genus is AT-rich[6], 3) it is located peripherically in the chromocenters[6], and 4) is not completely identical with the satellite DNA fraction[6]. The satellite DNA is composed of 2 components, which are reiterated respectively 2.2×10^5 and 1.4×10^5 times[10]. This is, with respect to the conditions of hybridization not enough to yield the silver grain number as shown in the figure. In addition, these reiteration values may represent an over-estimation, because they were obtained through analysis of the DNA extracted from nuclei regardless of their content of amplified DNA. Without knowing the genome organization in Cymbidium, it is difficult to say what kind of DNA is amplified. At present we know that it is a AT-rich fraction and probably a repetitive sequence (because of its location in heterochromatin).

The functional significance of the amplification of AT-rich DNA in somatic nuclei of Cymbidium is not yet clear. With respect to a hypothesis put forward earlier[11], it may be argued that this 'lateral reiteration' must substitute for too low 'tandem reiteration' during the evolution of non-coding DNA, which is probably involved in the control of gene activity and cytodifferentiation[12]. A similar interpretation has recently been given for the amplification of intermediately repetitive DNA in chicken cartilage cell differentiation[13], and is very likely to apply to the situation in Cymbidium, as phytohormone-controlled changes in the amplification process are accompanied by significant changes in morphogenesis[5].

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Effects of γ-rays on DNA-cholesterol complex

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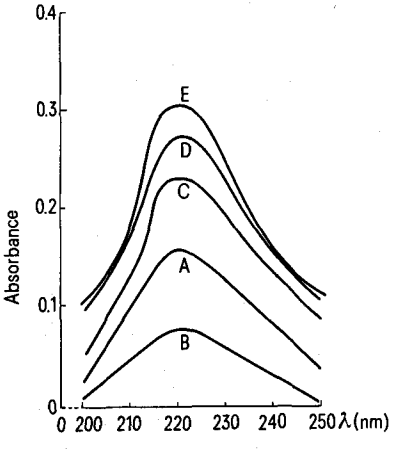
Summary. Effects of γ-rays on the DNA-cholesterol complex have been studied. Radiation-induced changes are found and compared with those on DNA-dye or drug complexes.

The effects or γ-rays on the complexes of DNA with multiferious dyes and drugs[2,3] have been studied. It has been known that cholesterol, which is a lipid, is complexed with DNA. Moreover steroid hormone[4] binds to cytoplasmic receptor proteins. To investigate the effects on the complex with an agent (with probable different binding mechanism), dissimilar to that of earlier studies[2,3], the effects of γ-rays on the DNA-cholesterol complex, formed under varied conditions have been studied spectrophotometrically.

Materials and methods. Highly polymerized calf thymus DNA (Sigma), as sodium salt, cholesterol (Merck) and for solvent 0.01 M Tris-HCl buffer at pH-7.4[5], found free from imparting radiation effects were used, unless otherwise specified. Utilizing the property of slight[6] solubility of cholesterol in water, solutions in water and by the same method in Tris-HCl buffer were made: $\times 5$ mg of cholesterol was stirred with 100 ml of water or buffer and heated for 10 min on water-bath. Part of the sample did not dissolve and was filtered off. For verification, part of the filtrate was evaporated to dryness on water-bath; the residues were dissolved in chloroform, and presence of cholesterol was confirmed by Lieberman-Burchard reaction. Cholesterol solution of arbitrary concentration, having OD (stated later), was thus prepared in buffer to use as parent solution. The spectrum of the parent solution, mixed with equal volume of the solvent, served as reference, and that with equal volumes of DNA and the parent solutions as control. All measurements, including control, were carried out between 200 and 250 nm, using the concentration of reference. For test samples, equal volumes of 1.5×10^{-5} M, 3×10^{-5} M, 6×10^{-5} M, 9×10^{-5} M, 12×10^{-5} M and 18×10^{-5} M DNA and parent solutions were mixed. The complexes formed after incubation at room temperature for 30 min were irradiated to different doses, in CS^{137} source at a dose rate of 245 R/min; for the slow oxidation[7] of cholesterol, in presence of air and light,



Effect of γ-rays on DNA-cholesterol Complex. A-cholesterol; B-reference; C – 9×10^{-5} M DNA + cholesterol; D-solution of C + 9000 R. E – Solution of C + 15,000 R.

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fresh solutions were used with precautions and after-effects were not measured. All experiments concerning each set were carried out from the same stock. The parent Stock for all sets showed similar optical density. Hilger spectrophotometer and 1 cm quartz cuvettes were used. Changes are estimated against reference spectra.

Results and discussion. The parent solution in buffer displays an OD of 0.150 in peak at 220 nm and that in water of 0.263 in peak at 215 nm, showing no other peak. The displacement of the peak indicates the state of the molecule[8] in different medium.

