The data are thus evidence that sarcolysin has an inhibitory action on the synthetic phase of the DNA repair process. The depth of inhibition is dose-dependent. The ability of sarcolysin to induce a rapidly increasing number of irreparable breaks in DNA molecules, disturbing its template function, may perhaps also be responsible for its high antitumor activity, which may be exhibited after a single administration of the maximally tolerated dose.

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INDUCTION OF MICROFILAMENT BUNDLES IN INTERPHASE NUCLEI OF RAT NEURINOMA CELLS BY DIMETHYL SULFOXIDE

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Interest in the study of the biological action of the polyfunctional compound dimethyl sulfoxide (DMSO) on widely different cells of living organisms has recently increased considerably. It is widely used as a cryoprotector, growth stimulator, and chemotherapeutic agent, and is known in medicine as dimexide. A unique property of DMSO is its high transportability. At the same time, it is actively used as an inducer of cell differentiation. The effect of DMSO on cell cultures depends essentially on its concentration, the temperature and duration of action, the degree of differentiation of the cells, and so on [3]. In somatic hybridization experiments DMSO is used as an additional agent which greatly improves the process of cell fusion [9]. In our own experiments on fusion of membranes of tumor cells in the nervous system with the aid of polyethylene-glycol (PEG, mol. wt. 1000) addition of 15% DMSO led to a high yield of polykarya, without any appreciable cytotoxic effect [1]. Meanwhile the morphologic aspects of the action of different DMSO concentrations at the subcellular level have been inadequately studied.

Interest has accordingly increased in the study of the action of a 15% concentration of DMSO, used in fusion experiments, on the ultrastructure of tumor cells, and the investigation described below was undertaken to investigate the problem.

EXPERIMENTAL METHOD

Experiments were carried out on transplantable cell line RGGN-1 obtained from a neurinoma of the rat Gasserian ganglion. Cells of the RGGN-1 line were cultured in Eagle's MEM

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medium with the addition of 20% calf blood serum and 5% chick embryonic extract. A culture in the logarithmic phase of growth was used in the experiments, and the 15% DMSO solution (Serva, West Germany) was made up in medium RPMI without the addition of serum. DMSO was added to the culture in accordance with the scheme worked out by the writers previously in experiments on somatic hybridization of cells of the nervous system [1]. Strict observance of the order of the operations in accordance with this scheme was essential so as to differentiate between the effects of PEG and DMSO on the RGGN-1 cells. Cells for electron-microscopic investigation were taken after incubation for 15, 30, and 60 min in growth medium with DMSO at 37°C. Material was fixed in 1.5% glutaraldehyde solution in 0.1 M cacodylate buffer, dehydrated, stained with 0.5% uranyl acetate solution in 70° alcohol, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-III Ultratome, stained with lead citrate by Reynolds' method, and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the RGGN-1 tumor cells incubated in medium with DMSO revealed fibriallary bundles in the interphase nuclei 30 and 60 min after addition of the compound. These structures, 0.1-1 μ thick and 0.5-2.5 μ long, were composed of separate thin slightly undulating fibrils about 6-7 nm in diameter (Figs. 1 and 2a, b). They were distributed locally in one part of the nucleus (Fig. 1) or they were found as a number of loci distributed throughout its area (Fig. 2a). Close to these bands of fibrils regions of heterochromatin were observed (Fig. 1a, b and Fig. 2a). The appearance of these bundles was preceded by definite changes in the density and structure of the nuclear matrix. After 15-30 min discrete areas of karyoplasm became translucent and structures composed of delicate fibrils and granules appeared; later, these evidently joined together to form long fibrillary bundles. The nuclei of cells in which the fibrillary bundles formed became round. Under the influence of this concentration of DMSO the rough endoplasmic reticulum was widened and vacuolation of the cytoplasm increased (Fig. 1a).

