NH_4OH and lyophilized. Peptide T_2 was further purified by preparative paper electrophoresis[13] at pH 6.5. It was finally chromatographed on a Sephadex G-50 column (2.5×190 cm) with $0.1\,M$ NH_4OH.

The peptide (T_2), thus obtained, reacted positively with the PAULY, SAKAGUCHI, EHRLICH, ninhydrin, and chlorine-KI-starch reagents. Amino terminal analysis by the Dansyl-method[14] gave a single valine residue. The homogeneity of peptide T_2 was further corroborated by two dimensional electrophoresis-chromatography which showed a single spot. Amino acid analysis[15] gave (46 residues): $Arg_{1.0}$CM-$Cys_{1.5}Asx_{5.5}Thr_{4.3}Ser_{4.1}Glx_{2.0}Pro_{1.1}Gly_{6.1}Ala_{9.0}Val_{6.2}Leu_{3.1}Tyr_{1.0}Phe_{1.0}$ and $Trp_{1.0}$. Tryptophan was determined spectrophotometrically[16]. Manual subtractive EDMAN degradation[17], carried out essentially according to the prodecure described by BLOMBÄCK et al.[18], gave the N-terminal tripeptide sequence Val-Ala-Gly-. Sequence analysis in the automated protein sequenator (BECKMAN, model 890), following previously described methods[10,11], gave the following result in twice repeated experiments: Val–Ala–Gly–Ala–Gly–Leu–Gln–Ala–Gly–Thr–Ala–Tyr–Asp–Val–Gly–Gln–Cys(CM)–Ala–( )–Val–Asn–Thr–Gly–Val–Leu–. No residue could be definitely identified at step 19 in either degradation, possibly due to the presence at this position of an amino acid (such as serine) giving rise to a labile thiohydantoin derivative. No other difficulties were observed in the sequenator study. Work on the complete sequence of neocarzinostatin is in progress and will be published elsewhere[19].

Zusammenfassung. Das antitumoraktive Protein-Antibiotikum Neocarzinostatin wurde mit Dithiothreit in flüssigem Ammoniak reduziert und mit Chloressigsäure alkyliert. Tryptische Spaltung des tetra-S-carboxymethylierten Proteins ergab 5 Fragmente. Die Sequenz von 25 Aminosäureresten im tyrosinhaltigen Fragment H_3 wurde durch EDMAN-Abbau im automatisierten Sequenator ermittelt.

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The Influence of Vitamin E on the Expenditure of Vitamin A from the Liver

Since the first communication on the subject by MOORE[1] it is generally recognized that vitamin E is necessary for optimal utilization of vitamin A.

Studies with chicks and rats receiving vitamin A in the form of cod liver oil (DAM et al.[2,3]) showed that more vitamin A was stored in the liver when the diet contained D, L-α-tocopheryl acetate than when the diet was vitamin E deficient, and, further, that methylene blue and other redox dyes had an effect on the storage of vitamin A in the liver similar to the effect of vitamin E. It was, therefore, held likely that the sparing action on vitamin A exerted by vitamin E is simply due to inhibition of the autoxidation of polyunsaturated fatty acids which, if unchecked, would lead to destruction of vitamin A.

The present studies, the details of which will be reported elsewhere, show that vitamin E has a sparing effect on the vitamin A content also when the diet does not contain fatty acids.

53-day-old chicks received for 13 days a 'starter ration' containing no vitamins A and E. During the last 6 days of this period, each chick received by mouth equal doses of retinyl acetate. Analysis (method as in reference[4]) of the livers of 10 chicks killed on the 14th day showed that the average amount of retinol per liver was 8.37 mg. The remaining 43 chicks were divided into 4 groups and given the vitamin A deficient diets indicated in Table I for a period of 4 weeks, whereafter they were killed and their livers assayed for vitamin A. The average amounts of vitamin A (retinol) found in the livers from each group of chicks after the 4-weeks depletion period are indicated in Table I as percent of the average amount (8.37 mg) present immediately before the depletion period.

