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Changes in the Organization of Non-Epithelial Intermediate Filaments Induced by Triethyl Lead Chloride

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The in vivo effect of triethyl lead chloride (TriEL) (10^{-6}–10^{-8} M) on the organization of non-epithelial intermediate filaments (vimentin and desmin filaments) was studied by indirect immunofluorescence microscopy employing different mammalian cell lines. The in vitro effect of TriEL on filament formation as well as on the structure of preformed filaments was investigated by electron microscopy. TriEL induces perinuclear coil formation of intermediate filaments in SV40-transformed human fibroblasts and baby hamster kidney (BHK21) cells. The rearrangements observed are not correlated with significant changes in the microtubular system as tested by double labelling of both filament systems. The effect of TriEL is reversible. Assembly of intermediate filaments in vitro is disturbed in the presence of TriEL such that only short filaments and various kinds of fragments are formed. When preformed filaments are incubated in the presence of TriEL, unravelling of fibres into protofilamentous strands is observed. Possible mechanisms of TriEL–filament interaction are discussed.

Intermediate filaments (IFs) represent one major component of the cytoskeleton of the cells of vertebrates. To date, there are no chemicals available that interact specifically with the protein components of IFs. Two recent reports have been published concerning the rearrangement of keratin filaments induced by treatment of cells either with chemicals acting on either microtubules or microfilaments [1] or with acrylamide at rather high concentrations [2]. In both reports, changes in the distribution of filaments were demonstrated by immunofluorescence microscopy.

Ultrastructural data, however, with respect to the direct effect of these chemicals on IF proteins were not presented. Thus, it cannot be excluded that they only exerted an indirect effect on IF organization, for instance, via rearrangement of microtubules. It is well known that the vimentin filament system is rather sensitive to treatment of cells with classical inhibitors of microtubule assembly such as colchicine and nocodazole [3, 4].

We were interested in testing whether this is also the case for TriEL, a newly discovered inhibitor of microtubule formation. TriEL is a degradation product of tetraethyl lead, an 'antiknock' additive in motor fuel. It interacts with the

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microtubular system of mammalian cells and induces its breakdown at concentrations of 10^{-6} M [5]. Here we show that substantially lower concentrations of TriEL are able to bring about drastic changes in the organization of the IF system of mammalian cells, despite the persistence of an intact microtubular system.

MATERIALS AND METHODS

Cells

SV40 virus-transformed human fibroblasts (WI38 VA13 subline 2RA) and baby hamster kidney (BHK21) cells were used for immunofluorescence microscopy. The cells were cultivated according to procedures specified by the American Type Culture Collection (Catalogue of Strains II, second edition, 1979).

Chemicals

Triethyl lead chloride (TriEL, 96% pure) was kindly supplied by the Associated Octel Co. Ltd (UK) and freshly diluted from a 10 mM aqueous stock solution.

Antibodies

Affinity-purified rabbit antibodies directed against porcine brain tubulin [6] and monoclonal antibodies directed against vimentin and desmin (Boehringer-Mannheim, Mannheim, FRG) were employed. Tetramethyl rhodamine (TRITC)-labelled goat anti-rabbit immunoglobulins (IgG) (Bio-Yeda, Rehovot, Israel) and fluorescein (FITC)-labelled sheep anti-mouse IgGs (Boehringer) were both used as a second antibody.

Immunofluorescence Microscopy

Cells grown on coverslips were fixed with cold methanol for 10 min, followed by a brief treatment with cold (−10°C) acetone, and processed further as described previously [6]. For double-labelling experiments, the cells were treated according to the method published by Blose [7]. The procedures used for immunofluorescence microscopy have been described in detail elsewhere [5, 6].

Isolation of IFs and Their Subunit Proteins

Vimentin and desmin from mouse Ehrlich ascites tumour cells and porcine smooth muscle, respectively, were isolated according to published procedures [8, 9] and stored frozen (−80°C) in 10 mM Tris-HCl, pH 7.6, 3 mM EDTA, 6 mM 2-mercaptoethanol (buffer I).

