motes keratinization. It is well known that some chemicals, such as vitamin A, influence epithelial differentiation: addition of vitamin A to organ cultures of hamster trachea after development of keratinized squamous lesions causes reversal of the process of keratinization[7]. We are now investigating the problem of whether the effect of MNNG on keratinization can be modulated by such chemicals.

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The distribution of carcinogens, 4-nitroquinoline-1-oxide and 4-hydroxyaminoquinoline-1-oxide, in the nervous system and its possible neurotoxicological significance[1]

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Summary. 4-NQO-^{14}C can enter the grey matter parenchyma of the central nervous system of mice after i.v. injection. The level of its uptake by the central grey is higher than that taken up by the central white and by the trigeminal and spinal dorsal root ganglia. This pattern of distribution is strikingly different from that obtained after i.v. injection of 4-HAQO-^{14}C, suggesting the possible occurrence of 4-NQO encephalomyelopathy having entirely different sites of lesions from those of 4-HAQO neuropathy.

4-Nitroquinoline-1-oxide (4-NQO) is a well-known carcinogenic agent[2]. In animal bodies it is converted to its reduced form, 4-hydroxyaminoquinoline-1-oxide (4-HAQO), before acting as a carcinogen[3].

In 1971 Hayashi, Hasegawa and Toyoshima[4] reported in this journal on the degeneration of axons observed in the posterior column, as well as in the spinal tract of the trigeminal nerve, in rats after repeated i.v. injection of 4-HAQO. In 1977 Takahashi, Agari and Nakamura[5] have reconfirmed the facts. One of the most interesting points in this dying-back type of neuropathy is the progressive nature of degeneration recognized after completion of the administration of the agent, and this nature has been ascribed to the conceivably irreversible changes occurring in the DNA of the nerve cells[4,5]. On the one hand, no reports have ever appeared on the nervous disorders induced by 4-NQO.

Recently the author of this communication has observed the distribution of both these agents in mice. It has been concluded from these experiments that the pattern of distribution of 4-NQO-^{14}C in the nervous system is strikingly different from that of 4-HAQO-^{14}C after their i.v. injection. This finding suggests the possible occurrence of an encephalomyelopathy by 4-NQO having entirely different sites of lesions from those of 4-HAQO neuropathy.

Materials and methods. Male adult mice of ddy strain, around 10 weeks in age, were given i.v. either 4-NQO-5, 6, 7, 8, 9 and 10-^{14}C (6.2 mCi/mmole) suspended or 4-HAQO-5, 6, 7, 8, 9 and 10-^{14}C hydrochloride (3.2 mCi/mmole) dissolved in hydrochloric acidic saline. The doses were 9.5 and 9.1 μCi/mouse, respectively. After a certain length of time (i.e. 1, 5 or 24 h), the animals were sacrificed by chloroform inhalation, and whole-body macroautoradiograms were made, according to Matsuoka's modification of Ullberg's method, from the 40-μm-thick section of the frozen mice. The density distribution developed in the industrial X-ray films were examined by naked eye, aided by densitometry of the films. The details of the method were described elsewhere[6].

Results and discussion. The results were remarkable. Thus, it was in the grey matter of the central nervous system (CNS) that the level of 4-NQO-^{14}C uptake after 5 h was highest of all the parenchymal organs in the body (figure 1a). Differing from the case of methyl mercury[7], the level was relatively low in the cerebellar cortex and white matter, and its uptake by the spinal dorsal root ganglia was at quite a low level compared with that by the central grey (figure 2a). To the author's knowledge, there has never appeared a report on the distribution of 4-NQO in the nervous system.

On the one hand, the uptake of 4-HAQO-^{14}C by the brain and spinal cord was at a relatively low level in the body (figure 1b), and the level of its uptake by the spinal dorsal root ganglia was definitely high in comparison with that by the CNS (figure 2b). These results seem to be compatible with the occurrence of a sensory neuropathy of dying-back type in rats[4,5], involving primary sensory neuron. The level of its uptake by the trigeminal ganglia was also higher than that by the CNS and this finding agrees with Hayashi et al.'s with ^{3}H-4-HAQO only briefly mentioned in their review[8].

