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].

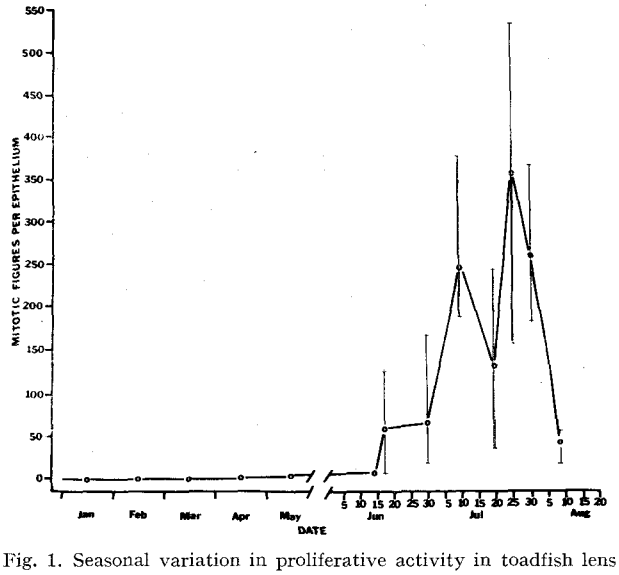

Fig. 1. Seasonal variation in proliferative activity in toadfish lens epithelium. Animals were killed periodically, as indicated, and lens epithelium wholemounts were scored for mitosis. Mean values for total division figures per lens preparation are plotted. Highest and lowest scores per interval are 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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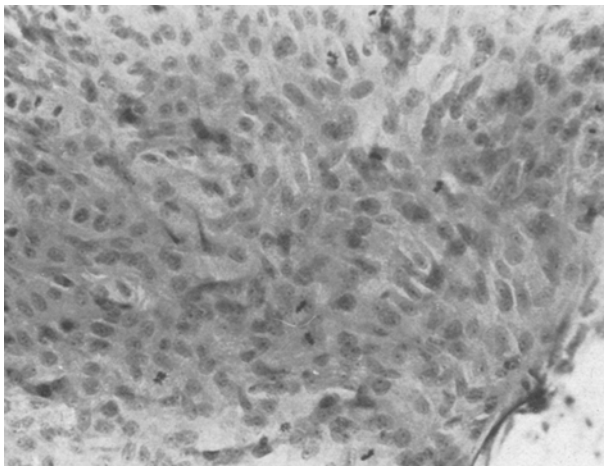

Fig. 2. Whole-mount preparation obtained 96 h after injury. Note the disorganization of the epithelial layer and the numerous mitotic figures. The injury is in the lower right hand corner (Hematoxylin).

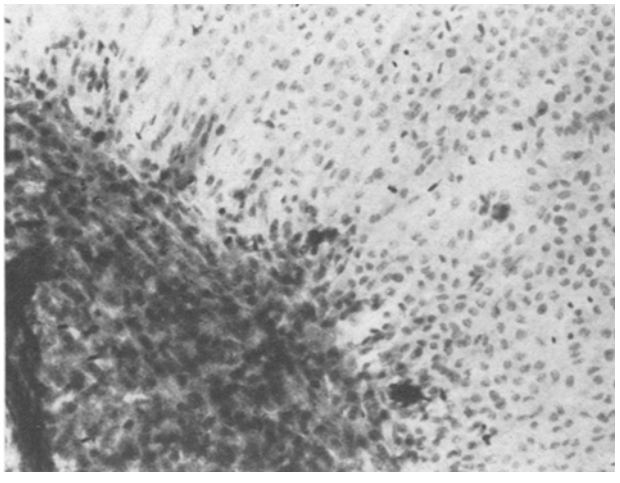

Fig. 3. Whole-mount preparation obtained 7 days post-injury. Note regions of cellular accumulation at the site of the wound and mitotic figures located in areas of cell sparsity (Hematoxylin).

