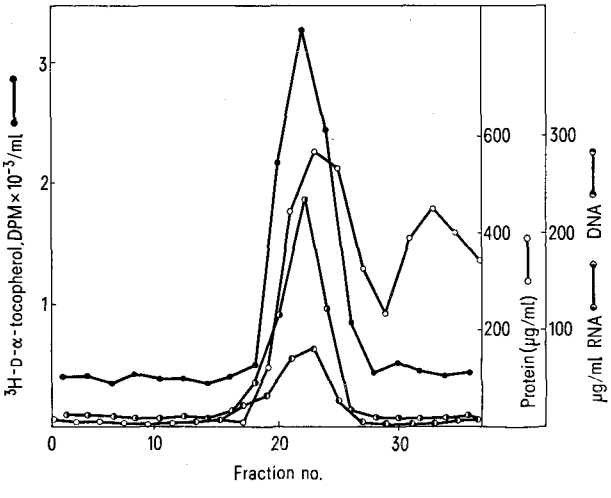
Protein content was determined by the method of LOWRY[9]. DNA and RNA content were determined by the methods of LEVY et al.[10], BURTON[11] and DISCHE[12]. Radioactivity was assayed either by using a dioxane based liquid scintillation cocktail[13], or with toluene containing liquifluor, after solubilizing 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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Table I. ^{3}H-thymidine incorporation by epidermal cells $2^1/_2$ and 6 h after injection of skin extracts

10 Mice in each group	$2^1/_2$ h after 1st injection		6 h after 1st injection	
	dpm/mg Epidermis (Mean ± SE)	Depression (%)	dpm/mg Epidermis (Mean ± SE)	Depression (%)
Control	6900 ± 500	–	8100 ± 620	–
Ethanol fraction	6670 ± 390	3	4750 ± 450	41 [a]
G_2 chalone	8030 ± 590	0	8640 ± 550	0

[a] $p < 0.01$.

Methods. Lyophilized ethanol fraction[9] (70–80% precipitate of pig skin extract, 55% protein, 4% RNA) was dissolved in distilled water and dialyzed against distilled water for 48 h at 5 °C. The supernatant, following centrifugation at 50,000 *g* (0 °C, $^1/_2$ h) to remove a fine sediment, was the test material. Electrophoretically purified, lyophilized G_2 chalone[9] was dissolved in distilled water. At 07.30 h the ethanol fraction (80 µg/g body weight) was injected s.c. into 20 male Swiss S mice which were $3^1/_2$ months old and had been previously housed 5 per box for 1 month under constant temperature and lighting conditions. At the same time G_2 chalone (1.2 µg/g body weight) was injected into 20 mice and saline into 20 control mice. At 09.30 h 10 mice from each group received an injection of methyl ^{3}H-thymidine (2.5 µCi/g body weight, specific activity 5 Ci/mmol, Radiochemical Centre, Amersham, England) and were killed at 10.10 h. The remaining 10 mice in each group received a further dose of ethanol fraction, G_2 chalone or saline at 10.30 h, ^{3}H-thymidine at 13.00 h, and were killed at 13.40 h. The ears were rapidly depilated with Immac (Anne French, N.Y.) before removal and storage on ice. Two 5 mm punches were taken from both ears of each animal and briefly immersed in an ice-cold solution of 1.2 *M* NaBr and 10^{-3} EDTA (pH 7.0). The epidermis was then removed as a sheet from the dermis using fine forceps and a dissecting microscope. Sebaceous glands and hair follicle cells were not removed with the epidermis during the process so that tissue specificity of the test was ensured. (This check on tissue specificity has not been a feature of previously described epidermal chalone assay systems). The epidermal sheet was placed on a glass fibre disc of predetermined weight, washed twice with ice-cold 0.4 *N* and 0.2 *N* perchloric acid, and twice with ethanol and ether, and then oven-dried overnight. The weights of the epidermal samples were determined to the nearest 2 µg and the samples placed in 1 ml Soluene (Packard & Co. Inc.). Counting was done in a Packard Tri-Carb Liquid Scintillation Spectrometer and the results expressed as decays per min/mg dry weight of epidermis. The small intestine formed a nonspecific target tissue to test for non-specific inhibitors or cytotoxicity of the test substances. Portion of the duodenum was removed from the animals, washed well in saline and homogenized in 4.0 ml distilled water. 0.2 ml concentrated perchloric acid was added and the samples placed on ice for 30 min. The amount of ^{3}H-thymidine incorporated into DNA was then estimated employing the method described by MARKS[6], who has examined a similar ethanol fraction for non-specific inhibitors of DNA synthesis.

The portion of tissue separating the two punch holes in each ear was fixed in alcoholic Bouin, sectioned at 3 µm and prepared for autoradiography. The labelling index in each of the 120 samples was determined by counting the number of ^{3}H-thymidine labelled cells per 10 mm length of epidermis. In this way an estimate of ^{3}H-thymidine incorporation and labelling index was obtained from the same area of ear epidermis.

At 11.30 h 10 mice were injected with ethanol fraction, 10 with G_2 chalone and 10 with saline. Each animal then received 4 µg/g body weight colcemid and was left undisturbed until sacrifice at 15.30 h. Samples of epidermis from the left and right ears of these 30 animals were sectioned at 7 µm and the number of arrested metaphase plates per 10 mm length of epidermis (mitotic index) was counted.