After 24 h, the basic pattern of distribution of 4-NQO-^{14}C and 4-HAQO-^{14}C remained unchanged although the level of their uptake was generally reduced. The most interesting findings seem to be the predilection of 4-NQO-^{14}C for the CNS and the striking difference in distribution found between 4-NQO-^{14}C and 4-HAQO-^{14}C, as if 1 of the 2 were the positive or negative of the other.

What should be stressed first is the fact that the uptake of 4-NQO-^{14}C by the grey matter of the CNS was much higher than that by the blood. This indicates that the concentration of 4-NQO-^{14}C in the central grey parenchyma must have been much higher than in the blood.

Second to be emphasized is the fact that the level of 4-NQO-^{14}C uptake by the cerebral cortex and by the spinal grey matter, for example, was definitely higher than that by the corpus callosum and by the spinal white matter, respec-

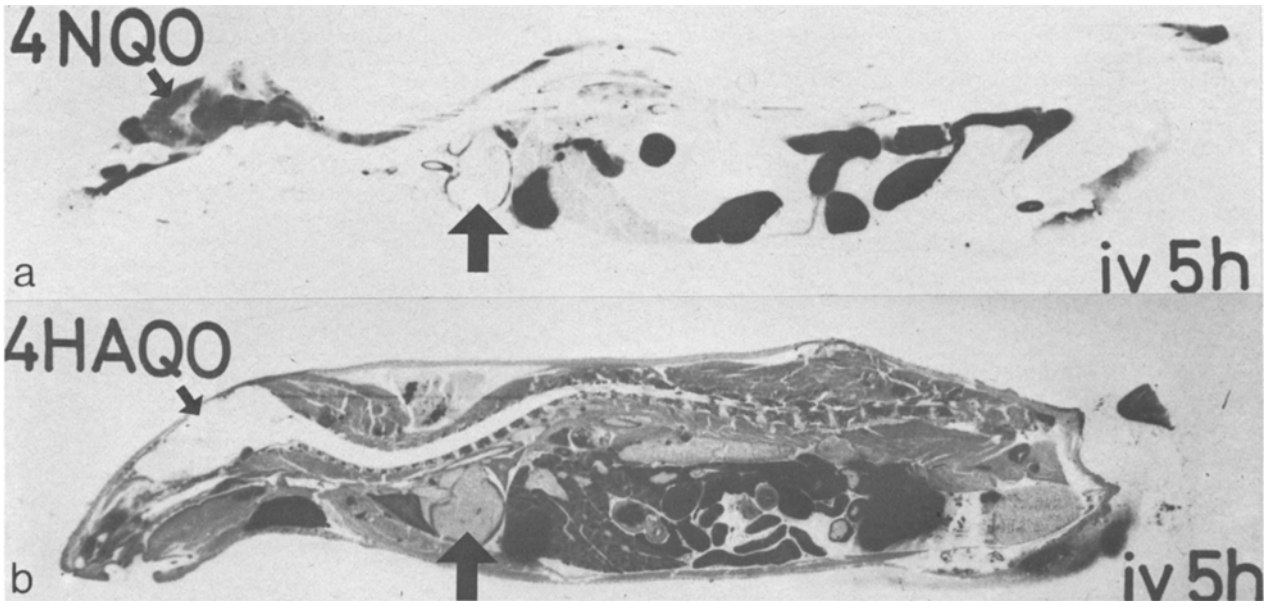

Fig. 1. The uptake of 4-NQO-^{14}C by the CNS, particularly by its grey matter, of a mouse in contrast to that of 4-HAQO-^{14}C by the same organ of another mouse. Both pictures show a sagittal section near midline with the mouse's head placed on the left. It was noted that with 4-NQO-^{14}C (a) the density of the film part corresponding to the cerebral cortex (small arrow) and other central grey was much higher than that of the one corresponding to any other parenchymal organs of the body, including the cardiac blood (large arrow), while the former was much lower than the latter with 4-HAQO-^{14}C (b). Both mice were sacrificed 5 h after i.v. injection of the respective compounds.

tively; on the whole its distribution predominated in the grey matter and not in the white. Since the grey matter is characterized by the presence of the neuronal cell bodies with their dendrites and the axon terminals ending on them, it can be conceived that a considerable part of 4-NQO-^{14}C having entered the CNS tissue parenchyma has been taken up by one or the other portion of those structures.

