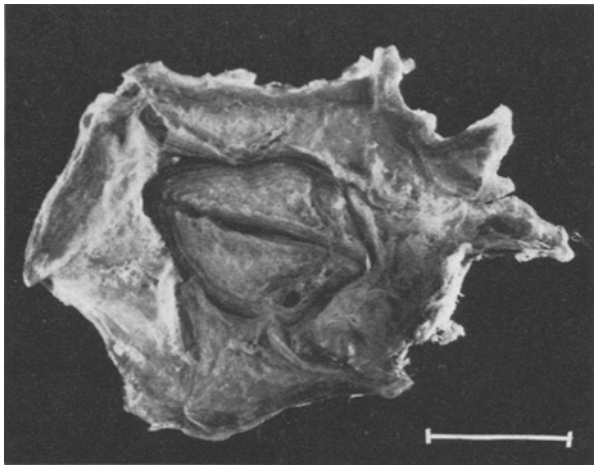

Fig. 2. *Chthamalus depressus* (Poli): same animal viewed from above; opercular valves and wall plates normal; shell eroded indicating exposed habitat; scale bar: 3 mm.

logical processes and differentiations. CRISP[5] has also found occasional abnormal development in the ova of *Balanus balanoides* (L.); part of the vegetative pole of a normal egg became detached and the remainder gave rise to a miniature embryo which did not, however, give a viable nauplius. Partially viable, abnormal larvae are also formed as the result of development in hypertonic media; such abnormalities are largely confined to the limbs but do give larvae, which are viable for a limited time (BARNES and BARNES[6]).

CHEUNG[7] has described abnormalities resulting from the treatment of cyprids with 20-hydroxyecdysone; abnormal larvae are produced but the adult shell remains unaffected and TIGHE-FORD[8] found that analogies of an insect juvenile hormone induced abnormalities in the nauplii and cyprids of *Elminius modestus* Darwin – gross extension of the anterior region bearing the antenna; there was no evidence of a duplication of structures.

Dr. H. BARNES informs me that Dr. P. CHEUNG, in examining the exuvia of some experimental animals, has found one with 2 complete penes on the exuvia: unfortunately the animal itself was not retained. It seems unlikely that the abnormality found in the adult *C. depressus* had originated and been carried through from the larval stage but rather that it had arisen at, or subsequent to, metamorphosis from the cyprid to the adult. It seems clear that it would have to give rise to an exuvia in which both 'twin' parts could have been recognized.

Zusammenfassung. Eine bemerkenswerte Abnormität wird von einem adulten *Chthamalus depressus* (Poli), gefunden in der oberen Litoralzone von Rovinj, Jugoslawien, gemeldet. Der cirrentragende Körperabschnitt war verzwillingt, wobei die Cirren selbst am kleineren Teil nur unvollständig entwickelt waren. Das Tier lebte. Frühere Meldungen von Abnormitäten an Cirripediern beziehen sich immer auf Larven unter Experimentierbedingungen.

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Binding of D-α-Tocopherol to Rat Liver Nuclear Components

Previous studies have shown that significant amounts of vitamin E are associated with the nuclear apparatus in mammalian cells[1,2]. Although binding of this vitamin to various native proteins like serum albumin and egg albumin under in vitro conditions[3], and to a cytosolic lipoprotein under in vivo conditions[4], has been observed, information is lacking with respect to the binding of this vitamin to intranuclear components. The present report describes the identification of a nucleoprotein receptor complex that binds D-α-tocopherol when it is administered to vitamin E deficient rats.

Materials and methods. Weanling male rats of the Sprague-Dawley strain maintained on a vitamin E deficient diet[5] for 10 to 12 weeks, were injected i.v. with specified amounts of ^{3}H-D-α-tocopherol (3.7 Ci/mmole, The Radiochemical Center, Amersham, England) dissolved in a compatible vehicle (0.5 – 1.0 ml of a mixture of dimethylsulfoxide, saline and tween 80, 1:0.9:0.1). The rats were sacrificed 3 h after administration of labelled tocopherol. Highly purified nuclei were isolated from liver[6,7].

The nuclei associated receptor complex was solubilized by homogenization of the labelled nuclei in a hypotonic *tris* buffer using a glass-teflon homogenizer, and the homogenate was extracted with varying concentrations of sodium chloride, essentially as described by BRUCHOVSKY and WILSON[8]. Each milliliter of homogenate contained 29,000 dpm of ^{3}H-D-α-tocopherol, 6.8 mg of protein, 1.0 mg of DNA and 0.34 mg of RNA. After completion of the extraction, each sample was centrifuged and both supernatant and the pellet were analyzed for radioactivity, protein, DNA and RNA.

The total nuclear extract prepared by the method described above, containing 0.6 *M* NaCl, was fractionated through a Sephadex G-200 column (65 × 2.5 cm, i.d.) at 20 °C. Fractions of 2.5 ml each were collected and were analyzed for radioactivity, protein, DNA and RNA.

