and have been reported to be effective in their melanocytolytic action both in vivo and in vitro (*3–9*). It is clear, therefore, that melanocytolytic agents, if administered appropriately, may be helpful in the clinical management of malignant melanoma.

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## Somatomedin C: Restoration in vivo of Cycle Traverse in $G_0/G_1$ Blocked Cells of Hypophysectomized Animals

Abstract. *DNA synthesis and mitosis in frog lens epithelium are abolished by hypophysectomy and restored by somatomedin C. Both growth hormone and triiodothyronine also restore lens cell proliferation in vivo but not in vitro. Somatomedin-like activity in frog serum is diminished by hypophysectomy and is restored by growth hormone and triiodothyronine.*

The somatomedins (*1*) are a family of growth hormone-dependent peptides, ancestrally related to insulin (*2*) and capable of inducing DNA synthesis and mitosis in cell culture systems (*3*). There have been no reports that these effects could be achieved in vivo since (i) the somatomedins are available in limited quantities and (ii) there has been no animal model in which DNA synthesis and mitosis could be totally but reversibly eliminated.

Three weeks after frogs are subjected to hypophysectomy, both mitotic and DNA synthetic activity disappear completely in the lens epithelium (*4*). Microspectrophotometric and autoradiographic analyses have shown that in the hypophysis-deprived frog almost all of the epithelial cells come to reside in the $G_0/G_1$ segment of the cell cycle (*5*). In organ cultures of lenses from frogs the addition of 15 to 20 percent normal frog serum to the medium (Medium-199) triggers division (*5, 6*); however, the addition of serum from hypophysectomized frogs does not stimulate cell proliferation (*5*).

When administered in vivo, growth hormone, frog prolactin, or triiodothyronine ($T_3$) restore or maintain the proliferative process in lenses of hypophysectomized animals (*7, 8*). However, these hormones fail to promote cell division in organ culture (*5, 7*). Growth hormone injected into hypophysis-deprived frogs rebuilds the mitogenicity of the serum, as judged by lens organ culture tests (*5*). The foregoing information suggests that a pituitary-dependent mitogen, such as somatomedin C (*2*), may be effective in frog lens epithelium. Since no division or thymidine incorporation occur 3 to 4 weeks after hypophysectomy and since every cell in a whole-mount preparation of the tissue can be observed by light microscopy, the frog lens is an ideal system with which to evaluate pertinent growth factors.

To assess the effects of human somatomedin C, we used newly postmetamorphic *Rana catesbeiana* (8 to 21 g); the blood volume of each frog was estimated to be 0.25 to 0.40 ml. For assays of somatomedin-like activity of serum, we used adult bullfrogs (*R. catesbeiana*) and leopard frogs (*R. pipiens*). We estimated the somatomedin-C content of various frog serums by a heterologous radioimmunoassay using a rabbit antiserum against human somatomedin C and human $^{125}$I-labeled somatomedin C as the tracer (*9*). For the receptor assays of somatomedin C we used $^{125}$I-labeled somatomedin C as the tracer and a human placental cell membrane preparation as the source of receptor (*10*).

Somatomedin C was purified from Cohn fraction IV of human serum (*11*). The weight of somatomedin C in this preparation was based on the somatomedin C content determined by radioimmunoassay and the known specific activity of the pure peptide. Surgical hypophysectomy was carried out by the procedure of Hogben (*12*). Human growth hormone (Calbiochem), purified somatomedin C, or Earle's balanced salt solution (the medium in which the hormones were dissolved) and [$^3$H]thymidine (specific activity 6.7 Ci/mmole) were injected into the dorsal lymph sac in a volume of 100 $\mu$l according to schedules to be detailed. On the day after the final injection the animals were killed and the enucleated eyes were fixed in Carnoy's fluid. Whole-mount preparations were made from the lenses (*13*). The preparations were autoradiographed (Kodak NTB3) and after 14 days of exposure were stained with Harris hematoxylin. Corneal endothelium was separated from the stroma and corneal epithelium. Both corneal endothelium and lens epithelium are avascular and share the aqueous humor as their principal, if not sole, source of nutrients. The serum for radioimmunoassay was derived from *R. catesbeiana* by puncture of the conus arteriosus or ventricle. The blood was allowed to clot and was then centrifuged; the samples were frozen at −20°C.