DMSO had a similar effect in cell nuclei of Amoeba proteus and Dictyostelium mucoroides when exposed for 30 min to a 10% concentration at 22°C, and in HeLa cell nuclei after the same exposure at 37°C. Special investigations showed that the intranuclear structures observed are actin [7]. Thin actin fibrils also were revealed by the aid of DMSO in Tetrahymena pyriformis cell nuclei, despite the small number of them contained in the cells of this organism [10]. The appearance of actin paracrystals was induced in interphase nuclei of kangaroo rat cells not only by DMSO, but also by the ionophore A 23187, in the presence of high magnesium ion concentrations [12]. The cell morphology and the distribution of actin-containing microfilaments were shown to vary significantly depending on the concentration of DMSO applied and the duration of exposure. With a concentration of 10-15% cell retraction and the formation of nuclear actin paracrystals were clearly visible. After termination of the exposure to DMSO no fibrillary bundles could be seen, i.e., the effect was completely reversible and the cells remained viable. DMSO in concentrations above 20% was less effective in inducing bundles of nuclear microfilaments, and caused death of the cells [7, 12].

The similarity of the morphology, the size of the microfilaments, and also the specificity of the conditions of their appearance suggested that the structures we found are also evidently actin in nature. We know that the contribution of actin to the total nuclear protein may differ. In malignantly transformed cells the total actin content is almost twice as high as normal, just as it is also in normal cutaneous fibroblasts of patients with dominantly inherited neuroblastoma, rectal polyposis, and with basal-cell nevus carcinoma [2].

Several views have been expressed on the function of this protein. It is suggested that actin is necessary to maintain nuclear tone and it participates in changes in shape of the nucleus [6]. An active role of polymerized actin also has been noted in chromatin condensation [13, 14]. Nuclear actin is involved in the process of contraction of the nucleus during amitosis in Dictyostelium mucoroides [8] and it is incorporated into the mitotic spindle during division of mammalian cells [4]. Investigations of the properties and functions of nuclear and cytoplasmic actin are continuing. It has been shown that nuclear actin in Amoeba proteus is more highly polymerized than plasma actin [13].

Some investigators have demonstrated translocation of cytoplasmic actin into the nucleus under the influence of DMSO [12]. It has been suggested that this phenomenon is a more vivid manifestation of the process taking place in the natural state. Actin freely moving

