INTRANUCLEAR INCLUSIONS IN BRAIN NEURONS OF HENS WITH EXPERIMENTAL HYGROMYCIN B POISONING

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Hygromycin B, when injected into hens as a single dose of 100 000-350 000 units/kg body weight, causes the formation of intranuclear inclusions in the neurons of the medulla and mesencephalon, cerebral hemispheres, and cerebellum. These inclusions are very rich in ribonucleoproteins but do not contain desoxyribonucleoproteins. This indicates the possible effect of high doses of this compound in stimulating RNA synthesis by the neurons. In addition, signs of hydropic degeneration and neuronophagy develop in the brain and the number of gliocytes is increased.

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Cytoplasmic and intranuclear inclusions and also inclusions located simultaneously in both cytoplasm and nucleus, have been found in 90% of cases during virus diseases of man, animals, and plants and in certain types of poisoning [7].

Hygromycin B is produced by the fungus Actinomyces hygroscopicus and is used in poultry farming as an anthelminthic [5]. Since no data regarding tolerance to single doses of the compound were found in the literature, an experimental investigation of this problem was undertaken.

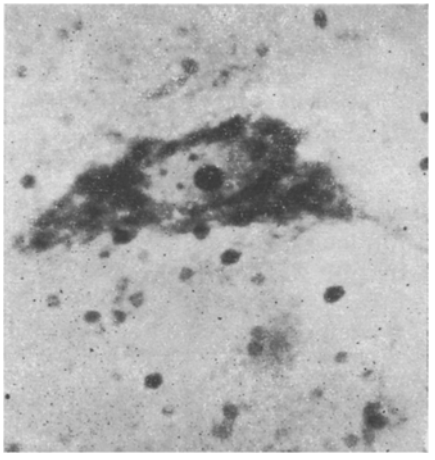

Fig. 1. Neurons from Deiters' nucleus. Vacuolation of cytoplasm, Nissl's substance concentrated in conglomerates. Four intranuclear inclusions can be seen in the enlarged nucleus. Nissl's stain (cresyl violet), immersion, 900×.

EXPERIMENTAL METHOD

Experiments were carried out on 5 groups of hens, 5 birds in each group. The antibiotic was administered to the birds as a 2% solution in a single dose of 50 000, 100 000, 200 000, 300 000, and 350 000 units/kg. Birds receiving 50 000 units/kg showed no clinical or pathological evidence of poisoning. Death of the birds in the other groups began after 24 and 40 h and 6-7 days.

Pieces of the cerebral hemispheres, mesencephalon, medulla, and cerebellum were fixed in 10% neutral formalin solution, Carnoy's fluid, Lillie's fixative, and Brodskii's fixing mixture. Sections were stained with hematoxylin-eosin, picrofuchsin, azocamine, fuchselin, and by Nissl's method. Desoxyribonucleoproteins (DNP) were detected by the Feulgen–Rossenbeck reaction. Control sections were stained after incubation in a solution of crystalline desoxyribonuclease in a concentration of 1000 units/ml in 0.025M veronal buffer (pH 7.5), containing 0.003M $MgSO_4$, for 12 h at 37° and after treatment with 5% TCA solution, while some sections were stained without preliminary hydrolysis. Ribonucleoproteins (RNP) were detected by Brachet's methyl green–pyronine method. Control sections were treated after incubation in a solution of crystalline ribonuclease (1 mg/ml) for 1 h at 37°. Some sections were stained after preliminary treatment with 5% TCA solution for 5 min at 90°.

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EXPERIMENTAL RESULTS

Medulla. The large cells of Deiters' nucleus were deformed and their cytoplasm contained vacuoles. The Nissl's substance had lost its usual granular appearance and was aggregated into strongly basophilic, structureless conglomerates, located nearer the cell membrane. The enlarged, translucent, eccentrically situated nucleus, its usual shape modified, displaced the basophilic substance toward the periphery of the neuron. The outline of these cells was uneven and ragged. The nucleolus was enlarged and strongly basophilic. Neurons with two nucleoli were frequently seen. Besides the nucleolus, two or three strongly basophilic inclusions were found in the karyoplasm, about one-third the size of the nucleolus (Fig. 1). The inclusions were found either close to the nucleolus or distant from it, in most cases along the nuclear membrane. Inclusions were usually round in shape, without a pale border. Frequently smaller basophilic dots could be seen, not grouped together into a conglomerate, also located along the nuclear membrane. After staining by Brachet's method the basophilic parts of the cytoplasm, the inclusions, and the nucleolus were stained with pyronine a bright crimson color and tinged with violet.

Cerebral hemispheres. Besides hydropic degeneration of the cytoplasm of the large pyramidal cells, strongly basophilic intranuclear inclusions were also found in them, some being round, others triangular, polygonal, or trident-shaped. Where the inclusions lay close to the nuclear membrane, they projected through it into the cytoplasm. Similar inclusions were also found in neurons of the mesencephalon and in the Purkinje cells of the cerebellum. The cytoplasm of some degenerating neurons of the mesencephalon also contained relatively large, round basophilic structures. The Nissl's substance of these neurons had disappeared, the nuclei could not be seen, and the edges of the cells were irregular and ragged. These structures, like the nuclei of the modified cells, were strongly pyroninophilic when stained by Brachet's method, indicating that they contained a high concentration of RNP. In all parts of the brain the number of gliocytes was increased, and their nuclei contained strongly basophilic punctate inclusions, rich in RNP, and also the characteristic figures of neuronophagy. Schiff's reagent did not stain the inclusions, demonstrating the absence of DNP in them and confirming their nonnuclear origin.

Neurons in various parts of the brain of hens receiving hygromycin B in a dose of 100 000 units/kg or more revealed increased pyroninophilia of the nucleus and tigroid of the cytoplasm and also contained pyroninophilic intranuclear inclusions which were Feulgen-negative. The high basophilia of the tigroid and the pyroninophilia of the cell components demonstrated their high content of RNP [4, 8, 9, 12, 14]. Physiological and chemical stimulation, as well as administration of certain antibiotics, can produce changes in the RNA concentration in neurons [1, 11, 12]. The nucleolus is the most important, if not the only site of RNA synthesis [6], and most of the cell RNA is nuclear in origin and is transported into the cytoplasm [2, 3, 10, 15].

When hygromycin B enters the body it is evidently incorporated into metabolism and stimulates RNA synthesis in the nucleus of the neurons. As a result the RNP concentration is increased, its movement into the cytoplasm in disturbed, and an excess remains in the nucleus of the neurons, as confirmed by the intense pyroninophilia of the nucleolus and of the inclusions.

The inclusions were Feulgen-negative, demonstrating that they contain no DNP, thus confirming data in the literature describing the absence of DNP in chemotoxic cell inclusions [13].

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