transferred to Warburg flasks containing 2 ml of minimum essential medium-based amphibian organ culture medium[13]. (^{14}C)-DMN was added to each flask to give a final concentration of 60 µg and 0.1 µCi. The flasks were then shaken at 25 °C for 90 min in an air atmosphere. Radioactive CO_2 was trapped by NaOH in the centre well of each flask and its radioactivity determined by scintillation counting after conversion to (^{14}C)-$BaCO_3$ according to the method of Swann[14].

The results (table 2) show a considerable variation in the ability of the livers of the amphibian species tested to metabolize DMN. Liver from *Rana temporaria,* the species used by Khudoley[11] produced significantly more radioactive CO_2 than that from any other species, whereas *Xenopus* liver metabolized less DMN than liver from any other species.

3. *The effects of oxygen on DMN metabolism in vitro.* Montesano et al.[9] used an oxygen atmosphere in their work on DMN metabolism by fish and amphibian liver slices, but in our previous work on amphibian liver organ culture we have always used a zwitterionic buffered medium in free gaseous exchange with the atmosphere[13]. In a further series of experiments we therefore compared DMN metabolism by liver and kidney from a 200 g adult male Wistar rat and liver from *Xenopus* and 2 other amphibian species in Warburg flasks either filled with air or gassed with a 95% O_2/5% CO_2 mixture at the beginning of the test period.The results (table 3) show that the use of an oxygen atmosphere significantly increased the ability of the rat tissues, particularly the liver, to metabolize DMN in vitro, but had no significant effects on radioactive CO_2 production by liver from the amphibians. In addition, there were no significant differences in oxygen consumption by the 2 types of amphibian preparation (measured by Warburg manometry[15]), but the rat tissues incubated in 95% O_2/5% CO_2 used twice as much oxygen as those in air.

These results show that *Xenopus* eliminate injected DMN very rapidly when placed in the relatively large volumes of water in which these fully aquatic amphibians are normally maintained. Although some injected DMN appears to be retained in the body when *Xenopus* are kept in very small volumes of water, the comparatively very low rate of DMN metabolism by *Xenopus* tissues in vitro suggests that insufficient active metabolite would be produced for alkylation of cellular components to occur at the levels associated with toxic damage and/or tumour induction in rats[16]. Differences in rates of metabolism of DMN provide an explanation for our failure to induce tumours in *Xenopus* using procedures which, according to Ingram[10] and Khudoley[11], resulted in tumour induction in *Triturus helveticus* and in *Rana temporaria.*

1 This work was supported by a grant from the Cancer Research Campaign. We are also grateful to Dr P.F. Swann, both for the supply of DMN and DEN and for many useful discussions.
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Chromatin fluorescence by pyronin staining[1]

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9 March 1979

Summary. Human, chicken and mouse cells from different tissues show a bright red-orange fluorescence of the chromatin after staining with pyronin Y. The possibility that intercalation of the dye into double helical nucleic acids accounts for this fluorescence pattern is briefly discussed.

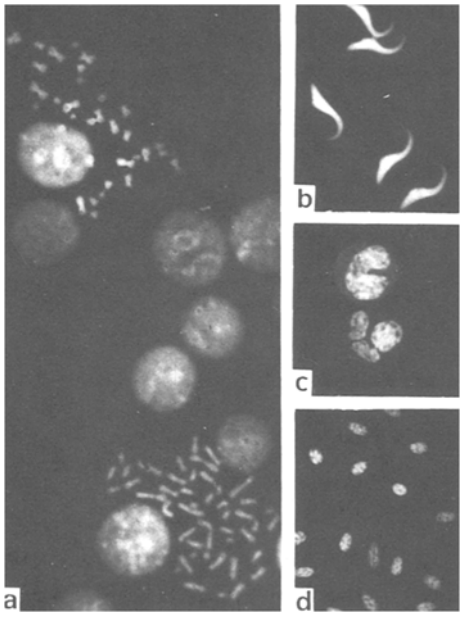
Pyronin Y (G) is a basic dye of the xanthene group, which is of considerable importance for nucleic acid cytochemistry[2-5]. Although this stain seems to be only relatively specific for RNA – e.g. see Kasten[6] and Pearse[4] –, its use in combination with methyl green as well as in the Brachet test[7] have proved very suitable for analyzing the occurrence and distribution of RNA in light microscopy[4,8]. During the course of cytological studies on the staining properties of pyronin Y, we have obtained unexpected results which are the subject of the present communication.

Smears of human and chicken blood were fixed in methanol for 2 min and air dried. Metaphase cells from human leukocyte cultures, and meiotic cells and spermatozoa from mouse testes were prepared according to the air drying method as usual. Staining was performed at room temperature, by using solutions of pyronin Y (Gurr) in distilled water at different concentrations, ranging from 0.74×10^{-1} M, (2%) to 0.74×10^{-7} M. Staining time was 5 min, after which slides were briefly washed in distilled water and air dried. Observations and photography were carried out using a Zeiss Photomicroscope III equipped with an epi-fluorescence condenser III RS. Preparations were analyzed under oil immersion using ×40 and ×100 objectives and green excitation filters.

After staining with considerably diluted pyronin Y solutions (0.74×10^{-5} M), nuclei in all preparations show a bright red-orange fluorescence (figure). The cytoplasm of blood cells with high RNA content (lymphocytes, monocytes) shows a scarce pale orange fluorescence. Mounting of preparations in different media (water, immersion oil, or

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.

1 This work was partially supported by a grant from the Comisión Asesora de Investigación Científica y Técnica, Spain.
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Divalent cation-phospholipid complexes and tumor growth inhibition

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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