As shown in Fig. 1, normal (intact) adult bullfrog serum is only about 5 percent as potent as is human serum in competing with $^{125}$I-labeled somatomedin C for binding to antibody. Furthermore, bullfrog and human serum give nonparallel displacement curves, although displacement curves of various samples of frog serum are parallel to each other. Serum from animals that had been hypophysectomized 1 month previously was 50 percent as potent as normal frog


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serum, whereas serum from hypophysectomized bullfrogs treated with bovine growth hormone (5 μg/g) for 4 days was 1.7 times as potent as that of normal bullfrogs.

Similar quantities of cross-reacting materials were present in the blood of normal leopard frogs, with a 33 percent decrease by day 49 after hypophysectomy. Replacement therapy with $T_3$ (0.5 μg per gram of body weight), beginning on day 28 and administered every other day until day 49, resulted in an increase in somatomedin-like activity to 1.2 times the concentration of normal serum.

Normal bullfrog serum also competes with $^{125}$I-labeled somatomedin C for binding to the somatomedin receptor in human placental membrane preparations. The potency of frog serum relative to human serum is somewhat greater in this less specific assay, but, again, the curves of competition are not parallel. The lack of parallelism between human and frog serum in both assays suggests that there are structural differences between frog somatomedin and human somatomedin C. For this reason, the absolute concentrations of somatomedin in frog serum relative to human serum may be substantially different from the relative potency suggested by these comparisons. Nonetheless, the growth hormone dependency of these somatomedin-like substances in frog serum is clearly evident. So far, amphibia represent the most primitive class of vertebrates in which a somatomedin C-like substance has been detected. No such materials could be found in the blood of the carp or Atlantic bluefish (*9*, *14*). The foregoing findings make reasonable the suggestion that a somatomedin C-like substance controls proliferation in frog lens epithelium. This suggestion was strengthened by experiments in vivo.

Experiments on postmetamorphic bullfrogs were begun between 5 to 12 weeks after they were hypophysectomized. Previous studies have repeatedly shown that in adult bullfrogs mitosis and DNA synthesis subside entirely by day 28 after hypophysectomy, and that they are restored by administration of growth hormones (*4*, *5*, *15*).

Injections of human growth hormone (0.25 μg per gram of body weight per injection; 65 μg, total) were given over a 13-day period between days 36 and 49 after hypophysectomy. Because the availability of highly purified somatomedin C is very limited, only two animals were treated with this growth factor. Beginning on days 75 and 85 after the operation, respectively, one animal received

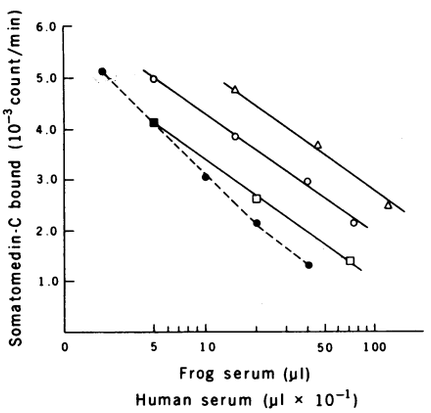


Fig. 1. Radioimmunoassay of somatomedin C in bullfrog serum. A rabbit antiserum against human somatomedin C and human $^{125}$I-labeled somatomedin C was used as the tracer. Incubations were carried out in a total volume of 500 μl by the nonequilibrium technique of Furlanetto *et al.* (*9*). Symbols: ●, normal human serum; ○, normal frog serum; △, hypophysectomized frog serum; □, serum from hypophysectomized frogs treated with bovine growth hormone.

0.03 μg of somatomedin C per gram of body weight (5 μg, total) in ten injections over a 13-day period, and the other 0.076 μg/g (7 μg, total) in similar fashion. The froglet serving as the untreated hypophysectomized control received only Earle's balanced salt solution by the same schedule. All animals received four injections of [$^3$H]thymidine, 1 μCi per gram of body weight, on days 4, 7, 10, and 13 after the beginning of each treatment period. Also, an intact froglet was given [$^3$H]thymidine at the same dose and time intervals. Sham operations were not included in these studies since the procedure was without effect in previous experiments.

