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.

Ions with radii only a few pm larger or smaller are substantially more effective inhibitors of the twitch tension. The peak coincides precisely with the ionic radius of 6 coordinate Ca(II)[14]. This is significant because Ca(II) has been shown to be necessary for the maintenance of the resting membrane potential and for the propagation of the action potential[16].

Electron microscopy. We have already shown that Gd(III) is an effective tracer element in electron microscopy[6]. We therefore used Gd(III) to determine whether it could penetrate the sarcolemma. Fibres were incubated with 2.5 mM Gd(III) for 5–10 min, washed briefly with Ringers solution containing 3 mM inorganic phosphate which resulted in the formation of insoluble $GdPO_4$. Fibres were then fixed in 2% glutaraldehyde, dehydrated and embedded[17]. Deposits of Gd(III) were observed on the outside of the sarcolemma and within its associated caveolae but none were seen within the fibre. When the sarcolemma was experimentally damaged by fixation with glutaraldehyde prior to incubation with Gd(III), the electron dense Gd(III) deposits were seen both inside and outside the muscle fibres. We therefore conclude that unless the sarcolemma is damaged, it is impermeable to Gd(III).

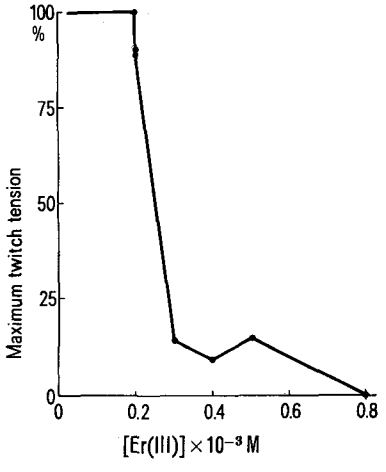

Fig. 1. The effect of increasing concentrations of Er(III) on the twitch response of a bundle of 3 toad semitendinosus fibres. 50% inhibition is achieved with 0.28 mM Er(III).

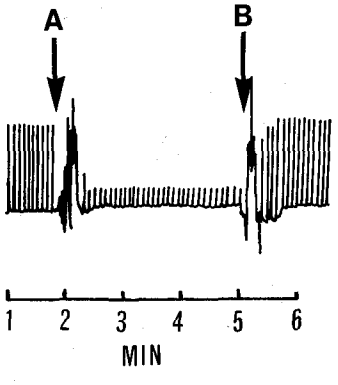

Fig. 2. The effects of 80 μM Nd(III) in Ringers solution (A) on the twitch response of 4 semitendinosus muscle fibres. Note the uniform inhibition of twitch immediately following the disturbance caused by changing the bath solution. Recovery of twitch after return to normal Ringers conditions (B) is complete.

Discussion. These data are consistent with the observations of others[3,5,6] that the lanthanide ions do not penetrate intact cell membranes. The data presented above indicated that even though the sarcolemma is selectively affected by ions whose radii are approximately 100 pm (Tm = 99; Ho = 102 pm), the trivalent lanthanide ions inhibit while bivalent Ca(II) is needed for excitation. Presumably therefore, the sarcolemma is sensitive to the increased charge density of the lanthanide ions. Additionally, we have carried out experiments on glycerinated

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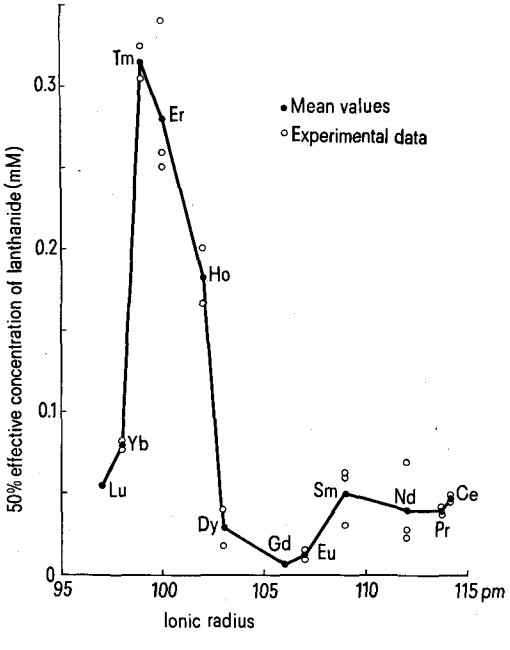

Fig. 3. Plot of the concentration of lanthanide ion required to produce 50% inhibition of twitch tension versus the crystal ionic radius. Open circles are actual data points; closed circles are the means for each lanthanide ion.

skeletal muscle fibres[18] which show that the contractile mechanism is unaffected by lanthanide ion concentrations below 0.3 mM and that with up to 1 mM there appears to be an activation rather than an inhibition of contraction. These results could only be understood if the Gd(III) was prevented from interacting with contractile proteins in the live fibre experiments.