Samples of both proteins were either used directly for in vitro assembly of IFs (protein concentration: 150 μg/ml; 30 min, 37°C) in the presence of 150 mM KCl [10], or after the addition of solid urea (ultrapure, Bethesda Research Laboratories) to yield an 8 M solution and extensive dialysis (15 h, 4°C) against buffer I or 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA. In order to determine whether TriEL (10^{-8} and 2×10^{-7} M) interfered with filament assembly, the drug was added immediately after the addition of the appropriate volume of 4 M KCl. To test whether TriEL affected preformed IFs, filament formation was allowed to proceed for 30 min prior to the addition of TriEL.

Electron Microscopy

Carbon-coated pieces of mica were dipped for 45 sec into a 20 μl drop of the respective filament solution, washed with double distilled water and stained for 30 sec with a 1% aqueous solution of uranyl acetate. The films were taken up on 200-mesh Formvar-coated grids and surplus stain solution was sucked off with filter paper. The specimens were viewed in a Siemens microscope (EM I or EM 102).

RESULTS

Immunofluorescence Microscopy

The rearrangement of vimentin and desmin filaments in various mammalian cells in the presence of low concentrations (10^{-8} M) of TriEL was examined by indirect immunofluorescence microscopy.

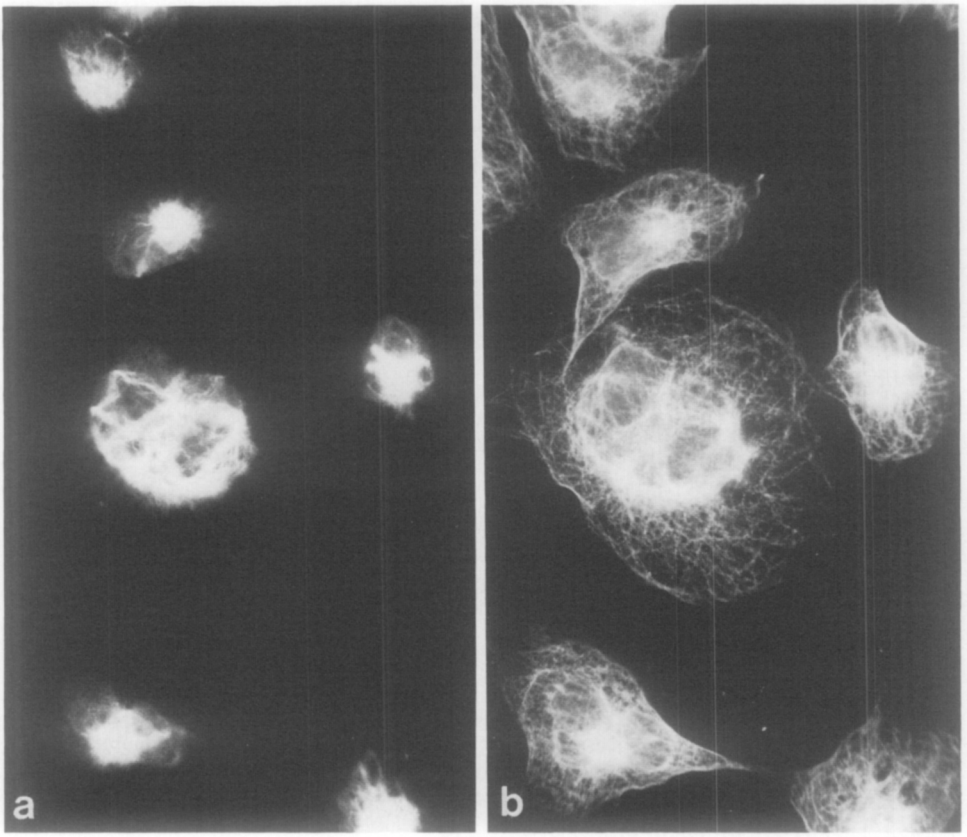

Fig. 1. Double immuno-labelling of SV40-transformed WI38 cells after incubation with 10^{-8} M TriEL for 2 h. Labelling with (*a*) anti-vimentin antibody—the vimentin filaments are collapsed into large bundles and coils surrounding the nucleus; (*b*) anti-tubulin antibody—the microtubular network is still preserved in these cells after treatment with TriEL at low concentration. ×600.

