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.

virus tests. Cell monolayers (10^6 cells/sample) were infected with 10 infectious units (IU) per cell and incubated at 20 °C for 1 h. Cells were then washed 3 times in Hank's base and incubated at 37 °C with 10 ml Eagle's MEM per culture. Thiols were added at time zero postinfection. Unless stated otherwise (see figures), 24 h later, whole cultures were frozen and thawed (− 70 and + 20 °C) 3 times and cleared of cell debris at 3000 rpm for 3 min. The yield in infectious units of Polio and VSV was measured by the plaque method[5], while the end point titration (6 stationary tubes per decimal dilution) was used for Vaccinia, HSV and EMC. The first method was found to have an error of less than 10%, the second of about 33%. Macromolecular synthesis and organization of Vaccinia virus were studied in HEp 2 cells (4×10^7 cell/sample) which, soon after infection, were incubated at 37 °C in Eagle's MEM containing 1/3 of the normal leucine supplement. Mercaptopropionylglycine (200 µg/ml) was added at time zero postinfection and, 2 h later, ^{3}H thymidine and ^{14}C leucine (2 µCi/ml). 24 h postinfection cell samples were detached from the glass by 0.25% trypsin (Difco, 1:250), washed 3 times in Hank's base, pelleted at 600 g for 5 min and resuspended by Vortex in 0.5 ml of hypotonic buffer (EDTA 0.05 M, Tris 0.01 M, NaCl 0.01 M) at 4 °C. After adding Nonidet P40 (up to 0.5%) cells were shaken again, reincubated at 4 °C for 10 more min and centrifuged at 600 g for 5 min. The supernatant fraction containing cytoplasmic extract deprived of nuclei was then separated. Part of it (0.1 ml) precipitated at 4 °C in 10% trichloroacetic acid (TCA) and (TCA) and solubilized in 0.5 ml Soluene (Packard), served to measure overall synthesis of proteins and viral DNA. Another part (0.3 ml) was layered onto 11 ml of preformed 40–20% sucrose gradients in the above buffer, centrifuged at 15,000 rpm for 60 min at 4 °C in Spinco 65 L (40SW Ti rotor) and then separated into 40 fractions in order to localize virus peak. In both cases, radioactivity was determined by Spectrometer (Packard, scintillation liquid: toluene ml 666, Triton X100 ml 333, PPO 7 g, dimethyl POPOP 0.1 g).

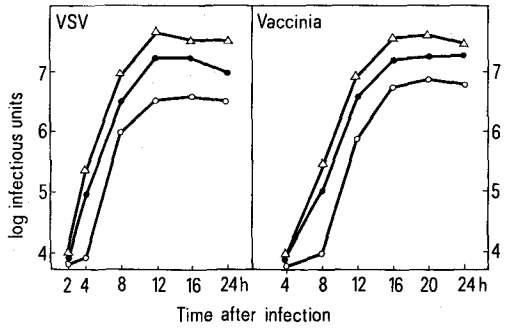

Fig. 1. Effect of thiols on growth of VSV and Vaccinia virus in HEp2 cells. Final yield (in IU/ml) of VSV and Vaccinia virus at 24 h postinfection in: thiol free Eagle's MEM (empty circles), Eagle's MEM containing Glutathione SH (800 µg/ml) (full circles), Eagle's MEM containing mercaptopropionylglycine (200 µg/ml) (triangles).

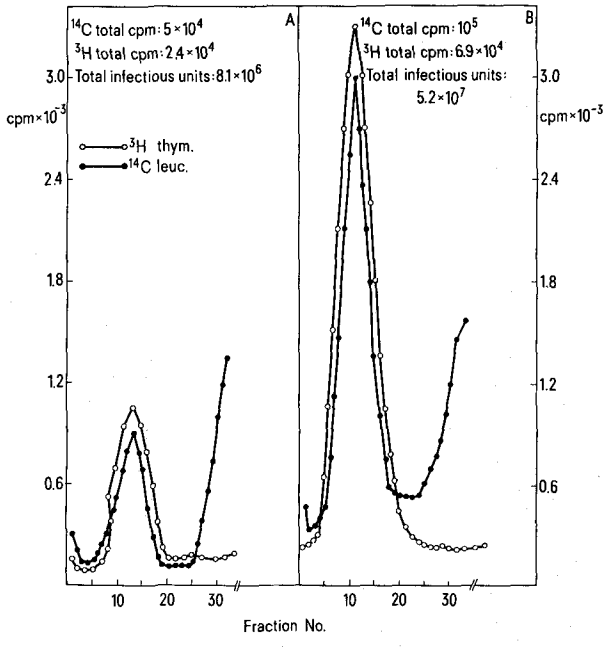

Fig. 2. Effect of mercaptopropionylglycine on Vaccina virus growth. Organization of complete vaccinia virus particles in thiol free Eagle's MEM (A) and in the presence of mercaptopropionylglycine (200 µg/ml) (B) 24 h postinfection. For more details see text.

Results. Data in the table show that aminated thiols may enhance virus growth considerably. Mercaptopropionylglycine and glutathione SH and SS are the most active, followed by cysteamine and cystamine and by cysteine and cystine. On the other hand, the only non-aminated thiol tested, 2-mercaptoethanol, has no effect on virus growth. As also shown in the table, promoting activity of thiols on virus growth depends both on cell substrates and, to a greater extent, on the type of virus tested: Vaccinia virus is the most stimulated, followed by VSV, HSV, EMC and Polio. Thiols also hasten virus synthesis, thus shortening the latency phase. As shown in figure 1, mercaptopropionylglycine and glutathione SH anticipate the onset of production of infectious particles by about 2 h.

Data on effect of thiols on synthesis at macromolecular level are referred to in figure 2. These data show that, in cells infected with vaccinia virus, 2-mercaptopropionylglycine raises overall protein synthesis 2fold, viral DNA replication about 2.8fold, while it increases the assembly of virus particles about 3fold and production of infectious units more than 6fold. Whether the last event simply depends on enhanced synthesis of macromolecules, or also on better assembly of infectious units, mediated by thiols, remains to be determined. Research is in progress to verify these possibilities and to determine the mechanism(s) by which both reduced and oxidized thiols, known to play different roles in polypeptide synthesis and protein organization[6,7], are able to stimulate virus growth.

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