Andersson and Edman[4] investigated the effect of lanthanum on isometric tetanus. Lanthanum had no apparent effect on tetanus amplitude, but at concentrations producing action potential prolongation and thereby twitch potentiation, the fibres' ability to repond to repetitive stimulation was reduced, resulting in breakdown of the tetanic plateau. We might have used the inhibition of the tetanic response to study ionic radius discrimination by skeletal muscle, using as data points the concentration at which the plateau began to break down. However, tetani are subject to experimental complications that we preferred to avoid, namely the short duration of tetanic contractions and the lengthy periods necessary to determine whether the muscle had fully recovered. Furthermore, there is a short life expectancy of a muscle preparation subjected to tetanus and it is known that the smooth tetanic contraction can become discontinuous even in normal Ringers solution[4]. Only twitch responses could provide us with a rapid analysis of concentration dependence.

The extreme sensitivity of these skeletal muscle membranes to the radius of the active cation suggests that other membranes may show a similar sensitivity. This notion is currently under examination using isolated skeletal muscle sarcoplasmic reticulum. A more pressing question concerns the nature of the observed specificity. At present we can shed little light on this problem.

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Enhancement of virus growth produced by thiols and disulphides[1]

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Summary. Several thiols and disulphides have been found able both to shorten the latency phase and to increase the growth of several virus strains in cell cultures.

Previous studies have shown that the antiviral effect of bichlorinated pyrimidines is potentiated by certain thiols and antagonized by others[2,3]. Since bichlorinated pyrimidines act on virus growth by impairing assembly of virus constituents into infectious particles, interest has been given to evaluating the effects of thiols on virus synthesis and organization.

Materials and methods. Cysteine HCl, Cystine, Cysteamine HCl, Cystamine 2 HCl, glutathione SH and SS were purchased from Schuchardt; 2-mercaptoethanol from Eastman; 2-mercaptopropionylglycine from Fluka; ^{3}H thymidine (21 Ci/mMol) and ^{14}C leucine (280 mCi/mMol) from Amersham. Virus strains used were Polio 1 Brunenders, Encephalomyocarditis (EMC), Vesicular stomatitis (VSV), Vaccinia and Herpes simplex virus (HSV). Human aneuploid HEp 2 cells and primary mouse embryo cells, both grown in Eagle's MEM (Hank's base, pH 7.3), supplemented with 7% calf serum, were also used.

Experiments were carried out on 16 h old cell monolayers maintained in Eagle's MEM (Earle's base, pH 7.3) supplemented with 2% calf serum. The maximum non-cytotoxic dose (MNCTD) of thiols was determined by incubating cell monolayers in the presence of decreasing drug concentrations. After 60 h at 37 °C, cytotoxicity was determined both by examining cells at low magnification and measuring intracellular incorporation of neutral red at 530 nm[4]; 2/3 of MNCTD thus established was used in

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Effect of thiols on virus growth

Thiol in Eagle's MEM (μg/ml)*		Enhancement produced on virus yield (in IU/ml of medium) Polio 1 (H)**	EMC (H)	VSV (H)	HSV (H)	(M)**	Vaccinia (H)	(M)
(Reference data)		7.6×10^{7}	2.1×10^{7}	6.1×10^{6}	1.8×10^{7}	9×10^{6}	7.1×10^{6}	3.5×10^{6}
Cysteine	300	×1.8	×1.6	×2.3	×2.3	×2.5	×3.5	×4.2
Cystine	300	×1.6	×1.4	×3.1	×3.0	×2.8	×3.6	×3.6
Cysteamine	40	×2.3	×2.8	×3.1	×3.6	×2.9	×4.1	×4.0
Cystamine	40	×2.4	×2.6	×3.3	×3.4	×3.2	×4.0	×4.3
Glutathione SH	800	×2.8	×2.7	×4.6	×4.1	×4.2	×8.4	×6.2
Glutathione SS	800	×2.3	×2.9	×5.1	×3.9	×3.0	×8.5	×6.3
Mercaptopropionylglycine	300	×2.6	×3.1	×6.2	×4.9	×3.2	×9.6	×5.7
2-Mercaptoethanol	2	×1.2	×0.9	×1.2	×1.0	×1.2	×1.3	×1.1

* Thiols were tested at 2/3 of the MNCTD; ** H, in HEp2 cells; M, in mouse embryo cells.