The complexes of 1.5×10^{-5} M also with 3×10^{-5} M DNA show increases of 0.3 and 0.6 unit respectively in peak. The peaks increased further on irradiation to 6000 R and 9000 R. The complex with 6×10^{-5} M DNA shows increase of 1.1 unit in peak, standing above that of the parent solution by 0.5 unit. On irradiation to 9000 R and 15,000 R the peak rises farther systematically. The complex with 9×10^{-5} M DNA (figure) exhibits an increase of 1.7 unit in peak, standing above that of the parent solution by 0.75 unit. At doses of 9000 R and 15,000 R, the peak shows systematic rise. The complex with 12×10^{-5} M DNA exhibits an increase of 2.2 unit in peak, standing above that of the parent solution by 1.1. unit. On irradiation to 6000 R and 9000 R no change occurs. At a dose of 15,000 R, the peak increased. The increase is less than the counter cases of the irradiated complexes of 6×10^{-5}M and 9×10^{-5} MDNA. The complex of 18×10^{-5} M DNA shows similar increase of 3 units in peak, standing above that of the parent solution by 2 units. On irradiation to 9000 R and 15,000 R, it exhibits no effect. In no case was the peak shifted from 220 nm.

The type of complex seems to be similar at all concentrations of DNA, since the rise in peak is proportional to DNA content. It indicates a single type of binding mechanism, acting in all cases. In comparison, the mechanism[9,10] varies with DNA content in many cases with dyes and drugs[2,3]. Radiation effect on the present complexes varies with DNA content. The increase in effect begins at lower DNA content, attains the maximum at intermediate DNA content and minimises at higher DNA content. In most cases of dyes[2,3] and drugs, pronounced radiation effect occurred with low DNA content. In the present cases, radiation increased the binding, as indicated by the rise in peaks. Similar increased binding was noted in many cases with dyes[2,3] and drugs. The observations could not be explained at this stage. No change was noted in the parent solution, when irradiated to 9000 R; Around 220 nm DNA spectrum also does not contribute significantly towards the observed changes, when checked. Furthermore, the peaks of the complexes with 1.5×10^{-5} M and 3×10^{-5} M DNA, are below that of the parent solution and above that of the reference, indicating no additive effect due to DNA. The observed rise in the spectra of the complexes thus appears to be due to radiation effects on the complexes.

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Responses of skeletal muscle fibres to lanthanide ions. Dependence of the twitch response on ionic radii[1]

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Summary. A study of the effects of the lanthanide series of ions on toad skeletal muscle fibres reveals that they a) inhibit the twitch response, b) exhibit a marked dependence on ionic radius, and c) apparently exert their effects on the sarcolemma.

The lanthanide ions have been used to investigate Ca(II) binding in biological membranes[2] and they apparently exert their effect by displacing Ca(II). Evidence suggests that they do not pass through cell membranes[3] such as skeletal muscle sarcolemma[4,5] or even isolated sarcoplasmic reticulum vesicles[6]. Lanthanide ionic radius-dependent Ca-displacement effects have been observed for smooth muscle contraction[7], conversion of trypsinogen to trypsin[8], activation of prothrombin[9] and activation of α-amylase[10,11]. However, some studies using muscle fibres failed to detect any such dependence[12].

We dissected 3–5 fibres of semitendinosus from Bufo marinus in a Hepes-buffered Ringers solution (NaCl 115 mM, KCl 2.5 mM, $CaCl_2$ 1.8 mM, Hepes 5 mM, pH 7.0). Twitch responses were recorded[13] every 6 sec. Supramaximal stimuli were used. Fibres showing more than 10% reduction in tension at the end of a series of tests were discarded. Test solutions were replaced by flushing the bath (3 ml) with 60 ml of the next solution. Lanthanide ions were dissolved and their concentrations were determined as described previously[6].

Effect of lanthanide ions on the twitch response. The concentration-dependence of the twitch response with Er(III) exhibits (figure 1) a sharp inhibition with a 50% effective Er(III) concentration of 0.28 mM. This 50% point was determined at least twice for each of the 12 lanthanide ions tested. In every case, the decline in the twitch tension was completed within 30 sec after the solution change. The inhibitory effects were completely reversed in about the same time (figure 2). The same results were obtained using either increasing or decreasing concentrations of the lanthanide ions.

Figure 3 illustrates the relationship between the concentration of lanthanide ions required to produce 50% inhibition of the twitch response and the crystal ionic radius[14] of those ions. These radii are based on a coordination number of 8 which appears to be appropriate under the experimental conditions employed here[15]. The open circles represent separate experiments and the line joins the means of these data. The outstanding feature of this curve is the sharp peak obtained with Tm(III), Er(III) and Ho(III), which centre on an ionic radius of 100 pm.