Thirdly it could be pointed out that, compared with the level of its uptake by the central grey, the uptake by all other parenchymal organs in the body were remarkably low. This feature was retained at 24 h after i.v. injection as well, but at early hours (for example, at 1 h) the levels of its uptake by the CNS and by other organs were closer to each other. From these facts it seems appropriate to conceive that the 4-NQO-^{14}C, having distributed in general organs, would be excreted relatively rapidly, while that having entered the central grey, would remain there for a relatively long period.

In a striking contrast to the behaviour of 4-NQO-^{14}C specified above, strict limitations existed to the entry of 4-HAQO-^{14}C into the CNS (socalled blood-brain barrier phenomena). This finding suggests that 4-HAQO and its metabolic products in the blood cannot easily pass into the CNS tissue parenchyma.

Enzymatic conversion of 4-NQO to 4-HAQO and of the latter to 4-aminoquinoline-1-oxide, first proved with rat liver homogenate, was later demonstrated with homogenates of various organs, including brain, of many species of animals (mice, rats etc)[3,9]. Based on these facts, it is conceivable that at least part of 4-NQO having entered the brains of the mice in this study would be enzymatically converted to 4-HAQO in loco. However, it remains to be demonstrated that such reductive processes actually occur in the brains of the mice, to which 4-NQO has been given. The relationship of the 2 compounds, 4-NQO and 4-HAQO, seems comparable with those of l-dopa and dopamine or of 5-hydroxytryptophan and serotonin in that, in all these settings, the metabolic precursor can enter the brain easily, while its metabolic product cannot. It is noteworthy that dopamine or serotonin is a physiological substance acting as a transmitter, while 4-HAQO is a non-physiological one, which may badly affect the living mechanism of the cell irreversibly.

At the moment it remains entirely to be demonstrated whether the facts presented here can really have any further neurotoxicological or neurological meanings. Here, suffice it to say that if 4-NQO could induce a toxic disorder of the nervous tissues, the site of its pathological lesions might possibly be different from that in 4-HAQO neuropathy. Further experiments alone can give us an answer.

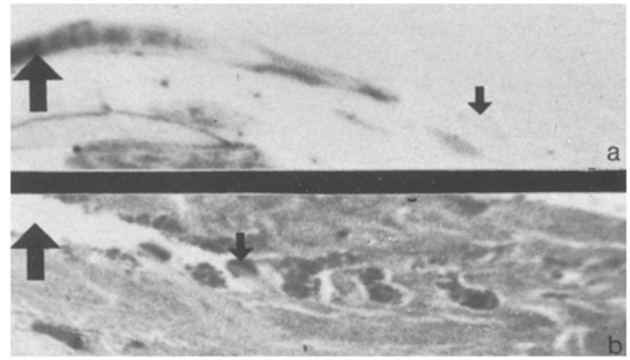

Fig. 2. The uptake of 4-NQO-^{14}C by the spinal dorsal root ganglia of a mouse in contrast to that of 4-HAQO-^{14}C by the same organs of another mouse. Both pictures show a sagittal section near midplane, only a portion of which is included in the figure. It was noted that with 4-NQO-^{14}C (a) the density of the film part corresponding to one of the spinal dorsal root ganglia (small arrow) was much lower than that of the one corresponding to the spinal cord grey matter (large arrow), while the former was much higher than the latter with 4-HAQO-^{14}C (b). Both mice were the same as the ones cited in figure 1.

1 This work was supported by a grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of the Japanese Government (No. 321304, 1978).
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Staining of microtubules of the electrocyte of *Electrophorus electricus* L. by alcian blue and lanthanum[1,2]

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Summary. Microtubules were observed in the cytoplasm of the *Electrophorus electricus* L. when the tissue was fixed in the presence of alcian blue and lanthanum nitrate.

The electric organs of *Electrophorus electricus* have been the object of several investigations at the Institute of Biophysics in Rio de Janeiro, Brasil, where they continue to be a subject of study[3]. In a previous paper we described the fine structure of the electrocyte[4] and showed that its surface presents tubular invaginations of the cell membrane and that a fibrous coat covers the outer face of the membrane penetrating the lumen of the invaginations[5]. The cytoplasm of the electrocyte has few organelles. We observed the presence of mitochondria, glycogen particles, dense bodies, Golgi complex, and a network of microfilaments. Microtubules were not observed.