Figure 2 shows that [$^3$H]thymidine was incorporated into the cells of all preparations except that derived from the hypophysectomized control (vehicle injected) specimen. Since the S period (DNA synthesis) in the germinative zone of the uninjured lens lasts 3 to 4 days (*5*, *16*), the [$^3$H]thymidine regimen approximates continuous labeling. The somatomedin-C injected frogs contained labeled nuclei only in the germinative zone—the region to which mitosis is normally restricted in adult organisms. Labeling in the intact control and the hypophysectomized froglet that received human growth hormone was heavy in the germinative zone but extended further toward the anterior pole of the lens than was the case in the somatomedin-C treated animals (*17*).

The corneal endothelium of neither the normal nor experimental animals showed any mitotic figures or thymidine uptake. These cells usually reside in a re-

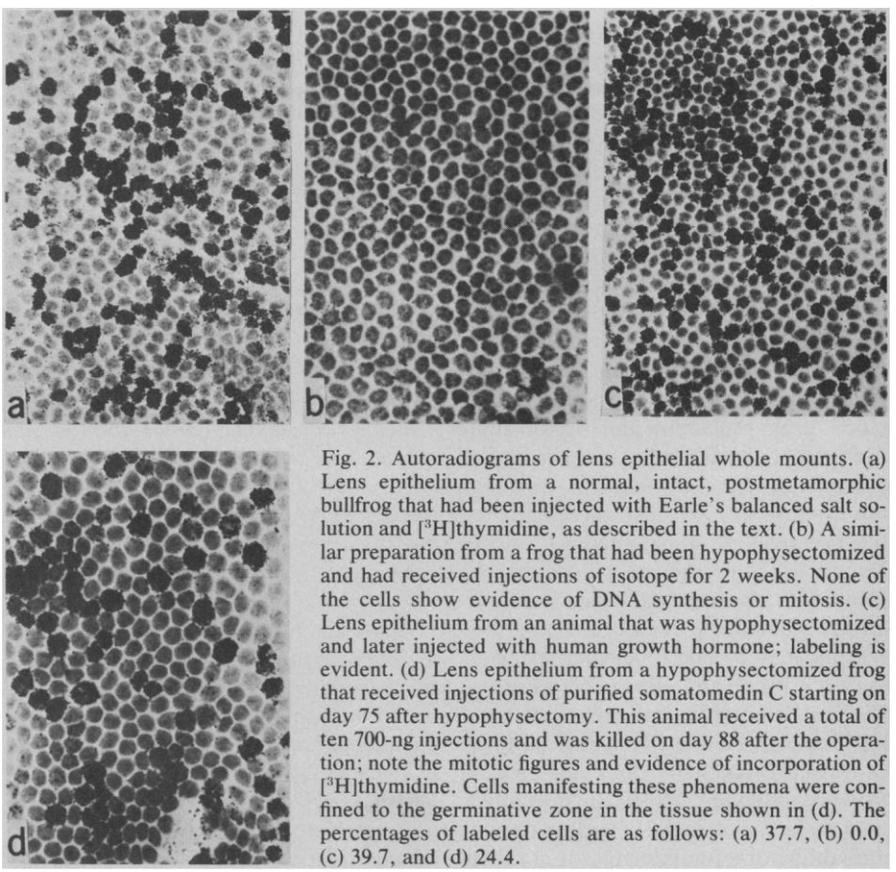


Fig. 2. Autoradiograms of lens epithelial whole mounts. (a) Lens epithelium from a normal, intact, postmetamorphic bullfrog that had been injected with Earle's balanced salt solution and [$^3$H]thymidine, as described in the text. (b) A similar preparation from a frog that had been hypophysectomized and had received injections of isotope for 2 weeks. None of the cells show evidence of DNA synthesis or mitosis. (c) Lens epithelium from an animal that was hypophysectomized and later injected with human growth hormone; labeling is evident. (d) Lens epithelium from a hypophysectomized frog that received injections of purified somatomedin C starting on day 75 after hypophysectomy. This animal received a total of ten 700-ng injections and was killed on day 88 after the operation; note the mitotic figures and evidence of incorporation of [$^3$H]thymidine. Cells manifesting these phenomena were confined to the germinative zone in the tissue shown in (d). The percentages of labeled cells are as follows: (a) 37.7, (b) 0.0, (c) 39.7, and (d) 24.4.

