Histochemical Studies of Phosphatases in the Nervous System of Thiamine Deficient Pigeons. (18309)

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(Introduced by E. W. Dempsey.)

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Thiamine deficiency is known to produce definite pathological alterations in the nervous system: capillary changes predominantly around the ventricular system(1), degeneration of the primary neurons of the proprioceptive nervous system and, finally, of the efferent nervous system(2) and corpus striatum(3). Since the changes in phosphatases in the nervous tissue in thiamine deficiency have not hitherto been investigated, it seemed desirable to do so.

Methods. The group of 14 pigeons used in this experiment was divided as follows: 3 served as normal controls, while 11 were fed on a thiamine deficient diet, consisting of cleaned rice thoroughly washed, to which 0.2 cc cod liver oil was added every 2 days. Some of the pigeons were killed after 14 days, but others were given the deficient diet for 25 to 29 days until typical opisthotonus appeared. Several of the latter were given daily injections of thiamine (15 to 45 gamma) for 3 days and 7 days. All of the pigeons were decapitated. Spinal ganglia and blocks of tissue obtained from the medulla oblongata, cerebellum and spinal cord were fixed in chilled acetone. Both alkaline and acid phosphatases were demonstrated histochemically by the Gomori-Takamatsu method as modified slightly by Shimizu and Arizono(4). Sodium glycerophosphate was used as substrate. The sections were incubated at pH 9.2 for 3 hours for alkaline phosphatase, and at pH 4.8 for 6 hours for acid phosphatase.

Results. Alkaline phosphatase. In the central nervous system of normal healthy pigeons alkaline phosphatase is distributed in a pattern similar to that seen in mammals, except that only traces of the enzyme occur in the walls of the blood vessels. In general, the grey matter presents a stronger reaction than the white matter. The neuropil of the grey substance reacts much more pronouncedly than the nerve cells (Fig. 1). Moreover, the enzyme content of the cerebral substance near the surface of the brain and cerebral ventricles is greater than that of the internal structures, as already reported by Shimizu(5).

Thiamine deficiency in pigeons causes characteristic changes in the distribution of alkaline phosphatase, which are most evident in the ventral horns of the spinal cord (Fig. 2). There is an apparent increase in the amount of enzyme in the ventral horn cells, accompanied by moderate shrinkage of the cell bodies and no change, or only a slight increase, in the reaction of the neuropil. As a consequence, the contrast between nerve cells and neuropil observed in the normal brain disappears. Following injection of thiamine, however, the reaction of the nerve cells decreases, whereas the reaction of the neuropil remains unchanged (Fig. 3), resulting in a nearly complete restoration of the normal condition.

In normal pigeons the grey matter in the floor of the fourth ventricle and outer surface of the brain reacts intensely, while the internal structures, as, for example, the formatio reticularis, exhibit only slight activity. In thiamine deficiency of maximal duration there is a general increase in enzymatic activity involving both neuropil and nerve cells, a change which is especially evident in the formatio reticularis. Following administration of thiamine, restoration to normal takes place in the same manner as in the spinal cord.