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Table I. Amount of vitamin A (as retinol) after the 4-weeks depletion period in livers of chicks reared on 4 different diets

Group No.	No. of chicks	Addition to the fat-free basal diet[a]	Vitamin A in whole liver	
			mg	Amount before depletion period (%)
1	12	none	2.64	32
2	11	0.01% all-rac.-α-tocopheryl acetate	7.45	89
3	10	10% cod liver oil freed from vitamins A and E	1.30	16
4	10	10% cod liver oil freed from vitamins A and E plus 0.01% all-rac.-α-tocopheryl acetate	7.59	91

[a] Basal diet: 30% casein, 3% gelatin, 5.17% salt mixture (ref.[6]), 0.1% vitamin B mixture (ref.[6]), 0.2% choline chloride, 61.53% sucrose, 1 mg/100 g vitamin substitute ('Synkavit'), 1.4 ppm selenium dioxide. Vitamin D_3 was given in the form of an aqueous solution twice a week, corresponding to 20 IU vitamin D_3 per day.

Table II. Amount of vitamin A (as retinol) after the 4-weeks depletion period in livers of chicks reared on fat-free diets with or without different antioxidants

Group No.	No. of chicks	Addition to the fat-free basal diet	Vitamin A in whole liver	
			mg	Amount before depletion period (%)
1	10	none	2.08	27
2	11	0.1% thiodiphenylamine	2.61	34
3	11	0.1% N,N'-diphenyl-*p*-phenylenediamine (DPPD)	2.09	27
4	10	0.1% ethoxyquin	4.44	58
5	11	0.5% ascorbic acid	2.11	27
6	11	0.01% all-rac.-α-tocopheryl acetate	7.22	94

These results show that with vitamin E, nearly the same high percentage (about 90) of the original depot of vitamin A was found in the liver after the depletion period whether the diet in the depletion period was fat-free or contained polyunsaturated fatty acids in the form of cod liver oil freed from vitamins A and E by treatment with filtrol as indicated in reference 5, whereas without vitamin E the decline of the vitamin A depot during the depletion period was considerable, and more marked when the diet contained filtrol-treated cod liver oil than when the diet was fat-free.

A second experiment was carried out in order to decide whether unphysiological antioxidants are able to influence the decline of a hepatic depot of vitamin A when the vitamin A-free diet is fat-free. In this experiment, 76 chicks received doses of retinyl acetate as in the first experiment, whereafter 12 of them were killed and the amount of vitamin A in the liver was determined. The amount was found to be 7.68 mg per liver.

The remaining 64 chicks were divided into 6 groups and given the fat-free vitamin A-free diet without or with supplements as indicated in Table II for 4 weeks, whereafter the chicks were killed and the amount of vitamin A in the liver determined. The results of the experiment are shown in the last column of Table II.

It is seen that thiodiphenylamine, DPPD and ascorbic acid had no influence on the decline of vitamin A in the liver. Ethoxyquin seems to have had some retarding influence on the decline of vitamin A, an effect which may be related to the partial structurel resemblance of this substance to α-tocopherol. In the group receiving 0.01% all-rac.-α-tocopheryl acetate, 94% of the initial amount of vitamin A was recovered after 4 weeks.

The inefficiency of the artificial antioxidants in delaying the expenditure of vitamin A from the liver when the vitamin A deficient diet is fat-free indicates that the ability of vitamin E to stabilize the vitamin A store in the liver during the fat-free depletion regimen is unrelated to its antioxidant effect.

The fact that in the first experiment the decline of the vitamin A store in the liver during the depletion period was more marked when the diet contained filtrol-treated cod liver oil than when the diet was fat-free, indicates that in this case the action of vitamin E was twofold, viz. an antioxidant effect superimposed on the effect seen with the fat-free diet.

In a third experiment, the above-mentioned antioxidants and all-rac.-α-tocopheryl acetate were given in the same amounts as in Table II as additions to diets containing 10% untreated cod liver oil as source of vitamin A. When these diets were given for 4 weeks to groups of chicks that had not received intitial doses of retinyl acetate, determination of vitamin A in the livers at the end of the feeding period showed that all the antioxidants except ascorbic acid had enhanced the accumulation of vitamin A in the liver considerably in accordance with the results of our previous study (reference[2]).

Zusammenfassung. Die Verminderung des Vitamin-A-Depots in Hühnchenleber wurde während einer Karenzzeit von 4 Wochen untersucht, einerseits bei fettfreier Nahrung, anderseits bei einer Nahrung mit 10% Vitamin-A- und E-freiem Dorschlebertran. Nur bei Zusatz von 0.01% all-rac.-α-Tocopheryl-acetat war nach Ablauf der Karenzzeit stets 89–91% des Vitamin-A-Depots noch vorhanden. Künstliche Antioxydantia waren bei der fettfreien Nahrung ohne Einfluss auf die Verminderung des Vitamin-A-Depots.

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