PATHOANATOMICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM AND IN THE INTERNAL ORGANS OF WHITE RATS WHEN VARYING DOSES OF BARBAMYL ARE USED

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Morphological studies of the central nervous system and the internal organs of animals either sacrificed or dying during the use of various doses of barbamyl must be of great interest not only for theroretical, but also for practical medicine, as revealing after the use of this preparation in humans and animals the observed harmful disturbances of function.

In the literature devoted to the study of hypnotics in psychiatrically ill patients [1, 2, 4, 6] and in hypertensive and malignant diseases [3, 7, 9, 10], there are indications that therapeutic sleep, specifically barbamyl-induced, not infrequently produces intoxications manifested by collapse, clouded sensorium, hallucinations, delirium, speech disturbances and difficulties in swallowing, digestion, urination, etc.

The wide use of barbiturates in the clinic for the production of therapeutic sleep stimulated observation of their effects under infectious-toxic conditions and during experiments.

On the basis of numerous experimental observations of V. A. Kozlov [5] and others it was determined that barbiturates differently affect the course of the same infection and the defense mechanisms of animal organisms.

The pathological morphology of the changes of the central nervous system and the internal organs in the course of drug-induced sleep have been studied very little. In the literature there are described only two cases of the death of psychiatrically ill patients from barbamyl intoxication; the histologic changes such as engorgement of the soft brain coverings, hemorrhagic foci in the brain, vacuolization of the oligodendroglia and cytolysis of brain cells [2] and Purkinje cells [8, 9] are discussed.

The absence of clear histological data on the changes occurring in those dying of barbamyl intoxication, and also the difficulty in excluding in these cases the influence of the basic disease upon the the central nervous system, led us to study the dynamics of the structural changes produced in the brain and internal organs of white rats subjected to various doses of barbamyl.

We attempted also to elucidate the similarities and differences in distinguishing the morphological changes in the various organs produced by hypnotic doses and to study the dynamics and consequences of the pathologic process in relation to the size of the dose and the length of time barbamyl was used.

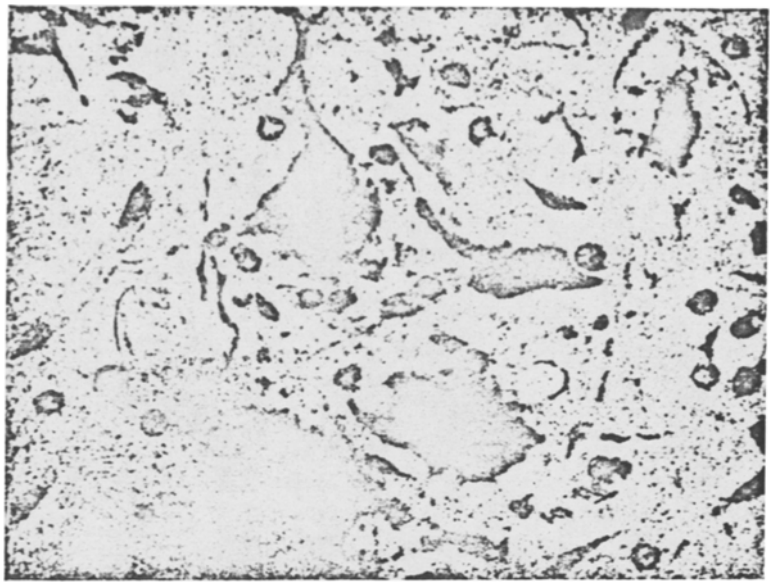

Fig. 1. Brain stem. Markedly expressed vacuolization seen in pale, as well as in darkly stained cells. Odd formations of the latter can be seen. Rat was killed on the 6th day of the experiment (2 g barbamyl per 100 g wt. of rat). Nissl stain.

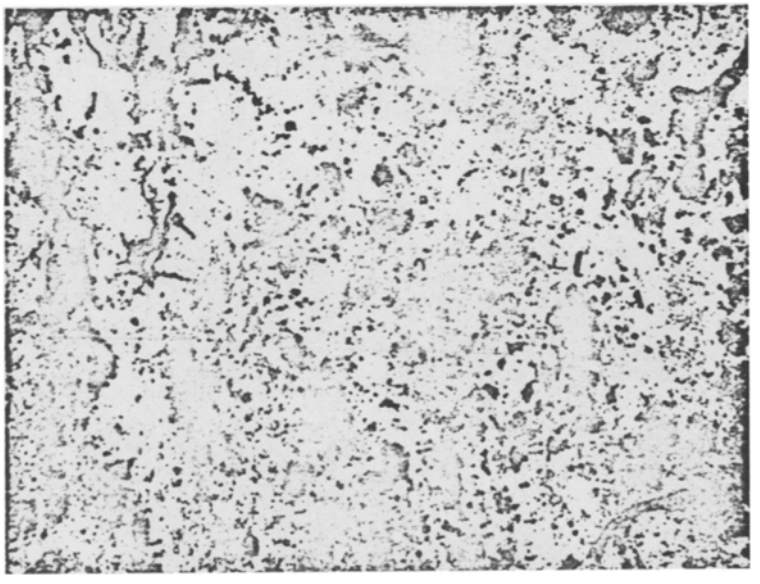

Fig. 2. Brain stem. Wrinkling of astrocytes, shortening of branches, curling of latter. Focal destruction of astrocytes. Rat killed on the 6th day of the experiment (2 g barbamyl per 100 g wt. of rat). Cajal stain.