The cerebellum of thiamine deficient pigeons

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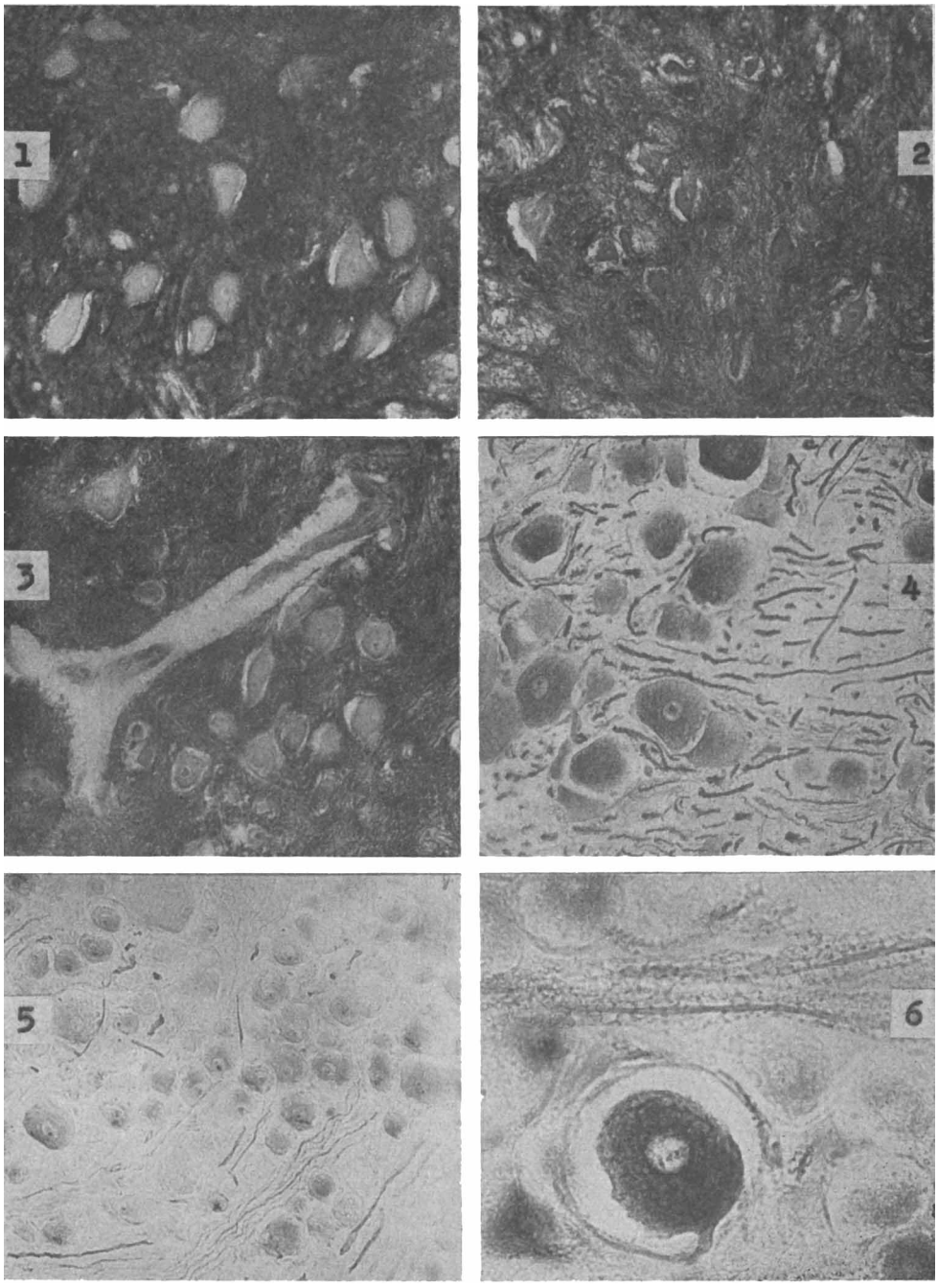

All of the figures illustrate the histochemical distribution of phosphatases in the nervous tissues of the pigeon. The blocks of tissue were fixed in chilled acetone. The sections were incubated in sodium glycerophosphate at pH 9.2 for 3 hours for alkaline phosphatase, and at pH 4.8 for 6 hours for acid phosphatase.

FIG. 1. Alkaline phosphatase reaction in the ventral horn of the spinal cord of a normal pigeon. Observe the marked reaction of the neuropil and the faint reaction of the nerve cells. × 130.

FIG. 2. Alkaline phosphatase reaction in the ventral horn of a pigeon fed on a thiamine

deficient diet for 29 days. Compare with Fig. 1. The shrunken ventral horn cells show a markedly increased phosphatase reaction, whereas the intensity of staining of the neuropil has not changed. × 130.

FIG. 3. Alkaline phosphatase reaction of ventral horn of a pigeon fed on a thiamine deficient diet for 29 days followed by daily injections of thiamine for 7 days. The reaction in the nerve cells apparently has diminished, so that the cells have been nearly restored to normal, while the neuropil exhibits a slightly increased response. × 130.

FIG. 4. Acid phosphatase reaction of a spinal ganglion of a normal pigeon. The nerve cells and axis cylinders show moderate activity. The pattern of the neurokeratin in the myelin sheaths is only faintly visible in few places. × 200.

FIG. 5. Acid phosphatase reaction of spinal ganglion of pigeon fed on thiamine deficient diet for 29 days. A marked decline in phosphatase has occurred in both nerve cells and axis cylinders. × 200.

