Pathogenetic Mechanism of Experimentally-induced Spongy Degeneration*

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Isonicotinic acid hydrazide (INH, Isoniazid) has been shown to be a neurotoxin to the Pekin duck (CARLTON et al.; CARLTON and KREUTZBERG). When fed to ducklings at the dietary level of 0.1% for four weeks, the drug induced a neurological syndrome dominated by intention type tremor and trunk ataxia. The most severe neuropathologic alterations occurred in the cerebellar white matter and consisted of vacuolization and demyelination, establishing the pattern of a spongy degeneration. After such a long period of feeding, the lesions observed were quite advanced making it difficult to establish guide lines as to the pattern of development.

In an effort to follow microscopically the development of the neural lesions, ducklings fed INH were sacrificed at daily intervals for study. The present communication details the neuropathologic changes induced by INH during the first two weeks of feeding.

Materials and Methods

Male Pekin ducks, received within 36 hours of hatching, were used. They were alloted at random into a control group of 40 ducks and an INH group of 60 ducks. Both groups were housed in electrically heated battery brooders and received feed and water ad libitum.

The control group received a 21% protein duck starter mash and the INH group received the mash supplemented with 1500 mg of Isoniazid per kg of diet (0.15%). The drug was incorporated into the mash diet by thorough mixing with a Hobart mixer. Diets were stored without refrigeration in plastic containers.

Two ducks from the control group and three ducks from the INH group were killed at daily intervals to obtain tissues for histological examination. Due to the high mortality in the INH group, ducks were available for only the first eight days of feeding (3 ducks survived for 13 days). Immediately after decapitation and exsanguination, the brains were exposed by removal of the cranial bones and the whole head fixed in 10% buffered formalin. Also taken at necropsy were the lumbar-sacral enlargement of the spinal cord and portions of the sciatic nerve. Paraffin sections of the brains, spinal cords, and sciatic nerves were stained with hematoxylin and eosin (H & E), with luxol fast blue-cresyl violet (Klüver-Barrera), with Einarson’s chrome alum gallocyanin, and by Szatmari’s modification of Bielschowsky’s silver impregnation method for axons. Selected sections were stained for fat by the Oil red 0 technique and by van Gieson’s method for connective tissue. Frozen sections of portions of the cerebellum, the optic lobes, the medulla oblongata and the cerebrum were stained by Cajal’s gold sublimate method for astrocytes.

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Results

Clinical Features

Isonicotinic acid hydrazide at the level of 0.15% of the diet was extremely toxic to ducklings resulting in a 50% mortality by the eighth day of feeding. Three ducks remained on the diet for 13 days at which time they were moribund and were killed. The clinical features of intoxication as described previously (Carlton and Kreutzberg) did not appear in many of the ducks before death or killing. Some ducks were found dead and the remaining ones appeared sleepy and would remain for long period of time resting on the cage bottom with their eyes closed. The INH ducks were smaller than the controls and some portions of this reduction in growth can be ascribed to the induced anorexia; the somnolent INH ducks failed to consume as much of the diet as the active controls.

Morphologic Findings

Animals killed after one day on the diet had no microscopic lesions in the central nervous system. (All ages in text refer to days on diet and not to chronological age.)

First alterations were observed in ducks fed INH for 2 days. The microscopic changes were most severe in the cerebellum around the 4th ventricle (Fig. 1). The tissue here had undergone a spongy change in the white matter, and the tissue appeared web-like and consisted principally of unaltered axis cylinders (partially demyelinated) and small cells with dark-staining nuclei (Fig. 2). These latter cells were partly subependymal glial cells and partly oligodendroglia cells. In the ependyma, occasionally, some of the epithelial cells had vacuoles in their cytoplasm. In some places the continuity of the ependymal lining was interrupted. Although this finding may be an artifact, it appeared that the spongy change was especially marked in the subependymal tissue under these interruptions.

When leaving the region of the subependymal tissue and progressing toward the deep roof nuclei of the cerebellum, a decrease in the severity of the spongy changes was noted. However, the variety of morphologic alterations within the cells increased and was especially conspicuous.

In H & E stained sections, on a background of uniformly stained tissue, there were many cells with lighter-staining cytoplasm (Fig. 3). The cells were round and sharply delineated, occasionally with processes (Fig. 4). The nuclei of these cells were large, round, with one or two nucleoli, poor in chromatin and with densely stained (Fig. 5a) nuclear membranes. They had morphological characteristics consistent with those of astrocytes.

These enlarged astrocytes appeared to undergo several changes which were demonstrable in a variety of forms in the brains of the test ducks. Swelling of these astrocytes was accompanied by the appearance of vacuoles and a consequent dilution and decreased stainability of the cytoplasm. The vacuoles displayed a tendency to become confluent and the larger vacuoles, displacing much of the cytoplasm, were confined to a single part of the cell, separated by a meniscus of cytoplasm (Fig. 4). The cytoplasm of such cells often had a reticulated pattern. The nuclei of such astrocytes were remarkably pale, extremely swollen and frequently were ruptured (Fig. 5b). The nuclei were often displaced peripherally by