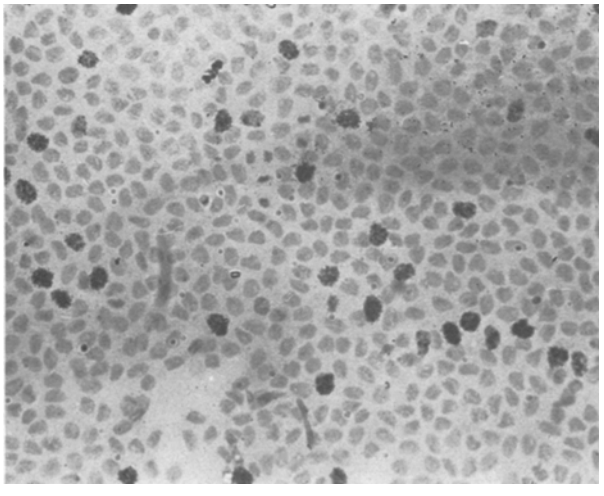

Fig. 4. Autoradiogram obtained 4 days after injury. The isolated lens was exposed to tritiated thymidine (5 Ci/ml; Spec. act. 19.5 Ci/mM) for 1 h prior to fixation. Note the admixture of labeled and dividing cells (Hematoxylin).

Materials and methods. Toadfish, *Opsanus tau*, 175–520 g, were killed during various months at noon ± 2 h and flat-mounts[14] of the lens epithelium were prepared. In some cases animals were injured unilaterally by the transcorneal insertion of a fine steel needle into the anterior pole of the lens. Certain lenses were incubated in teleost Ringer solution[15] containing ^{3}H-thymidine and prepared for autoradiography[16]. Experiments were conducted from January–August 1971 and from June–August 1972.

Results and discussion. Whole-mount preparations were essentially amitotic from January to May (Figure 1). Occasionally 1 or 2 dividing cells were found in specimens examined during the last week of May. In June the magnitude of proliferation increased and remained elevated through July and early August. Preparations obtained later in August showed diminished mitotic activity and represent the latest period for which data was obtained (Figure 1). The triggering of mitosis in the spring corresponded with the approximate time that toadfish move into shallow water for spawning under natural conditions[17]; water temperature at this time rose above 15 °C.

While individual variation in the level of mitosis was marked during the spring and summer months, levels of mitosis in contralateral lenses tended to be similar. In any case, the magnitude of mitosis noted throughout the spring and summer was clearly elevated above that detected from January to May.

Of added interst is the finding that during the June–August period mitotic figures were noted throughout the lens epithelium. In most adult vertebrates lenticular mitosis is confined to a narrow pre-equatorial zone. No distinct correlation between animal weight and level of mitosis was discernable.

The patterns of DNA synthesis and mitosis in lenses injured in July was determined. During the first day following needle injury there was cellular disorganization, but the level of mitosis was ostensibly similar to control preparations. By 72–96 h mitotic activity at the wound focus became elevated when compared to non-injured controls or to areas somewhat removed from the perforating injury itself. At 96 h the wound area was populated by cells having nuclei of varied size, cells elongated and oriented normal to the lesion and cells in mitosis (Figure 2). The proliferative activity was intense at 7 days, with areas of cell accumulation surrounded by fusiform cells residing in areas of cell sparsity (Figure 3).

Thymidine autoradiography of toadfish lens epithelium (Figure 4) showed an admixture of labeled and dividing cells similar to the pattern noted in the injured sea bass[18], frog[19], or guinea-pig lens[20], but unlike the wave-like geometric pattern described for the rabbit lens[4]. The mechanism(s) responsible for the wave-like pattern of proliferation in one species and its absence in others remains obscure.

