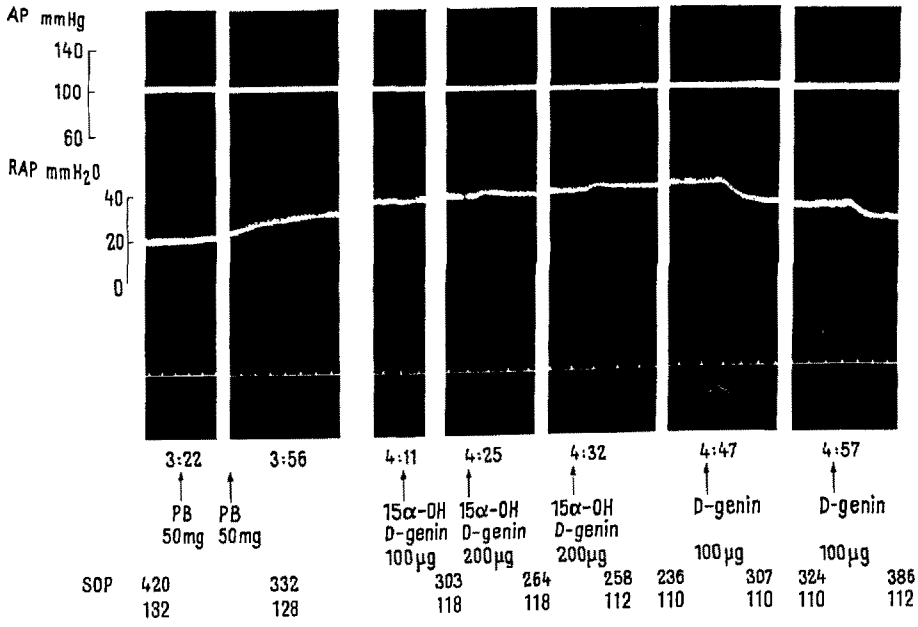

Effect of 15α-hydroxydigitoxigenin and digitoxigenin on the dog heart-lung preparation. The tracings are from top to bottom: arterial pressure (AP), right arterial pressure (RAP), systemic output recorded with a Weese stromuhr. PB = pentobarbital sodium, 15α-OH D-genin = 15α-hydroxydigitoxigenin, D-genin = digitoxigenin, SOP = systemic output in ml per min, HR = heart rate per min.

ducing the calcium concentration of the medium to half (0.8 m*M*). Under this condition, administration of 10^{-7} (w/v) of D-genin invariably produced an appreciable increase in heart contractile force. A higher concentration of D-genin showed a more remarkable effect and then caused a typical systolic arrest. In contrast, 15α-OH D-genin, even in a dose of as high as 10^{-5}, did not show any improvement in the heart contractility.

(2) *Heart-lung preparation of the dog.* In this preparation heart failure was produced by administering pentobarbital sodium. After a new steady state was reached, 15α-OH D-genin was injected into the rubber tubing leading to the venous cannula. As is shown in the Figure, even a cumulative dose of as high as 500 µg of 15α-OH D-genin could not produce any appreciable change in the heart contractile force. Then 100 µg of D-genin was injected *via* the same route. This caused a marked decrease in right atrial pressure and an increase in systemic output.

The results of these experiments suggest that the hydroxyl group introduced into 15α position somehow interferes with the stereochemical arrangement in the vicinity of the C and D rings, depriving the cardiotonic activity of the resulting compound[5].

Zusammenfassung. Es wurde die inotrope Wirkung von 15α-Hydroxy-Digitoxigenin am isolierten Herzen des Frosches und am Herz-Lungen-Präparat des Hundes untersucht. Abweichend von Digitoxigenin hat das 15α-Hydroxy-Derivat keinen Einfluss auf die Kontraktionskraft dieser Präparate.

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[5] 15α-OH D-genin used in this study was generously supplied by Dr. Masashi Okada of the Tokyo Biochemical Institute, Tokyo (Japan).

Canthaxanthin als Sekundär-Carotinoid einiger Grünalgen

In vielen Fällen ändert sich die Pigmentgarnitur von Grünalgen bei Nährstoffmangel, besonders bei Fehlen von Stickstoff[1]. Die dabei gelegentlich auftretenden Sekundär-Carotinoide sind mehrfach untersucht[1,2] und bei der Gattung Chlorella auf ihre Bedeutung als taxonomisches Merkmal geprüft worden[3]. Astaxanthin konnte als eines dieser Pigmente eindeutig identifiziert werden[1].

