The effect of sodium salicylate on the H^3-TdR incorporation in cultures with and without SRBC and on the number of direct and indirect PFCs. Mean suppression (%) ± S.E.M. in 5 experiments

Measured parameter	Day of culture				
	1	2	3	4	5
H^3-TdR SRBC	27± 2	54± 2	62± 2	67± 5	45±16
— H^3-TdR	10± 4	13± 7	26± 7	27± 5	−3±18
Direct PFCs	−34±76	79±13	93± 3	95± 2	86± 7
Indirect PFCs	6± 8	1±17	−17±37	56±10	60± 5

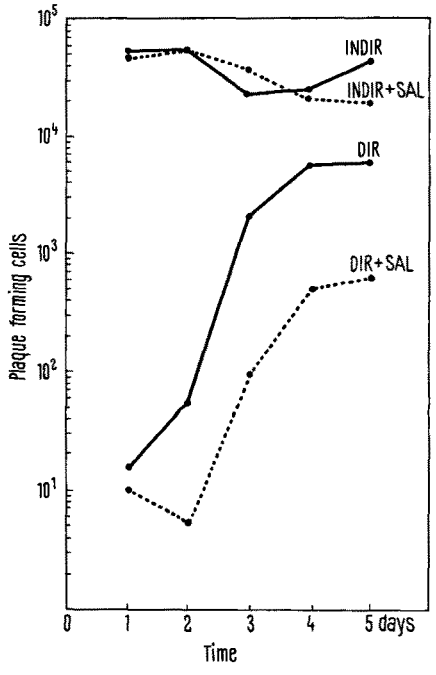

Fig. 2. The effect of sodium salicylate (SAL) on the number of direct (DIR) and indirect (INDIR) PFCs in cultures with SRBC.

rate of the increase was unaffected. In contrast the number of indirect PFCs (IgG) was much less affected by sodium salicylate. A suppressive effect was found on the last 2 days of the culture period.

The Table summarizes the effects of salicylate, expressed as per cent suppression, on the in vitro immune response to SRBC. The results indicate in general that sodium salicylate is an inhibitor of the in vitro anamnestic immune response. The drug suppressed the number of direct PFCs > antigen-induced H^3-TdR incorporation > the number of indirect PFCs > background H^3-TdR incorporation. This indicates a certain selectivity in its action.

Phytohemagglutinin-stimulated lymphocytes preparing for mitosis show an increased dependency on mitochondrial function[9]. Therefore the action of the salicylate may be a result of its uncoupling effect on oxidative phosphorylation and subsequent inhibition of the energy-consuming processes of proliferation and antibody synthesis. In addition, we habe been unable to demonstrate a direct inhibition of the secretion of preformed antibody from spleen cells incubated for up to 6 h with sodium salicylate (ALM and PACHMAN, unpublished results)[10].

Zusammenfassung. Na-Salicylat bewirkt in vitro eine Hemmung der sekundären Immunreaktion von Hühnchenmilzzellen gegen Schaferythrozyten.

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15 February 1971.

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Nucleolar Alterations of Peripheral Nerve Cells in Rats Following Administration of 4-Hydroxyaminoquinoline 1-Oxide

Neuronal injuries have been noted to occur in animals following administration of certain carcinogenic or carcinostatic agents. MCDONALD et al.[1] have demonstrated that brains of mice receiving an injection of nitrogen mustard exhibit neuronal shrinkage in the neocortex, pyriform cortex, hippocampus, cerebellum and medulla oblongata. Administration of cycasin to young mice has been reported to induce ataxia[2] associated with necrosis of granular cells of the cerebellum[3]. KOENIG et al.[4] have shown nucleolar-cap formation of the anterior motoneurones in cats given an intraperitoneal injection of actinomycin D. In the present study, we also found that an i.v. injection of 4-hydroxyaminoquinoline 1-oxide, a compound known as a potent carcinogen, produced nucleolar segregation of the peripheral nerve cells in rats.

Materials and methods. 40 mg of 4-hydroxyaminoquinoline 1-oxide hydrochloride (4HAQO × HCl) was dissolved in 1.0 ml of 0.1 *N* HCl and diluted to a volume of 20 ml with physiological saline. 24 five-week-old male Sprague-Dawley rats were given an i.v. injection of this solution into the tailvein in a dose of 10 mg of 4HAQO × HCl per kg body wt., and 4 rats each were then sacrificed 30 min, 2, 6, 18, 48 and 72 h after injection. As the control group, 5 rats were i.v. injected with 0.5 ml of 0.005 *N* HCl and sacrificed after 6 h. These animals were perfused through the ascending aorta with cacodylate-buffered 3% glutaraldehyde for 30 min at a pressure of 3 ft water under Nembutal anesthesia. After completion of the perfusion, the ganglia of L_4 and L_5, and trigeminal ganglia were excised. In some rats, the coeliac ganglia, supracervical ganglia and terminal ileum were also excised. Tissue samples were fixed again in cacodylate-buffered 3% glutaraldehyde for 30 min, post-fixed in cacodylate-buffered 2% osmium tetraoxide for 45 min, dehydrated through a series of alcoholic concentrations, and embedded in Epon 812. Sections were cut on an LKB ultramicrotome, stained with uranium acetate-lead citrate, and examined with a JEM 6C electron microscope.

