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MICROSCOPIC AND ULTRASTRUCTURAL CHANGES IN THE THYROID GLAND, RESULTING FROM EFFECTS OF THE INSECTICIDE SEVIN

One of the most promising insecticides of the carbamates is Sevin. Many aspects of its effect on the organisms of warm-blooded animals have been studied by toxicologists, biochemists and morphologists (Carpenter, et al., 1961; N. V. Khomich, 1962; Ye. I. Makovskaya, M. B. Rappoport, V. G. Pinchuk, T. P. Bycheva, 1963; Yu. A. Moreynis, I. M. Estrin, 1963; V. S. Yakim, 1966; A. Kh. Ostrovskaya, 1967; I. I. Pavlova, 1967; and others). However, its influence on the glands of the endocrine system, particularly on the thyroid gland, has not been adequately covered. In spite of this, the detrimental effects on this gland of pollutants of industrial environments are well known. This gland is, from the functional standpoint, one of the most important organs of the endocrine system. The gland takes an active part in regulating growth and development, in determining intensity of energy stimulation, and in metabolizing albumins, carbohydrates, fats, minerals and water. It also influences the nervous, cardiovascular and sexual systems (R. D. Gabovich, V. I. Bukhovets, N. V. Verzhikovskaya, 1960; I. M. Trakhtenberg, N. V. Verzhikovskaya, 1962). At present it is accepted that the hormone of the thyroid gland, containing thyroxin and triiodothyronine, in its functioning, affects the fermentative systems, localized in the mitochondria of cells of various organs (Ya. D. Kirshenblat, 1965). This is why it is of special interest to study the fine, microscopic and especially submicroscopic changes which occur in the given organ, as a result of the actions of harmful chemical pollutants of industrial environments.

Our previous research established that tension develops in the thyroid gland following repeated introduction of certain pesticides into the organism of warm-blooded animals.

A period of intensification is followed by a process of normalization, and then a decrease in the functioning of the organ.

The goal of the research was the study of microscopic and ultramicroscopic changes in the thyroid gland, occurring after repeated, weekly, intra-abdominal administration to test animals of Sevin, in a dose of 50 mg/kg (1/7 LD 50), over a period of three months.

150 ppm

This period was chosen for the morphological study because in the time indicated, according to radiometric computations, as certain chemicals are administered, hyperfunction of the thyroid gland is observed. This time of highest functional stress is considered most suitable for a careful study of the organ.

It was necessary to determine the nature and degree of development of the ultrastructural changes, which cannot be observed under normal light microscopy. No less important was the requirement to compare the data derived from electron microscopy with that obtained with a light microscope.

The tests involved ten white mice of the C_3NA strain, predominantly male, weighing 18-20 grams each. Five were used in the tests (were administered Sevin) and five were used as controls. All 10 were maintained under the same conditions and were fed common rations. It should be noted that neither the test, nor the control animals showed any signs of intoxication. Three months after the start of the experiment, at the end of the period of observation, the test and the control mice were killed by decapitation. The thyroid gland was excised in its entirety. A portion was set aside for electron microscopy, the remainder was to be used for the usual testing sequence and in preparation for the microtome. Histological sections were stained with hematoxylin-eosin and, selectively, according to Kraus, for colloids.

In preparing the thyroid gland for the ultra-microtome, segments were cut into small pieces and fixed in a chilled solution of osmium tetroxide. After dehydration in alcohol of ascending strength, the pieces were mixed with methacryls. The sections prepared on the Shestrand ultra-microtome were observed under a "Tesla" electron microscope.

Using light microscopy, thyroid glands of control mice showed follicles of average size. Single, large follicles were found only on the periphery of the organ. The epithelium of the follicles was cube-shaped. Only occasionally did the epithelium assume a prismatic form. The nuclei were circular in shape and were located in the central portion of the cell. Colloid in the gaps between the follicles reflected eosin only faintly. In the follicle gaps there was a modest quantity of vacuoles of colloidal resorption. The vessels of the organ were filled with blood.

Under electron microscopy of the thyroid gland of the control animals follicle cells were closely spaced and were separated by plasmatic membranes. Adjacent cells were divided by narrow inter-cellular spaces with low electron densities. Microfibers of approximately the same width, but different lengths, were found on that portion of the cell surface facing the follicle gaps. In the center of the cells, there was a single nucleus, round or oval in shape, with a regular configuration. The nuclei were surrounded by membranes of two layers separated by a narrow space. The membranes of the nuclei contained pores, arranged at equal distances from one another. In the nuclei of the cells were odd-shaped segments of chromatin with a high electron density. The cytoplasm of the cells contained an endoplasmatic net (endoplasmatic reticulum), more developed in the basal portion of the cell. Ribonucleic elements with a high electron density were disposed near the membranes. In places they appeared without links to the endoplasmatic net, as separate and not too large groupings in the cytoplasmic matrix.