Cytochemical studies were carried out to determine the nature of the fibrous coat of the electrocyte[5]. When alcian blue and lanthanum nitrate were used to detect acid polysaccharides, we could observe the presence of microtubules in the cytoplasm of the electrocyte. The results obtained are described in this report.

Materials and methods. Fragments of the main electric organ of *E. electricus* were removed close to the surface of adult fishes, near the head. Fixation was carried out in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 containing 0.5% alcian blue 8 GX (Allied Chemical, N.Y.) for 2 h. After rinsing in cacodylate buffer, the fragments were post-fixed in 1% OsO_4 in s-collidine buffer at pH 8.0, containing 1% lanthanum nitrate (British Drug Houses Ltd.) for 2 h[6]. Some fragments were fixed in the same way but without lanthanum nitrate in the post-fixation. In this case 0.1 M cacodylate buffer pH 7.2 was used. After post-fixation, all fragments were dehydrated through an ethanol series and embedded in epon. Ultrathin sections were obtained in a LKB Ultratome III ultramicrotome. After staining with uranyl acetate and lead citrate, they were observed in an AEI EM-6B electron microscope.

Results and discussion. When the electric organ is fixed with the fixatives usually used in electron microscopy, the cytoplasm of the electrocyte appears poor in organelles. Many nuclei can usually be seen, surrounded by Golgi complexes, multivesicular structures, dense bodies and mitochondria. Throughout the cytoplasm, only glycogen granules and filaments are observed (figure 1). The filaments are 7 nm thick. The following fixatives have been used during our studies on the fine structure of the electrocyte: a) double fixation with glutaraldehyde and osmium tetroxide; b) fix-

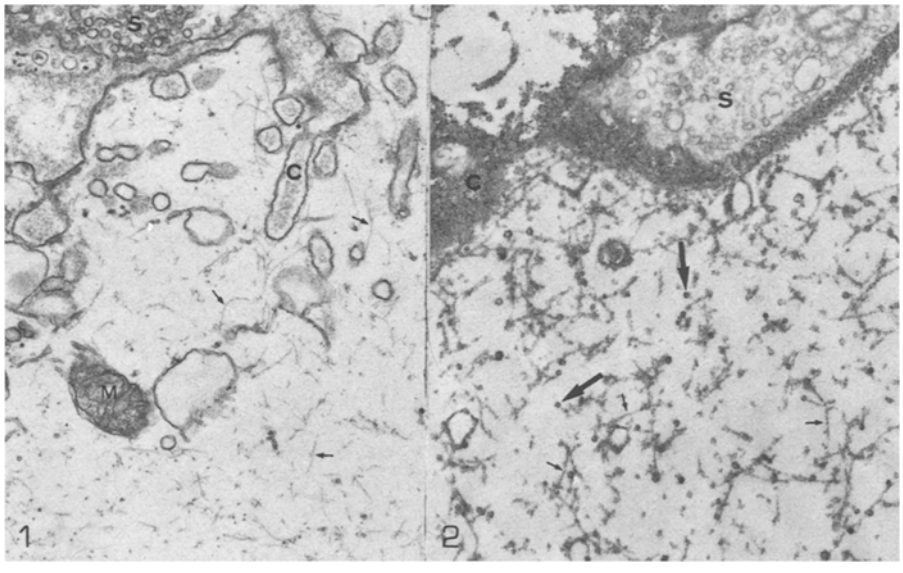

Fig. 1. Posterior surface of an electrocyte after ordinary fixation. A synapsis (S) is seen containing synaptic vesicles. The cell coat (C) is present as usual penetrating the invaginations. In the cytoplasm, a mitochondrion (M) and filaments (small arrows) can be pointed out. Microtubules are not distinct. × 23,400.

Fig. 2. Posterior surface of an electrocyte after fixation in presence of alcian blue and lanthanum nitrate. A synapsis (S) is seen. Note the cell coat (C) with a dense precipitate. In the cytoplasm, the filaments (small arrows) are more distinct and the microtubules (large arrows) are conspicuous. × 23,400.