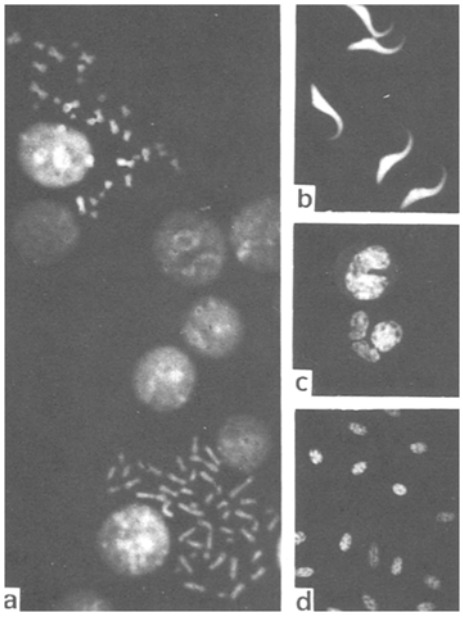
Euparal) does not modify the color or intensity of the fluorescence emission.

Chicken erythrocytes stained with pyronin Y at high or low concentrations always show a clear fluorescence of the chromatin. Interestingly, at a concentration of 0.74×10^{-1} M and under bright field illumination, pyronin Y stains erythrocyte nuclei in red; at lower concentrations (typically, 0.74×10^{-6} M) chromatin appears unstained, but it shows the highest intensity in the red-orange fluorescence.



Fluorescence pattern after pyronin Y staining: *a* Interphase nuclei and 2 metaphase plates from a human leukocyte culture; *b* Mouse spermatozoa; *c* Monocyte (top) and neutrophilic leukocyte (bottom) from human blood; *d* Chicken erythrocytes.

Studies on the affinity of pyronin Y for nucleic acids have revealed that in its interaction with RNA, the dye is not only bound by electrostatic forces, but also by hydrophobic interactions with the bases[9]. As far as we know, no report has described the fluorescence emission of nuclei and chromatin after staining with pyronin Y. Comparisons of the chemical structure of this dye with some thiazine and acridine dyes, such as methylene blue and acridine orange, show interesting similarities. Since the orthochromatic staining reaction of DNA by thiazine and acridine dyes involves intercalation between base pairs[10–12], it is tempting to speculate that pyronin Y interacts with double helical nucleic acids in a similar way. As happens with other intercalating dyes (i.e., ethidium bromide[13]), the fluorescence yield of pyronin could also be higher when located in a hydrophobic environment such as the stacked base pairs. Further investigations in order to analyze the binding mechanisms of this dye to nucleic acids and to explain the fluorescence pattern are being undertaken.

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Divalent cation-phospholipid complexes and tumor growth inhibition

L.J. Anghileri[1,2] and H. Delbrück

Service de Biophysique, Faculté de Médecine St.-Antoine, Université de Paris, Paris and Département de Virologie, Institut du Cancer, Villejuif (France), 14 March 1979

Summary. Growth inhibition of DS sarcomas provoked by calcitonin treatment is accompanied by an increase of calcium and magnesium in the phospholipid fraction. Changes in tumor cell membrane characteristics reflected in ionic or molecular transport modifications seem to be involved in the growth impairment phenomenon.

Calcium ions play an important role in the regulation of growth and cell division[3,4]. Some characteristics peculiar to tumor cell (e.g. glycolytic rate, crabtree effect, uncontrolled growth) seem to depend on an impairment of tumor cell calcium metabolism which can be related to an abnormal permeability of tumor cell membranes to calcium[5–7]. Calcitonin inhibits calcium efflux by depressing membrane transport of the ion[8–10]. In this communication we report the growth inhibitory effect of calcitonin on DS sarcoma.

Female Wistar rats (b.wt 120–140 g) were injected s.c. in the inguinal region with 2×10^7 viable DS sarcoma tumor cells suspended in 0.2 ml of saline. 24 h after inoculation the animals received s.c. 1 MRC U of salmon calcitonin (4700 MRC U/mg-Sandoz SA, Bâle, Switzerland) dissolved in 0.2 ml of isotonic phosphate buffer solution (pH 7.4) containing 0.2% of human serum albumin. Calcitonin administration was repeated daily for 9 days. A control group was injected s.c. with the same volume of buffer solution used to dissolve the calcitonin. All the animals were killed 13 days after tumor-cell inoculation and the tumors were excised and weighed. A part of the tissue was mineralized by ashing and the residue was dissolved in 1 N HCl. In order to isolate the phospholipid complexes of calcium and magnesium[11] another aliquot of tissue was extracted with methanol followed by methanol-chloroform (1:1, by volume). The extracts were evaporated and the residue mineralized by $HClO_4$ treatment. Calcium and magnesium were determined by atomic absorption spectrometry and phosphorus, as orthophosphate, by colorimetry[12]. To determine the level of significance the experimental values were subjected to statistical analysis using Wilcoxon's rank test. Both calcitonin and control groups presented the same

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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2 To whom reprint requests should be addressed. Service Hospitalier Fréderic Joliot, Hôpital d'Orsay, F-91400 Orsay (France).
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Effect of testosterone and 17-β estradiol on limb regeneration in the newt, *Notophthalmus viridescens*[1]

S.R. Scadding

Department of Zoology, University of Guelph, Guelph (Ontario N1G 2W1, Canada), 19 March 1979

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