Weight and composition of DS sarcoma tumors

	Group Calcitonin	Control
Number of tumors	23	16
Tumor weight (g)	9.7 ± 1.7[a] ($p<0.01$)[b]	14.6 ± 1.6[a]
Total Ca (mg/g tumor)	0.66 ± 0.12 (ns)	0.46 ± 0.04
Total Mg (mg/g tumor)	0.15 ± 0.01 (ns)	0.15 ± 0.01
Total PO_4^{3-} (mg/g tumor)	0.52 ± 0.04 (ns)	0.66 ± 0.06
Lipid Ca (µg/g tumor)	12.8 ± 1.8 ($p<0.01$)	6.3 ± 0.6
Lipid Mg (µg/g tumor)	3.1 ± 0.3 ($p<0.01$)	1.8 ± 0.2
Lipid PO_4^{3-} (µg/g tumor)	200.0 ± 2.1 (ns)	160.1 ± 19.6

[a] Mean value ± SEM; [b] Wilcoxon's rank test.

number of animals bearing a tumor (80%). As shown in the table the tumors of the animals treated with calcitonin were significantly smaller ($p < 0.01$). On the other hand total calcium, total magnesium and total phosphate showed differences which were statistically not significant. In contrast to this observation, the lipid fraction presented a highly significant difference between the means of calcium and magnesium concentrations measured in the calcitonin group and the control group $p < 0.01$). The phosphate concentration showed no significant difference.

Calcium and magnesium are known to influence membrane permeability and to reduce the absorption of solutes other than divalent cations[13,14]. Our experimental data indicate that calcitonin affects tumor growth by a mechanism which seems to be related to divalent cation-binding to the membranes (phospholipid fraction). The question now being studied is whether this growth inhibition is due to an alteration of divalent cation transport across cell membranes or to a decreased migration of the available nutrient molecules. In relation to these reported observations, it is worthwhile to point out that antitumor drugs such as vincristine, daunomycin and adriamycin show an inhibitory effect on cell calcium transport which seems related to changes in the binding capability of membrane phospholipids[15–17]. These observations indicate that the interrelationships between calcium transport and metabolism in the tumor, and cytostatic agents such as those mentioned above obviously merit investigation in this respect. Finally it should be mentioned that the DS sarcoma is relatively resistant to cytostatic agents such as bis-β-chlorethylmethyl amines or cyclophosphamide.

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Effect of testosterone and 17-β estradiol on limb regeneration in the newt, *Notophthalmus viridescens*[1]

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Summary. Gonadectomy, or injections of testosterone or 17-β estradiol, had no apparent effect on the rate of regeneration or histological appearance of limb regenerates in the newt, *Notophthalmus viridescens.* Neither promotion, nor inhibition of limb regeneration was observed.

It is clear that limb regeneration is dependent on hormones. Insulin, thyroxine, glucocorticoids, and one or more pituitary hormones seem to be required[2–4]. The role of the sex steroids, testosterone and estradiol, in limb regeneration, is not clear. Durand[5] reported that both ovariectomy and castration increased the rate of limb regeneration in *Triturus alpestris.* This is somewhat surprising since in mammals the sex steroids are usually associated with promotion of wound healing[6,7]. While the sex steroids are not essential for limb regeneration, they may, nevertheless, have some influence on the process. Bromley[4] has reported that estradiol accumulates in the regenerating newt limb. Precisely, what estradiol is doing in the regenerating limb is not known. Hence, this study was undertaken to determine if administration of testosterone or estradiol would have any influence on the rate or quality of limb regeneration.