versible $G_0$ phase, from which they can be removed by means of injury (*18*). Thus far, no manipulation of the pituitary (or hormones controlled by it) has been found to initiate corneal endothelial growth or to modify it where stimulated by injury (*8*). Since both populations of cells are bathed only by the aqueous humor and since just one cell type is responsive to injections of somatomedin C, these results suggest that proliferation in the two tissues is controlled by different mechanisms.

The lens epithelium, in contrast, is exquisitely sensitive to pituitary manipulation. The work reported here is the first, to our knowledge, in which a purified somatotropin-dependent mitogen has been found to restore growth that has been completely stopped in vivo.

The lens epithelial cell of the hypophysectomized frog has been shown to reside in a "deep" $G_0$ state. Such a cell requires about 50 percent longer (72 as opposed to 48 hours) to reach DNA synthesis after introduction of the organ into a suitable medium in vitro (*5*). Yet, somatomedin C is able to transpose such cells through the entire generative cycle. In Balb/c 3T3 cells, somatomedin C has been interpreted by Stiles *et al.* (*19*) to act as a progression factor—a factor whose presence is required after the "decision" to evacuate $G_0$ has been taken. Balb/c 3T3 cells must first be rendered competent (for example, by wounding or exposure to platelet-derived growth factor) before progression factors such as somatomedin C permit DNA synthesis to occur (*20*). The present studies do not clarify whether, in frog lens epithelial cells, somatomedin C works in concert with other endogenous non-growth hormone-dependent competence factors to permit DNA synthesis to occur or whether, in this tissue, induction of competence is unneccessary for the action of somatomedin C.

Three pituitary hormones (growth hormone, frog prolactin, and thyrotropin) can promote proliferation in the lenses of hypophysis-deprived frogs in vivo but not in vitro (*4, 7, 21*). All three enhance the synthesis of somatomedin C in the mammalian liver (the last, via the thyroid gland) (*22*). Bovine and ovine prolactin do not stimulate growth in the frog lens (*4*); this may be due to species specificity, since White and Nicoll (*23*) were unable to detect receptors to ovine prolactin in bullfrog liver. However we have shown (*23*) that hGH does not bind to frog hepatocytes. Insulin, insulinlike growth factors I and II, and epidermal growth factor stimulate growth in rabbit lens epithelium in vitro (*24*). Insulin exerts this effect on the epithelium of the organ-cultured frog lens (*25*). The effect of somatomedin C on the frog lens in vitro remains to be investigated.

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## Alcoholic Rhabdomyolysis: An Experimental Model in the Rat

Abstract. *The main features of alcoholic rhabdomyolysis—skeletal muscle necrosis, marked elevation of serum creatine phosphokinase, and myoglobinuria—were produced in rats by a combination of relatively prolonged (2 to 4 weeks) exposure to ethanol and a brief period of food deprivation. This observation suggests that fasting may similarly trigger muscle injury during binge drinking in man. The effect of fasting is in part related to an increase in blood alcohol due to reduced alcohol clearance and in part caused by a fasting-induced potentiation of the toxic effects of high concentrations of alcohol on skeletal muscle.*

Acute alcoholic myopathy, or alcoholic rhabdomyolysis, was first described by Hed and his co-workers and has since been increasingly recognized as a complication of severe alcoholism (*1*). It is manifested by rapidly evolving muscle destruction, with weakness, muscle pain, sudden and marked elevation of serum creatine phosphokinase (CPK), myoglobinuria, and histological evidence of muscle fiber necrosis, degeneration, and regeneration (*2*).

The pathogenesis of alcoholic rhabdomyolysis is unknown. It has not been reproduced experimentally (*3*), although administration of alcohol to human volunteers (*4*) or experimental rats (*5*) has been reported to cause mild elevation of


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