In order to study the influence of TriEL only on the vimentin filament system, SV40 virus-transformed human fibroblasts were used; these do not express any other IF proteins besides vimentin. Since TriEL interacts with intact microtubules and also interferes with microtubule assembly [5, 12], we applied TriEL at concentrations that do not affect the microtubular system.

SV40 virus-transformed human fibroblasts treated with 10^{-8} M TriEL for 2 h showed a rearrangement of vimentin filaments into large bundles or coils that often formed a juxtanuclear complex (fig. 1 *a*). The microtubular network remained intact (fig. 1 *b*). The vimentin filaments recovered their normal distribution pattern within 4 h of removal of TriEL. This proves that the TriEL-induced rearrangement is reversible and is not due to the considerable destruction of the protein(s) involved. It also shows that the restoration of the original vimentin distribution does not require microtubule assembly.

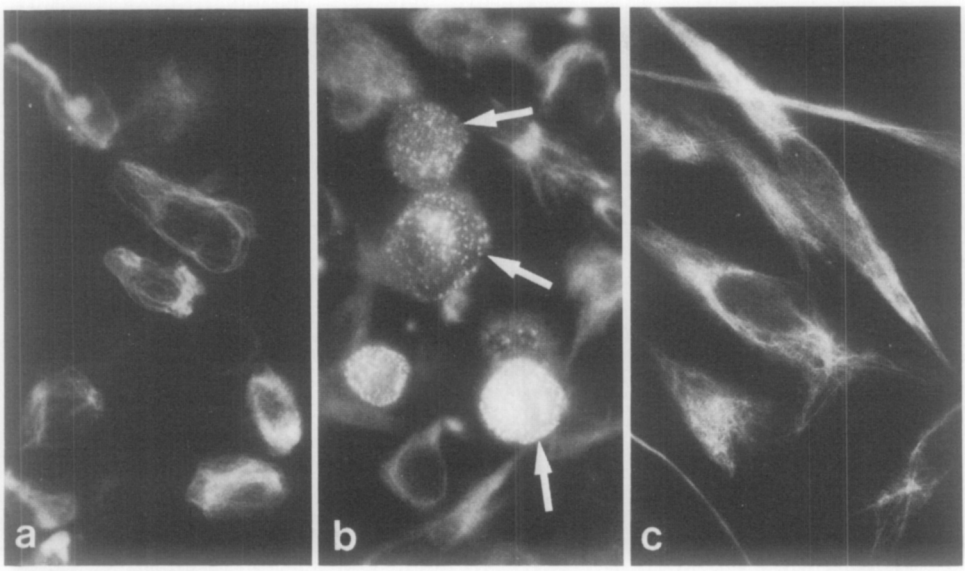

Fig. 2. Staining of BHK21 cells with monoclonal anti-desmin antibodies. BHK21 cells were incubated for 2 h with 10^{-8} M TriEL (*a, b*) and then fixed and labelled with monoclonal anti-desmin antibodies. The desmin filaments are rearranged into bundles and coils (*a*). Note the desmin-positive dots in mitotic BHK21 cells (*b, arrow*; cf [15–18]). (*c*) Fluorescence pattern of BHK21 cells 4 h after removal of TriEL; the labelling was carried out with antibodies directed against desmin. ×600.

Since there is no cell line available which contains desmin as the sole IF system, we tested the effect of TriEL on BHK21 cells containing mixed filaments of both vimentin and desmin [11]. BHK21 cells were incubated for 2 h in the presence of 10^{-8} M TriEL and were then labelled with monoclonal antibodies directed against desmin. The desmin filaments did rearrange under these conditions, forming bundles and/or coils (fig. 2*a*). After removal of the drug, the cells displayed the normal desmin pattern (fig. 2*c*). It should be mentioned that mitotic BHK21 cells showed a desmin-positive speckling (fig. 2*b*) instead of a filamentous desmin distribution and that this rearrangement occurred independently of TriEL treatment.

Electron Microscopy

In order to test whether changes in the IF pattern of living cells induced by TriEL might be correlated with changes in the filament structure itself, we investigated both (*i*) the assembly in vitro of IFs from their soluble subunits in the presence of TriEL and (*ii*) the influence of the drug on isolated or reconstituted IFs. The effect of TriEL on vimentin and desmin filaments assembled in vitro in the presence of TriEL appeared to be similar. The filaments were much shorter than those observed in the control preparations and they showed the phenomenon of unravelling (figs 3, 4). This occurred even when TriEL was added to in vitro preformed filaments.