Materials and methods. About 75 adult newts, *Notophthalmus viridescens,* were distributed into 5 groups, such that

Stage of regeneration 30 days after amputation

	Males	Females	Males and females together
Group 1: untreated controls	4.7±2.8 (7)	6.4±2.8 (6)	5.5±2.8 (13)
Group 2: gonadectomized	4.0±3.2 (4)	3.7±2.9 (6)	3.8±2.9 (10)
Group 3: oil injected controls	8.0±0.6 (5)	3.1±2.9 (5)	5.3±3.3 (11)
Group 4: testosterone injected	5.7±1.5 (3)	5.0±1.4 (2)	5.4±1.3 (5)
Group 5: estradiol injected	4.3±2.9 (7)	6.6±2.9 (5)	5.3±3.0 (12)

Each figure is the mean regeneration stage (±SD) reached 30 days after amputation. Number of cases is given in brackets. Analysis of variance indicated no significance differences ($p > 0.05$) in the results whether the sexes were considered separately ($F = 1.74$) or together ($F = 0.60$). Brief description of regeneration stages follows[10]: stage 1, stump flattened on end; stage 2, tip of stump becomes rounded; stage 3, central protusion of blastema apparent; stage 4, blastema becomes conical; stage 5, well defined cone; stage 6, elongated cone; stage 7, cone begins to flatten dorsoventrally; stage 8, paddle stage; stage 9, blood vessels apparent; stage 10, notch seen on edge of paddle.

each group had ½ males and ½ females. Each animal was anaesthetized in 0.1% tricaine methane sulphonate and had the right forelimb amputated through the humerus. Treatments were given as follows. Group 1: no further treatment. Group 2: these were gonadectomized at time of amputation by methods previously described[8,9]. Group 3: injected i.p. 3 times weekly with 0.05 ml olive oil. Group 4: injected with 500 µg of testosterone in 0.05 ml olive oil 3 times weekly. Group 5: injected with 500 µg 17-β estradiol in 0.05 ml olive oil 3 times weekly. The animals were maintained in the laboratory as previously described[8,9].

A few limbs were fixed at intervals early during the course of regeneration, but most animals were reanaesthetized and their limbs removed and fixed in Bouin's fluid 30 days after amputation. The limbs were decalcified in 5% trichloroacetic acid for several days, embedded in paraffin, sectioned serially at 5 µm, stained, and examined histologically. The stage of regeneration was then recorded following the stage descriptions of Pritchett and Dent[10].

Results. The histological study indicated no apparent differences in regeneration among the 5 groups. Although there was considerable variation, especially in size and extent of regeneration among individual limb regenerates, there were no observable differences between the 5 groups. Further, when the stage of regeneration was compared between groups (table), there were no statistically significant differences in stage reached by 30 days whether the sexes were considered independently or together. The results then indicate no detectable difference in the rate of regeneration.

Conclusion and discussion. The sex steroids seem to have no apparent influence on limb regeneration. Injection of large non-physiological doses of testosterone or estradiol appears to have no effect on limb regeneration. On the other hand, reduction of these hormones by castration or ovariectomy also had no observable effect on limb regeneration. The possible effect at physiological levels is not known, but these results suggest that the sex steroids are not of great importance for limb regeneration. The present observations on *Notophthalmus viridescens* differ from those of Durand on *Triturus alpestris,* who reported an acceleration of regeneration by gonadectomy and an inhibition of regeneration by norethandrolone (an anabolic and androgenic steroid)[5]. Since estradiol appears to have no influence on limb regeneration in *Notophthalmus viridescens,* its accumulation in regenerating limbs[4] is puzzling.

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Intraperitoneal growth pattern of murine teratocarcinomas

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Summary. Ascitic teratocarcinomas showed a unique growth pattern, suggesting size regulation in the peritoneal cavity of syngeneic 129/Sv mice.

Ascitic teratocarcinomas are called as embryoid bodies (EBs) from the resemblance to the early mouse embryo[1]. These aggregates are composed of an outer endoderm cell layer and a core of embryonal carcinoma (EC) cells. They can differentiate to various types of tissues after attachment to the substrata in vitro and in vivo, but in suspension they are in an undifferentiated state[2]. In suspension, they can maintain their multipotency in forming EBs for many years. How do they hold the capacity of multiple differentiation for a long time? This report deals with the growth pattern of EBs in the peritoneal cavity of syngeneic mice.

Materials and methods. Murine teratocarcinomas OTT6050 were used in this study. These tumors were maintained as ascitic EBs in syngeneic 129/Sv male mice by transplantation every 3 weeks. Although this tumor line was established in 1967 and transferred to the ascitic form[3], EBs can now express multiple differentiation in vivo and in vitro.

EBs were fractinated with nylon mesh according to the size.