The results of the present study show that the toadfish lens epithelium undergoes seasonal variation in mitosis. The onset of proliferation detected in the spring and the diminution observed in the fall is in broad agreement with the findings of Rosenbaum and Rothstein[12],

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who detected circannual patterns of mitosis in lens epithelium of 2 species of frog. The possible factors that may modulate the onset of mitosis in the spring are numerous, e.g., nutritional levels, day length, temperature, and various hormones. Previous studies have shown that temperature can regulate the kinetics of the cell cycle in the frog lens[21,22]. In addition, growth hormone can stimulate and hypophysectomy can curtail the level of mitosis in the normal frog lens[23]. Moreover, the hypophysectomized frog failed to show a mitotic response when challenged by either mechanical or chemical insult[24]. Thyroxin and triiodothyronine alter the locus of proliferation in the frog lens[25], extending it into the polar epithelium, resulting in patterns of DNA synthesis and mitosis comparable to those noted here in spring-summer toadfish. With respect to other species, perch reportedly do not grow in the winter when the pituitary contains little growth hormone (GH)[26]. Whether growth in the toadfish lens is under constraints similar to those noted in the frog and perch remains to be documented. More importantly however, the current study provides a base of information which permits such questions to be considered.

Summary. The toadfish lens epithelium is essentially amitotic from January to May. From June to August mitotic activity is noted. The onset of proliferation approximately corresponds to the time of year when the fish enter the shallow water to spawn. The epithelial mitotic response to needle injury is not propagated in a wave-like manner from the site of insult.

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High-Frequency Fusion of Fungal Protoplasts

In a previous communication[1], we described a method for obtaining an increased fusion frequency (2.5×10^{-3}) of *Aspergillus nidulans* protoplasts, based on spontaneous aggregation of protoplasts in inorganic osmotic stabilizers. This method was later further improved by using KCl as stabilizer, Ca ions in the concentration range of 10 to 100 m*M* and high pH. However, the frequency of protoplast fusion, measured via the complementation of nutritionally deficient *Aspergillus nidulans* mutants, remained of the same order of magnitude.

In a search for highly efficient procedures of protoplast fusion, with the aim of bringing about biochemical complementation of genetically distant fungal species, we found that higher molecular weight polyethylene glycols (PEG), known agents for stabilizing bacterial protoplasts[2] and aggregating and fusing higher plant protoplasts[3–8], can satisfy the requirements.

We describe here a simple technique which results in high-frequency intraspecific protoplast fusion and complementation of auxotrophic mutants of *Aspergillus* (*A.*) *flavus*, *A. nidulans*, *A. niger*, *Penicillium* (*P.*) *frequentans* and *P. ramigena*. The method presented may also be suitable as a means of interspecific genetic transfer.

Materials and methods. Large numbers of nutritionally-deficient stable UV-mutants of the strains *A. flavus* (SzMC 0552), *A. nidulans*[9,10] (pabal, y, ts6) *A. niger* (SzMC 0145), *P. frequentans* (SzMC 0531) and *P. ramigena* (SzMC 0519) were produced, and the mutant pairs requiring lysine (Lys) and methionine (Met) were used throughout.

Maintenance and cultivation of the mutants, and the protoplast formation were carried out as previously described[1], with the exception that protoplast formation was performed in 0.6 *M* KCl for *Aspergilli* and in 0.8 *M* KCl for *Penicillia*, at pH 6, in both cases unbuffered. After collection of protoplasts and removal of the enzymes by repeated centrifugation with isoosmotic KCl solution, protoplast suspensions (about 1 million/ml) of the Lys mutants were mixed with those of the corresponding Met mutants. The mixed suspensions were again centrifuged, the supernatant was removed by suction.

One ml solutions of PEG (m. w. 4000) of different concentrations, containing 10 m*M* $CaCl_2$, were added to the protoplasts to find the optimum concentration of the fusion agent. The suspensions were stirred vigorously for a few sec. No additional osmotic stabilizer was used in the experiments reported in this paper. Samples were taken at 15 min intervals, mixed with osmotically-stabilized minimal medium, plated for regeneration, and then checked for segregetion as described earlier[1]. Osmotically-stabilized yeast-extract medium served as control to determine both the stabilizing effect of PEG at various concentrations and the ratio of complemented protoplasts. In the stabilized yeast-extract medium, practically 100% protoplast regeneration could be attained.

In other experiments, the PEG concentration was kept on the level found to be optimum and the Ca concentration was varied.

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