FIG. 6. Acid phosphatase reaction of spinal ganglion following daily injection of thiamine for 7 days to thiamine deficient pigeon. The nerve cells and axis cylinders have reacquired acid phosphatase. Note the appearance of a neurokeratin-like network around the axis cylinders. × 500.

also presents a general increase in activity involving a pronounced reaction of the shrunken Purkinje cells.

In the spinal ganglia the ganglion cells exhibit a moderate increase in the amount of enzyme, while the capsular cells, in most instances, show a decrease.

Acid phosphatase. Moderate amounts of acid phosphatase are demonstrable uniformly in both axis cylinders and nerve cells in healthy pigeons (Fig. 4). Although the neurofibrils of nerve fibers contain acid phosphatase, as demonstrated by Wolf, Kabat and Newman(6), the neurofibrils of the nerve cell bodies are not always positive; instead, nerve cells may contain coarser networks resembling a Golgi apparatus. A network resembling the pattern of neurokeratin is occasionally barely perceptible in the myelin sheaths surrounding some of the nerve fibers.

In thiamine deficient pigeons a decrease in acid phosphatase takes place, more apparent in the axis cylinders than in the nerve cells (Fig. 5). This characteristic change is evident in all regions examined. Following administration of thiamine, the enzymatic activity of the nerve fibers is restored to a moderate degree, while that of the nerve cells is variably restored, being slight in some cells and very pronounced in others.

After injection of thiamine, it is observed that the network in the myelin sheaths, resembling that of neurokeratin, sometimes becomes more distinct than normal, as, for example, in the nerve fibers of the spinal cord (Fig. 6).

The Purkinje cells of the cerebellum normally show a moderate cytoplasmic reaction and a faint nuclear one. However, in thiamine deficiency the reaction of the cytoplasm is diminished, whereas that of the nuclei is increased. These changes are also observed, although less markedly so, in other types of neurones.

Comment. In the mammalian central nervous system alkaline phosphatase occurs mainly in the walls of the blood vessels at the site of the blood brain barrier(7). In pigeons there is much less present in the blood vessel walls, the major reaction being in the neuropil. The reaction in the nerve cells of normal pigeons is relatively slight, but in thiamine deficiency the amount of enzyme in these cells becomes greatly increased, so that the contrast with the surrounding neuropil diminishes.

Acid phosphatase of nerve tissue is apparently reduced by thiamine deficiency, a result related possibly to the observation of Hard and Lassek(8) that this enzyme disappears from axis cylinders after removal of their cells of origin. Following thiamine administration to thiamine deficient pigeons, the acid phosphatase reaction sometimes brings out a network in the myelin sheaths resembling the pattern of neurokeratin. This is of interest since acid phosphatase is assumed by some(8) to participate in the

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maintenance of the myelin sheaths.

Summary. Nervous tissues of thiamine deficient and control pigeons were examined histochemically in respect to both alkaline and acid phosphatases. In this deficiency the nerve cells shrink and acquire increased amounts of alkaline phosphatase, whereas the neuropil shows only a slightly increased reaction. Acid phosphatase of the axis cylinders and cytoplasm of the nerve cells is diminished in amount. After administration of thiamine to the deficient pigeons, a considerable return to normal is observed. Accompanying restoration of the acid phosphatase a network, similar to the pattern of "neurokeratin", becomes evident in the myelin sheaths.

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Failure to Demonstrate Antibody in Feces of Monkeys Vaccinated with Poliomyelitis Virus, Lansing Strain.*† (18310)

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There is evidence that antibodies for various infectious agents occur in certain of the secretions and excretions of humans and animals. For example, antibodies have been found for influenza virus(1-3) and the Lansing strain of poliomyelitis virus(4-6) in nasal secretions. In feces, antibodies have been reported for cholera(7,8), several enteric bacilli(9-11) and possibly for one strain of the Lansing type of poliomyelitis virus(12).

The present study was made in order to determine whether antibodies occurred in the feces of monkeys during and after vaccination with the Lansing strain of poliomyelitis virus. Monkeys were chosen for this study since it has been shown that high antibody titers may be obtained in their sera by vaccination(13,14).

Materials and Methods. Eight young Macaca mulatta monkeys were used. The monkeys were examined daily, and daily rectal temperatures were recorded. For titration of the virus-neutralizing capacity of stool suspensions and serum specimens, white Swiss mice 3-4 weeks of age and 12-16 g in weight were used; these were examined daily for a month. The day of onset of paralysis, the character of the paralysis and the day of death were recorded. At the end of one

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