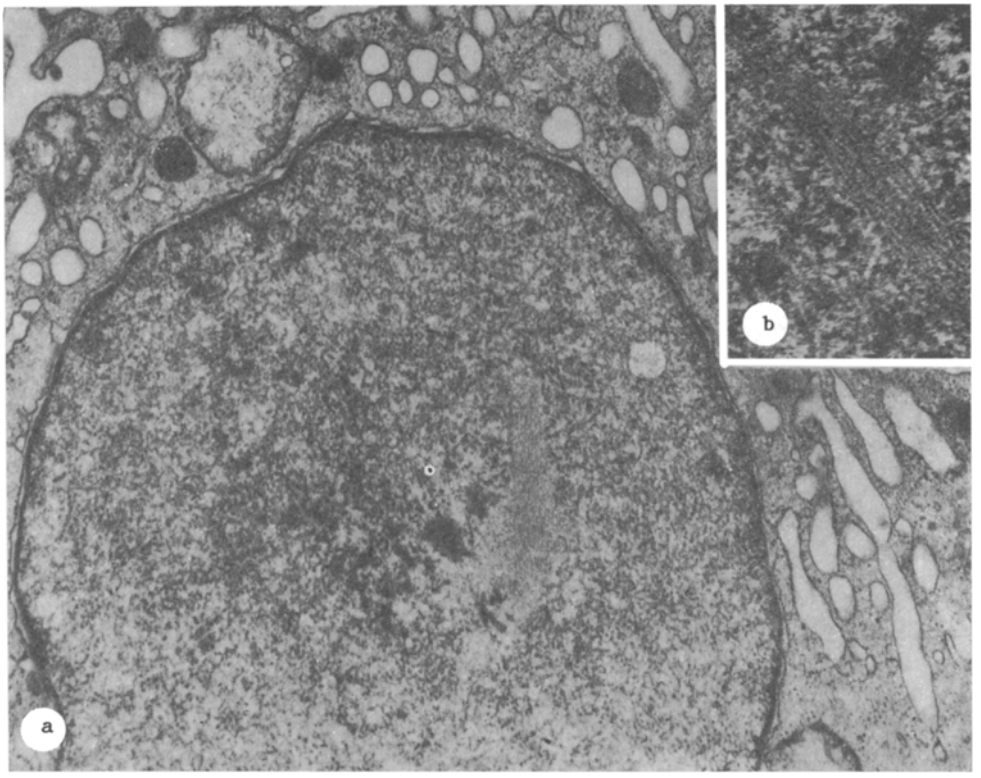

Fig. 1. RGGN-1 cell in culture 30 min after treatment with 15% DMSO: a) band of single microfilaments, near which regions of heterochromatin are visible, in nucleus; rough endoplasmic reticulum widened; cytoplasm vacuolated (30,000×); b) fragment of fibrillary intranuclear bundle (66,000×).

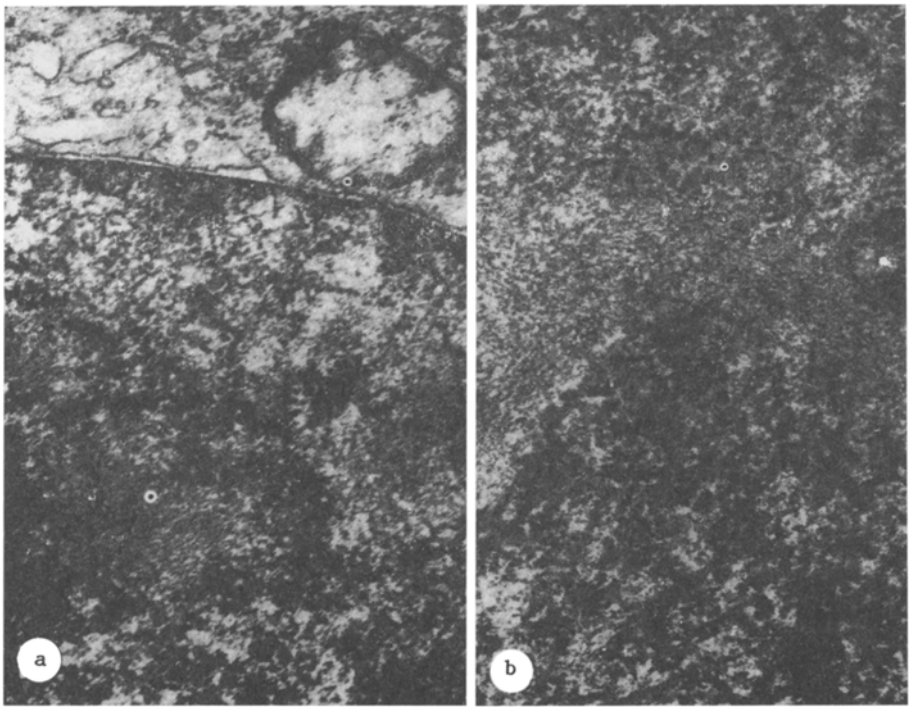

Fig. 2. RGGN-1 tumor cell 1 h after treatment with 15% DMSO: a) several loci of bundle of microfilaments in nucleus (40,000×); b) curved configuration of fibrillary structures (66,000×).

between nucleus and cytoplasm has been demonstrated in Xenopus [5] and Amoeba proteus [13]. These observations suggest that the nuclear membrane is not an obstacle for this protein.

There have been several investigations of actin polymerization [7, 10, 12, 13]. It is considered that actin exists in interphase cell nuclei in the low-polymer state and that it is found quite rarely in the form of fibrillary bundles. In certain situations the conditions are evidently favorable for polymerizatin of nuclear actin. For instance, intranuclear inclusions, similar to bundles induced by DMSO, have been found in cells of the rat hypothalamus [11], and in cat sympathetic neurons [15] without exposure to any experimental factors.

Intranuclear bundles of microfilaments were thus discovered electron-microscopically by the use of 5-15% DMSO under temperature conditions optimally physiological for each type of and organism. Consequently DMSO, besides its already well known functions, also performs the role of inducer of the structural organization of actin and can serve as an instrument for its localization and discovery in the nuclei of cells of different origin.

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