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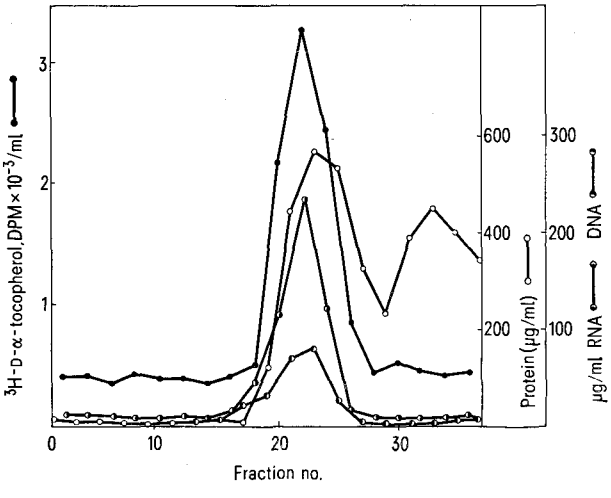
Protein content was determined by the method of LOWRY[9]. DNA and RNA content were determined by the methods of LEVY et al.[10], BURTON[11] and DISCHE[12]. Radioactivity was assayed either by using a dioxane based liquid scintillation cocktail[13], or with toluene containing liquifluor, after solubilizing the samples with NCS.

Results and discussion. The nuclear associated receptor of D-α-tocopherol can be rendered soluble by extraction of highly purified rat liver nuclei with a buffer containing high concentrations of sodium choride. With increasing concentrations of sodium chloride in the buffer, correspondingly higher amounts of radioactivity were extracted. It was also observed that an increase in the solubilization of the radioactivity was associated with a simultaneous proportional increase in the solubilization of protein, DNA and RNA. More than 70% of the total radioactivity could be extracted by raising the salt concentration up to 2.0 *M*.

In order to examine the physical nature of the tocopherol extracted by high salt solution, the solubilized nuclear extract (0.6 *M* NaCl) was fractionated on a Sephadex G-200 column. As shown in the Figure, all of the radioactivity were eluted in the void volume of the column and the labelled vitamin was associated with protein, DNA and RNA. Although several other protein species were eluted after the void volume, no radioactivity was associated with any one of them. Thus the receptor of this vitamin is associated with a nucleoprotein complex of high molecular weight.



Fractionation of the toal nuclear extract on Sephadex G-200. Rats were injected with ^{3}H-D-α-tocopherol (25 μCi; 3.7 Ci/mmole) for 3 h.

The radioactivity associated with the purified nuclei, as well as in the nucleoprotein compelx, was extracted with ethanol and subjected to thin layer chromatography (TLC) on silica gel G using benzene:methanol, 98:2, as the mobile phase. The ethanol soluble radioactivity recovered from TLC appeared in the zone corresponding to standard α-tocopherol, showing that the radioactivity represented unmetabolized α-tocopherol.

Summary. When D-α-tocopherol is administered i.v. to vitamin E dificient rats, significant amounts of this vitamin are bound to a nucleoprotein complex in hepatic nuclei, and this complex can be solubilized by high concentrations of sodium chloride (0.6 *M*). The bound vitamin in this complex, extractable by ethanol, was found to be identical with authentic α-tocopherol by thin layer chromatography.

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Chalone Regulation of the Epidermal Cell Cycle

It has been established that crude extracts of epidermis produce two tissue-specific responses when injected into mice. These are a delayed inhibition of ^{3}H-thymidine incorporation into epidermal DNA and a rapid reduction in the number of epidermal cells entering M-phase (mitosis)[1-6]. This difference in the timing of these two responses has led, in the absence of substantial cell kinetic data ,to the assumption that chalones are cell cycle phase-specific and that the inhibitor of DNA synthesis in these extracts acts by blocking the influx into S-phase at some point during the G_1-phase[5,6], while the inhibitor of mitosis acts in the G_2-phase. However, the time lag in ^{3}H-thymidine incorporation, may well represent competition between a short-term stimulator[7] and a slower-acting inhibitor of DNA synthesis, or it may reflect an insensitire assay system.

From an ethanol-fractionated skin extract an inhibitor of epidermal DNA synthesis (G_1 chalone) has been purified 50,000-fold although it is not yet homogeneous[6]. Previously, a similar ethanol fraction yielded an inhibitor of mitosis (G_2 chalone) which has been purified 2,000 times[8]. Chemically these appear to be two different substances[6,8] but neither has been tested against other phases of the cell cycle. A preliminary analysis of the specificity of chalone action within the cell cycle is reported here. The effect of the ethanol skin fraction (containing both chalones) was compared with the effect of the purified G_2 chalone on DNA synthesis and mitosis in mouse epidermis in vivo.

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