Results and discussion. From the results summarized in Tables I and II it is evident that the ethanol fraction contained a factor which caused a 41% decrease in the amount of ^{3}H-thymidine incorporated into epidermal DNA. This depression apparently took effect somewhere between the first and second sampling times: that is, $2^1/_2$ to 6 h after the initial injection. Concomitantly, there was a 33% reduction in the number of cells labelled in autoradiographs. This combined effect may have been

[9] Donated by N.V. Organon, Oss, Holland.

Table II. Epidermal labelling index $2^1/_2$ and 6 h after injection of skin extracts

Same animals as in Table I	$2^1/_2$ h after 1st injection		6 h after 1st injection	
	No. labelled cells (Mean ± SE)	Depression (%)	No. labelled cells (Mean ± SE)	Depression (%)
Control	38.4 ± 1.2	–	42.1 ± 1.6	–
Ethanol fraction	37.9 ± 0.65	0	26.7 ± 0.90	33 [a]
G_2 chalone	38.4 ± 1.5	0	39.3 ± 0.96	−7

[a] $p < 0.01$.

Table III. Epidermal mitotic index 4 h after injection of skin extracts

10 Mice in each group	Mitotic index (Mean ± SE)	Depression (%)
Control	11.25 ± 0.63	—
Ethanol fraction	5.50 ± 0.20	51 [a]
G_2 chalone	6.10 ± 0.33	46 [a]

caused by the inhibitor of DNA synthesis (G_1 chalone) which MARKS[6] has isolated from this ethanol fraction. The purified G_2 chalone reduced neither the amount of incorporated ^{3}H-thymidine nor the labelling index, whilst exhibiting a powerful inhibitory effect on epidermal cells flowing into M-phase (Tables I, II and III). A repeat of this experiment assaying G_2 chalone at $1^1/_2$ and 4 h after a single dose (1.2 µg/g body weight) revealed neither an early, transient inhibition of ^{3}H-thymidine incorporation, nor a change in labelling index.

The possibility of the G_2 chalone having a strong inhibitory effect on the flow of cells into S-phase, or on epidermal cells engaged in genome replication, can be tentatively dismissed. The purified G_2 chalone is thus phase-specific in its inhibitory action. It has already been demonstrated that this inhibitor is tissue-specific, species nonspecific and that it inhibits mitosis reversibly in vivo and in vitro[10,11].

The results in Tables II and III show clearly that the ethanol fraction contained also the G_2 inhibitory factor and thus depressed DNA synthesis as well as mitosis. MARKS[6] has already shown that this ethanol fraction is free of non-specific inhibitors of DNA synthesis. Likewise, the incorporation of ^{3}H-thymidine by intestinal cells was not affected during the experiments described here.

Thus it can be concluded that the epidermal G_2 chalone inhibits only the flow of cells into M-phase. The purified G_1 chalone has yet to be analyzed to establish its exact point of action in the cell cycle. This knowledge is essential if the mechanism of chalone regulation of cell proliferation is to be understood.

Summary. Purified epidermal G_2 chalone does not inhibit DNA synthesis or influx of S-phase cells and is therefore cell cycle phase-specific, inhibiting only the flow of cells into M-phase.

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Seasonal Mitotic Activity and Wound Healing in a Teleost (*Opsanus tau*) Ocular Lens[1]

Cells throughout the central region of the adult vertebrate lens epithelium are normally quiescent relative to mitosis[2,3]. However they can be triggered to divide as a result of mechanical[4,5] or chemical[6-8] insult or by exposure of the cultured lens to serum[9] or insulin[10,11].

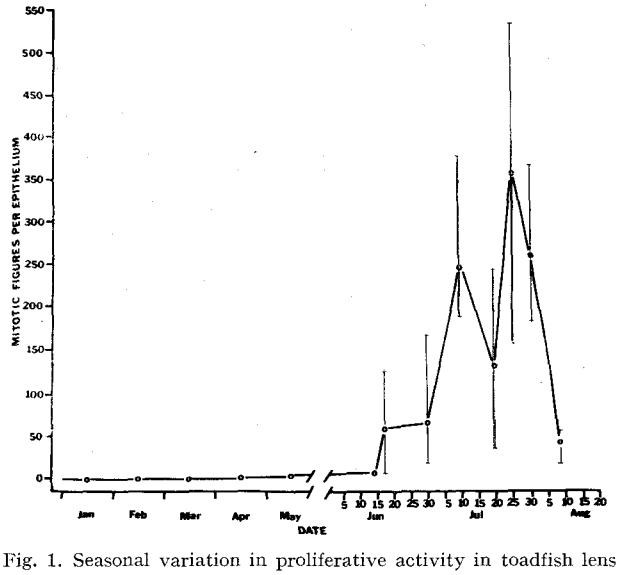

Fig. 1. Seasonal variation in proliferative activity in toadfish lens epithelium. Animals were killed periodically, as indicated, and lens epithelium wholemounts were scored for mitosis. Mean values for total division figures per lens preparation are plotted. Highest and lowest scores per interval are shown. Each point represents data from at least 6 lenses. Note the increase in mitosis from May to August.

The frog lens epithelium exhibits a circannual pattern of mitosis that may be under thermoendocrine control[12,13]. In order to show that the seasonal pattern of mitosis as documented in the amphibian lens is of general occurrence it will have to be demonstrated in several species. In this study we 1. characterize the pattern of mitosis in the toadfish lens, 2. explore seasonal variation in that pattern, and 3. determine the effect of needle injury on the pattern of DNA synthesis and cell division in the lenticular epithelium.

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