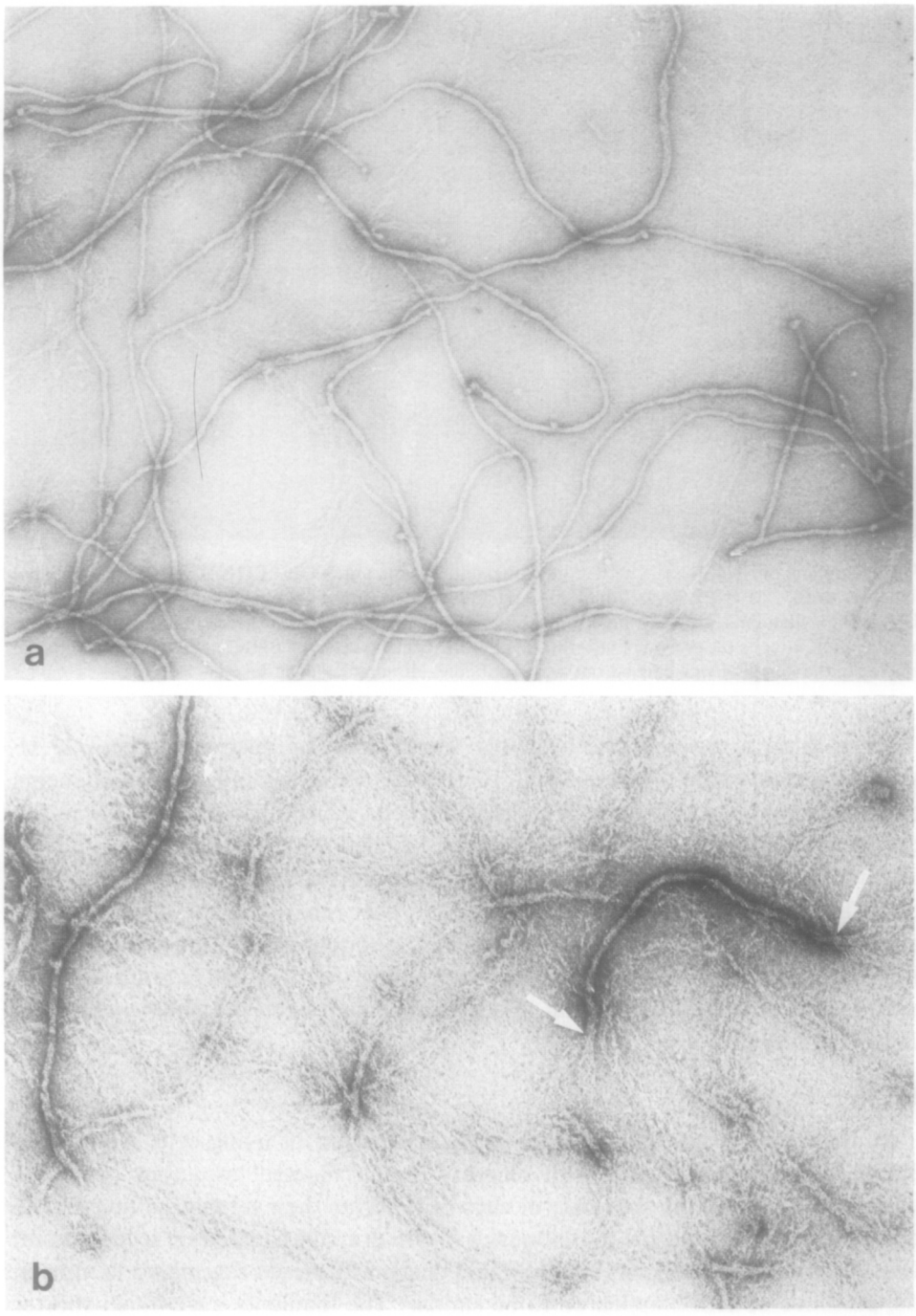

Fig. 3. Electron microscopy of vimentin filaments reconstituted in vitro. (*a*) Control; (*b*) same as (*a*), but incubated in the presence of 2×10^{-7} M TriEL for 30 min. The vimentin filaments show unravelling. The subfilaments (protofilament strands) seem to be untwisted. Note that an individual filament can appear normal at the mid-piece, yet it is unravelled at both ends (*arrows*). (*a*) ×60 000. (*b*) ×80 000.