Mitochandria were scattered irregularly throughout the entire cytoplasm of the cells. The membranes which envelop them consist of two layers. Interior membranes of the mitochandria are also distributed in pairs. Golgi apparatus more often appears in the apical part of the cell and consists of moderate accumulations of two-layered, thin, membranes.

Between the base of the cells of the follicle epithelium and the endothelium of the capillaries is a space occupied by two basal membranes. One of these, thinner, is located near the epithelium, the other, thicker, closer to the endothelium of the vessels.

These membranes have a high electron density. Endothelium cells were thin, contained a small number of mitochondria and a poorly developed endoplasmatic net. Elements of ribonuleic acid did not appear in these cells.

Under light microscopy of the thyroid gland of the test animals, follicles with a high prismatic epithelium, predominantly of small size, with a few of average size, were observed. Follicle gaps contained a light pink colloid and a large quantity of vacuoles of resorption of various sizes.

Vessels of the organ were filled with blood to a much higher degree than in the control mice. Under electron microscopy of the thyroid gland of test animals, it was noted that the follicle epithelium often acquired an irregular configuration and a polygonal form. Micro-fibers were somewhat longer, the quantity larger, than in the control mice.

Bubbles, droplets, appeared in the microfibers. A protrusion into the follicle gap of the entire apical portion was observed in separate cells. Plasmatic membranes of the cell base sometimes penetrated into the interior of the cytoplasm as if segmenting the basal portion.

In contrast to the regular configuration of the cells of the epithelium of the thyroid gland of the control mice, in the test animals the configuration was wavy. The membrane of the nucleus slightly thickened, its electron density increased and, in places, the two-layered membrane structure and porosity disappeared.

The chromatin substance of the nucleus was transformed into rough, irregularly scattered clumps. The latter, in places, were concentrated in the vicinity of the inner layer of the membrane of the nucleus. In the cytoplasm of the cells appeared granules, bubbles or tiny droplets, which accumulated predominantly in the apical portion of the cell elements. Drops and bubbles were round or oval, their electron density varied from low to high, and the structure was homogeneous. Mitochandria often assumed a spherical shape, losing the dual structure of the membrane. The mitochandrial crests were frequently destroyed.

Mitochandria, furthermore, at points cut at right angles displayed bubbles or vacuoles. The basic substance, as a rule, was unstructured and homogeneous. The Golgi apparatus was located in the apical portion of the cell and contained large vacuoles more numerous than in the control animals. Near the Golgi apparatus were accumulations of small, closely-spaced intrusions. Endoplasmatic reticulum also underwent modification. Compact ribonucleic elements appeared on the membranes of this net in larger numbers and in higher densities than in the control animals.

The distance between the follicle and the vessel membranes was reduced.

Summarizing the above, it should be noted that repeated weekly intra-abdominal administration of Sevin to test animals, in a dose of 50 mg/kg (1/7 LD_{50}) over a period of three months, brings about a series of morphological changes in the thyroid gland, reflecting hyperfunctioning of the organ.

Under electron microscopy of the thyroid gland of warm-blooded animals, aside from morphological signs indicating increased functioning of the organ, ultrastructural cellular modifications were found, reflecting the presence of dystrophic changes in the follicular epithelium (the disappearance of the dual structure of the membrane of the nucleus and cytoplasm, the disappearance of pores in the membrane of the nucleus, the loss of cristae of mitochandrias, etc.).

It can be concluded, therefore, that repeated administration of Sevin to warm-blooded animals gives rise not only to hyperfunctioning of the gland, but also to a series of inner-cellular structural modifications, indicating that the given insecticide together with an increase in the functioning of the thyroid gland causes dystrophic changes in the actively functioning cells of the organ.

There is no doubt that in extending the period of influence of Sevin, dystrophic cellular changes will multiply and in the end would bring about a functional exhaustion of the given endocrine organ.

Data obtained by us on an intracellular level, from all indications, will be useful in the differentiation of structural changes, based on the functional conditions of the gland (stability, increased or diminished functioning)and found within the limits of a norm, from these changes which should be classed with the pathology of the endocrine organ under study.

SUMMARY

Repeated weekly intra-abdominal administration of Sevin in experimental animals (dosage: 50 mg/kg, i.e., 1/7 LD) for three months caused several morphological changes in the thyroid gland resulting in hyperfunctioning of this organ.

Electron microscopial studies revealed changes indicating the presence of also dystrophic ch[illegible]ges in the follicular epithelium (loss

of double membrane structure of the nuclear and cytoplasma sheath, loss of mitochondria crists). These changes could not be revealed by light microscopy.