum par le hydrocortisone 17–19 h après l'opération. C'est pourquoi le foie en régénération est le plus sensible au hydrocortisone s'il est injecté pendant la période de la synthèse maximale de DNA.

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Fatty Acid Unsaturation and Cholesterol Content in Normal and Denervated Muscle

In the denervation atrophy of muscle, a marked increase of the extent of phospholipid labelling from P^{32}-ortophosphate occurs, together with an increased incorporation of C^{14}-acetate into lipids[1–3].

To evaluate differences in lipid composition between normal and denervated muscles, experiments have been carried out on the degree of unsaturation of fatty acids and the ratio cholesterol–phospholipid, which appear to be both relevant to the functional properties of membranes[4–6].

Wistar rats (200–250 g) were used in all experiments. They were maintained on stock diet and allowed free access to food. To produce atrophic muscles, the rats were anaesthetized with ether and the left sciatic nerve of each was cut. In others, the left gastrocnemius muscle was tenotomized. The animals were used 8–18 days after the operation.

Three to five atrophic or control muscles were pooled and homogenized in chloroform-methanol (2:1), the lipids were extracted (under nitrogen) according to FOLCH et al.[7] and redissolved in chloroform for the iodine number determination following the method of WIJS[8]. In parallel experiments the total cholesterol was determined (8 days after the operation) according to the procedure of BLOOR[9].

The results presented in Table I show that in the denervation atrophy the degree of fatty acid unsaturation is significantly lowered, whereas no change has been found in tenotomized muscles.

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