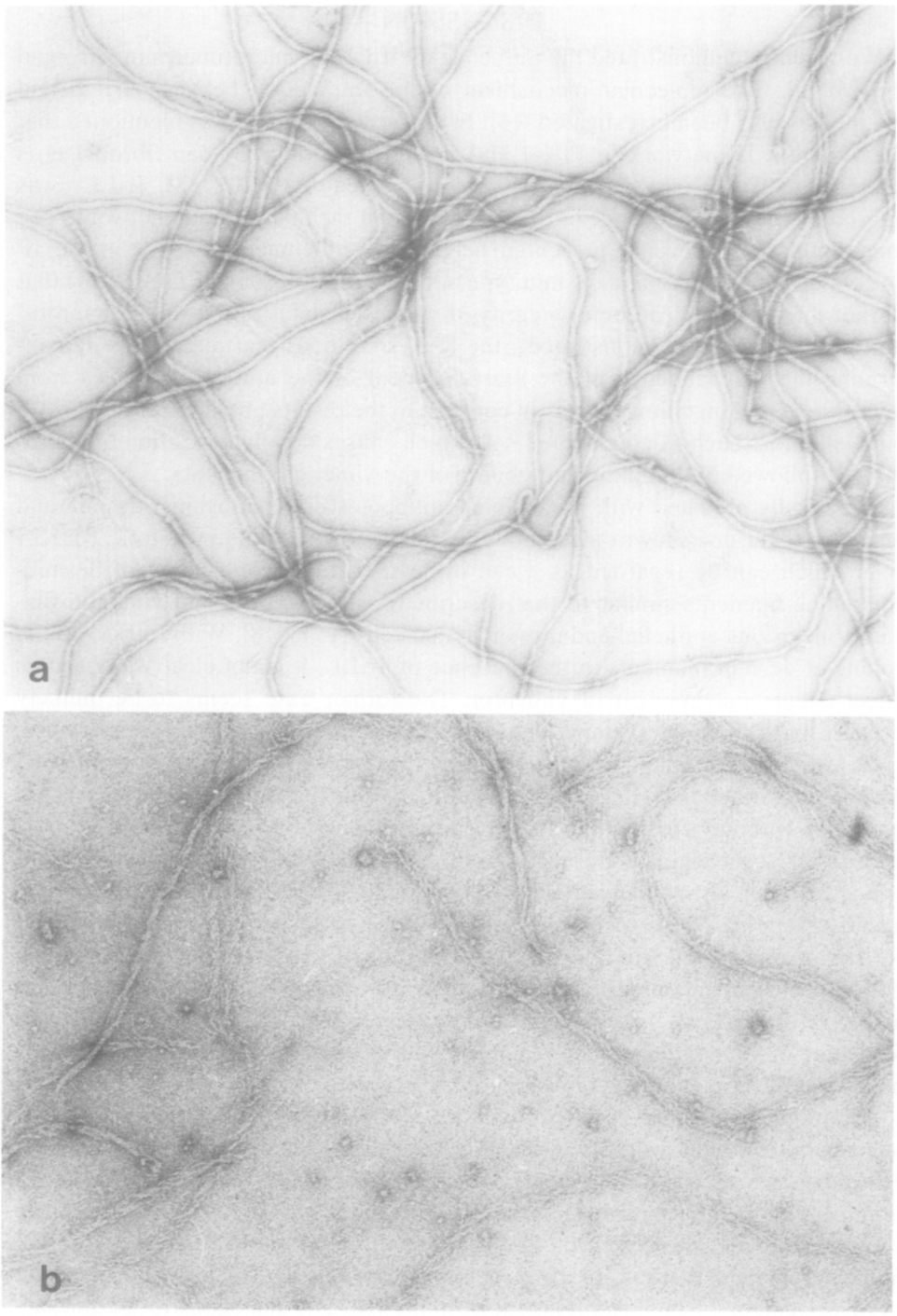

Fig. 4. Electron microscopy of desmin filaments reconstitued in vitro. (*a*) Control; (*b*) in the presence of 2×10^{-7} M TriEL for 30 min (cf fig. 3 *b*). ×60 000.