Während Untersuchungen über die Nitratreduktion[4] und über die Wirkung von Chloramphenicol auf die Farbstoffgarnitur einiger Grünalgen[5] standen uns grössere Mengen an N-Mangel-Algen zur Verfügung und boten uns Gelegenheit, weitere Pigmente genauer zu charakterisieren. Bereits Dersch[1] beschrieb ein auf Grund seiner Absorp-

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tionsmaxima dem Canthaxanthin ähnliches Pigment, das er wegen seines Vorkommens in Chlorella «Chlorellaxanthin» nannte. Wir konnten diesen Farbstoff aus *Chlorella pyrenoidosa* (Nr. 211–8b der Sammlung PRINGSHEIM, Göttingen) und aus zwei weiteren nicht näher identifizierten, von Baumstämmen in Marburg (Lahn) isolierten Chlorella-Stämmen nach dem üblichen Ausschüttelverfahren säulenchromatographisch gewinnen[1]. Aus einer Methanol/Benzol-Mischung kristallisiert das Pigment in dunkelroten Prismen, die bei 216–218°C (unkorr.) schmelzen. Die physikalischen Daten stimmen mit denen[6–8] von authentischem Canthaxanthin überein. Auch Mischchromatogramme ($CaCO_3$, Al_2O_3, Petroläther, Benzol) und Mischschmelzpunkt mit synthetischem Canthaxanthin beweisen die Identität beider Pigmente.

Damit ist Canthaxanthin neben Astaxanthin das zweite chemisch eindeutig bestimmte Sekundär-Carotinoid in grünen Algen. Dieses von HAXO[6] aus *Cantharellus cinnabarinus* und von SAPERSTEIN und STARR[7] aus einer farbigen Mutante von *Corynebacterium michiganense* isolierte Ketocarotinoid, dessen Struktur von PETRACEK und ZECHMEISTER[8] aufgeklärt wurde, konnte später von VÖLKER[9] in Vogelfedern und kürzlich von THOMMEN und WACKERNAGEL[10] in Daphnien nachgewiesen werden[11].

Summary. The ketonic carotenoid canthaxanthin has been isolated from three nitrogen-starved strains of *Chlorella*.

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[11] Der F. HOFFMANN-LA ROCHE AG, Basel, danke ich für authentisches Canthaxanthin. Mit freundlicher Genehmigung von Herrn Prof. Dr. H. DRAWERT wurden diese Untersuchungen im Botanischen Institut der Universität Marburg durchgeführt. Der Deutschen Forschungsgemeinschaft bin ich für ein Stipendium und für Sachbeihilfen zu Dank verpflichtet.

On the Presence of Sepiapterin Reductase Different from Folate and Dihydrofolate Reductase in Chicken Liver

Concerning the metabolism of sepiapterin (2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine)[1], it was reported that the pteridine was reduced in the presence of reduced nicotinamide dinucleotide phosphate (NADPH) by a folate reductase from chicken liver and Drosophila[2,3]. On the other hand, we showed that in a silkworm two distinct enzymes were responsible for attacking folate and sepiapterin, respectively[4].

In the present communication, we wish to present evidence indicating the presence of sepiapterin reductase different from folate and dihydrofolate reductase in chicken liver.

Experiments. Acetone powder of chicken liver was prepared as described by ZAKRZEWSKI[5]. All subsequent enzyme preparations were performed at 0–3°C, and centrifugation was carried out at 14,000 *g* for 20 min.

Acetone powder (5 g) was stirred for 1 h with 20 vol of cold water. After centrifugation, the supernatant (crude extract) was fractionated with ammonium sulphate. The fractions precipitating between 0.18 and 0.34 saturation (fraction 1) and 0.49 and 0.60 saturation (fraction 2), were collected, redissolved in 5 ml of water and dialysed overnight against distilled water. After removing insoluble materials by centrifugation, the supernatants were used for assay of enzyme. Dihydrofolate reductase was prepared by the method of SILVER et al.[6]. This fraction corresponds to 0.60–0.70 saturation of ammonium sulphate (fraction 3).

Sepiapterin reductase was assayed in the following reaction mixture: McIlvaine buffer (pH 6.0, 100 μmoles), sepiapterin (0.1 μmole), NADPH (0.1 μmole) and enzyme (0.2 ml), brought to a total volume of 0.5 ml. After incubation at 37°C for 30 min, the reaction was stopped by the addition of 0.5 ml of 16% trichloroacetic acid. After centrifugation, the supernatant was neutralized with 0.5 ml of 0.8 *M* tris-(hydroxymethyl)-amino-methane, and then optical density at 420 mμ of the mixture was measured to know the decrease in a concentration of sepiapterin.

Assay of folate reductase was performed in the mixture of citrate buffer (pH 5.0, 40 μmoles), NADPH (0.08 μmole), folate (0.05 μmole) and enzyme (0.3 ml), brought to a total volume of 2.5 ml. After incubation at 37°C for 30 min, the reaction was stopped by the addition of 0.5 ml of 30% trichloroacetic acid, and the amount of the resultant tetrahydrofolate (FAH_4) was determined by the method of BRATTON and MARSHALL[7].

Dihydrofolate reductase was assayed by measuring the decrease in optical density at 340 mμ of the reaction mixture of potassium phosphate buffer (pH 7.5, 50 μmoles), dihydrofolate (0.1 μmole), NADPH (0.1 μmole) and enzyme (0.2 ml), brought to a total volume of 1.2 ml.

Protein was determined by the method of LOWRY et al.[8]. NADPH was prepared enzymatically from NADP[5]. Dihydrofolate was prepared according to FUTTERMAN's method[9]. Purified sepiapterin was obtained from mutant sepia of Drosophila (unpublished work).

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