Results and discussion. In agreement with the findings by several authors[5,6], neuronal cells of the spinal and trigeminal ganglia from control rats were shown to possess large, irregulary contoured nucleoli consisting of granules, fibrils and amorphous materials. The granules were uniformly distributed constituting more than half of the total

nucleolar area, while the fibrils were closely packed to form a thread-like network (nucleolonema).

Within 30 min after injection of 4HAQO, the nucleoli were reduced in size, exhibiting a smooth profile. The nucleolonema had disappeared, and the granules were greatly diminished. Nucleolar bodies appeared to be composed of coalesced fibrils and small granular aggregates.

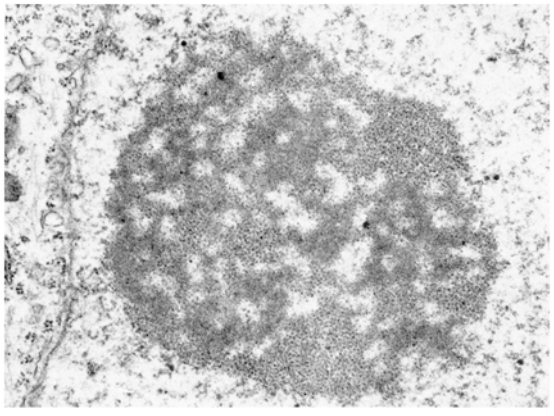

Fig. 1. A neuronal cell of the spinal ganglion from a control rat. The nucleous consists of granules, fibrils and amorphous materials. The fibrils appear to be closely packed, forming a thread-like network. × 19,000.

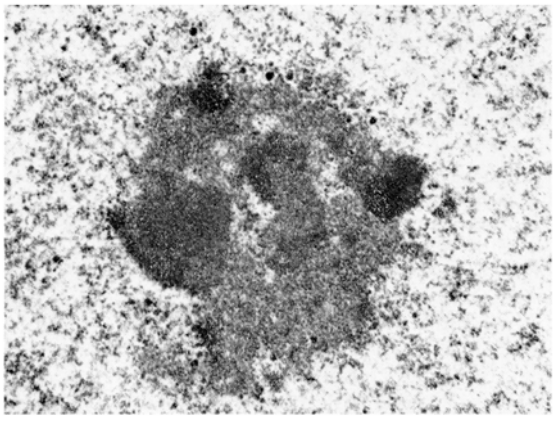

Fig. 2. Nucleous of a spinal ganglionic cell from a rat 30 min after an injection of 4HAQO. The fibrillar and granular components appears to be segregated into dense plaques. × 20,000.

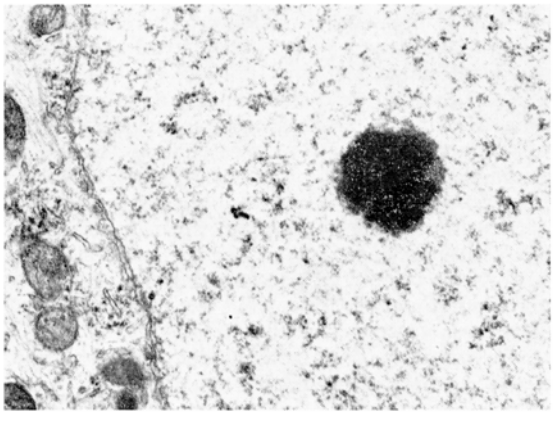

Fig. 3. A neuronal cell of the trigeminal ganglion from a rat 6 h after an injection of 4HAQO. A small nuclolar remnant consisting of coalesced fibrils is seen. × 20,000.

In some cells, nucleoli were disrupted into dense fibrillar clumps. These alterations occurred most prominently at 6 to 18 h and lasted till 48 h. By the 72nd h, nucleolonema had reappeared in association with an abundance of granules.

Similar nucleolar alterations were also noted in the neuronal cells of the coeliac ganglia, supracervical ganglia and Auerbach plexus in the treated rats. In contrast, neuronal cells of the central nerve including anterior horn cells of the spinal cord and Purkinje's cells of the cerebellum did not exhibit any nucleolar lesion. This may imply the possibility that 4HAQO is incapable of crossing the blood-brain barrier.

Nucleolar alterations characterized by disintegration of nucleolonema, and segregation of the granular and fibrillar components into separate zones are known to occur in a variety of in vitro and in vivo cell systems secondary to the action of various compounds, including actinomycin D[7], 4-nitroquinoline 1-oxide[8,9], aflatoxin[10] and proflavin[11]. These compounds also possess the biological properties[12,13] of forming complexes with DNA in some fashion such as nucleophilic substitution of the guanine residues, or intercalation between adjacent base pairs, and of interfering with DNA-directed RNA synthesis. In vivo interaction of 4HAQO with DNA has been demonstrated by means of fluorescence spectroscopy[14]. In a recent study using Ehrlich cancer cells treated with C^{14}-labelled 4-nitroquinoline 1-oxide, a metabolic precursor of 4HAQO, it was shown that almost all of the quinoline compound associated with DNA was bound to the purine bases[15]. ONO et al.[16] have reported that 4HAQO inactivates the transforming activity of *Bacillus subtilis* DNA. In consideration of these biochemical data, the present findings suggest that 4HAQO can interact in vivo with the nuclear DNA of the peripheral nerve cells in rats.

Zusammenfassung. Durch i.v. Injektion der besonders carcinogenen Substanz 4-Hydroxyaminochinolin 1-Oxid kam es bei Ratten zu nukleolären Veränderungen der peripheren Nervenzellen.

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