DISCUSSION

We recently demonstrated the influence of TriEL on microtubules in vitro and in vivo [5]. The molecular mechanism of the interaction between TriEL and tubulin has also been investigated [14]. In an earlier report, it was mentioned that the vimentin IF network in PtK-1 and SV40-transformed human fibroblasts is altered by TriEL treatment. This effect, caused by $\geq 10^{-6}$ M TriEL, was interpreted as a consequence of the destruction of the microtubular network. The immunofluorescence data presented here clearly document changes in the IF pattern of the vimentin and desmin type at concentrations of TriEL (10^{-8} M) that do not influence microtubule integrity. Even after stabilization of the microtubules by Taxol (not demonstrated), the IF system does rearrange, thus demonstrating the independence of the rearrangement of the non-epithelial IFs from microtubule organization. This is in contrast to the results obtained with microtubule inhibitors such as colchicine [3, 4] which causes depolymerization of microtubules followed by bundling and coiling of the vimentin filaments.

The results obtained with monoclonal antibodies directed against desmin and vimentin (data not shown) also showed desmin-positive dots in mitotic BHK21 cells which can be regarded as a cell cycle-specific rearrangement of desmin-containing filaments similar to that described for cytokeratin and vimentin filaments in various epithelial and mesenchymal cells [1, 15–18]. With respect to the coiling of desmin filaments in the presence of TriEL, it is not clear whether this effect occurs independent of vimentin. The former case seems to be unlikely because both filament systems behave in the same way in vitro. Furthermore, since Quinlan & Franke [11] demonstrated that heteropolymers of vimentin and desmin are formed in BHK21 cells, it is difficult to imagine how one partner could rearrange without affecting the IF system as a whole.

In order to investigate the direct interaction of TriEL with preformed non-epithelial IFs in vitro and its effect on their assembly from soluble protofilaments, negative staining of the respective samples was employed. The results obtained for IFs reconstructed from vimentin and desmin are very similar. It is obvious, however, that all filaments are fragmented by TriEL treatment, and that their assembly in vitro is interrupted in the presence of TriEL, resulting in the formation of filamentous structures much shorter than normal filaments. Furthermore, the phenomenon of unravelling is observed.

According to current concepts (see e.g. [19–21]), IF are composed of four helically interwound protofibrils, each of which consists of two helically twisted protofilaments. The protofilaments themselves represent longitudinally aligned tetramers of the respective IF protein. Unravelling of IF by TriEL could be caused either by weakening of the hydrophobic interaction between protofilaments or dimers thereof, or by specific interaction of TriEL with the single cysteine residue of the protofilament subunits [22–24], analogous to the interaction of TriEL with two thiol groups on the tubulin dimer [14]. This may induce a conformational change in the subunit proteins, thus causing the effect described.

In our in vitro experiments, IF proteins were employed at a concentration of approx. 3 μM, a 10- to 100-fold molar excess over TriEL. Close inspection of the ultramicrographs reveals that, at most, some 50% of all fibres remained unaffected by TriEL; they did not show untwisting, for example, of their helical array of protofilaments (e.g. fig. 3*b*, arrows). Therefore, this molar ratio implies that a single TriEL molecule exerts an effect along a distance of at least some 250 Å (in analogy to intercalating agents acting upon superhelical DNA). Furthermore, the selectivity indicates a cooperative action of TriEL. Studies with radioactive TriEL will be necessary to determine the binding site as well as the precise mode of action of the drug.

In summary, we have shown that TriEL changes the fluorescence pattern of desmin and vimentin filaments in various mammalian cells, independent of the organization of the microtubular system. TriEL alters the integrity of preformed non-epithelial IFs in vitro and also reduces their capacity to reconstitute normally. TriEL definitely interacts at concentrations of $\leq 10^{-6}$ M with IFs in vivo, but also with other targets [5, 25]. Its cytotoxicity may therefore be a cumulative effect. TriEL, when used at low concentrations which do not affect other targets, appears to be a very useful tool with which to study drug-dependent rearrangements as well as entirely unknown functional aspects of IFs.

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