Fig. 3. Brain stem. XII cranial nucleus. Wrinkling and atrophy of nerve cells. Rat sacrificed one month after the experiment (1.5 g barbamyl per 400 g wt. of rat). Nissl stain.

EXPERIMENTAL METHODS

We studied 38 rats weighing 150-200 g; three of them served as controls.

Barbamyl was introduced with a pipet perorally in a warm water solution. The animals were sacrificed by beheading.

We used as a base the dose established by L. E. Khozak for rats of 0.1 g of barbamyl per 100 g animal weight. This dosage in rats causes a rapid onset of natural sleep, during the course of which conditioned and unconditioned reflexes are preserved.

Such a dose was given 14 rats once daily for 5-10 and 18 days; 7 animals were sacrificed on the day following the last introduction of barbamyl, i. e., on the 6-11th and the 19th days of the experiment, and the remaining 7 on the 6th day after ceasing to administer the hypnotic mixture, i. e., on the 11-16th and 24th day of the experiment.

To determine the lethal dose of barbamyl, 19 rats were given 0.4, 0.8, 1, 1.5, and 2 g per 100 g weight. However, as clinical observations have already shown, in spite of the heavy nature of the sleep (narcotic sleep, bordering on the comatose), not one of the above indicated doses of barbamyl caused an animal's death.

The cited doses of barbamyl were given each animal just once; 10 rats were sacrificed on the 6th day after the experiment, 4 rats – after a month (with the aim of studying remote results); 5 animals were kept for study of their reflex activities.

A bit of brain and a bit of the internal organs of the sacrificed animals were fixed in a 10% formalin and in absolute alcohol. Stains were made with hematoxylin-eosin, and special ones – for fat and glycogen – with the preparations of Nissl, Cajal, Beletskov, Alexandrov, Spielmeyer, and Snesarev.

EXPERIMENTAL RESULTS

In rats receiving 0.1 g barbamyl per 100 g weight there were no macroscopic changes.

*A dose of barbamyl of 0.1 g per 100 g rat weight we arbitrarily designated as "therapeutic". Doses of 0.4 g and higher – as "tolerated-toxic" inasmuch as these, on the one hand were not lethal, and on the other hand – in distinction from the therapeutic dose – caused a profound sleep with appearance of cyanosis, twitchings, salivation, etc.

No histological changes were observed in the brain and internal organs of rats receiving therapeutic doses of barbamyl for 5-10 days. In animals receiving the same dose for 18 days and killed on the 19th day of the experiment, there was observed albuminous dystrophy of the liver, kidneys, adrenals, and myocardium. In rats sacrificed on the 6th day after stopping this hypnotic dose, i. e., on the 24th day of the experiment, these changes were either completely or almost gone, 6 days apparently being enough to restore slightly damaged organs.

As for the nervous system, hematoxylin-eosin and Nissl stains of rats which had received barbamyl for 18 days in 0.1 g doses revealedoccasional swelling of the cells in pyramidal layers III and V of the frontal and parietal lobes. The protoplasm of the cells was clouded, as if dusted. This appearance of the cell is designated by P. E. Snesarev as "cloudy swelling". In the optic lobe, variolar bridge, and stem of cell the edematous state is seen somewhat more frequently. It can still be seen on the 24th day of the experiment.

Passing to a description of the changes in the inner organs of the rats which had received tolerated-toxic doses of barbamyl (0.4-2 g per 100 g weight), it should be noted that on opening these animals on the 6th day of the experiment after a single indicated dose of barbamyl, hemorrhages are seen in the brain and its coverings and in the liver, the appearance of dystrophy.

Histologically there are noted marked vascular engorgement of the coverings and brain tissue itself, dilated veins and capillaries, and hemorrhages, especially in the subcortical stem regions. Nissl stain shows a considerable number of dark-stained cells in layers II-III and V of the cortex with hyperchromic nuclei, among which are seen poorly staining cells, and also cells having undergone dystrophy, properly termed "basophilic"

The basic peculiarity of these cells is the intensive basophilia of the protoplasm and nucleus. In numerous preparations there can be observed the destruction of protoplasm and the dissolved basophilic substances. In the poorly stained cells the tigroid substance and the nucleus are poorly differentiated, the protoplasm being frequently vacuolated. The vacuolization of individual cells and axons is so great that the cell frequently becomes separated from its branches.

In the visual lobe and variolar mount the pallor of the cells and this vacuolization is particularly marked.

In the brain stem, the medulla, and the cervical segments of the spinal cord this vacuolization of the cells is especially sharply marked and can be seen even in the darkly stained cells. These latter acquire strange shapes (Fig. 1). In individual cells there can be seen a fine granulation, which upon death of the cells seems to be lying free. Sometimes around the heavily damaged cells there appears neuronophagocytosis. However, in the zone of dying cells proliferation of satellites is not observed.

It must also be noted that there is marked diminution of pathological changes as we go caudally down the spinal cord.

The Purkinje cells undergo basically the same changes, but the vacuolization of their protoplasm is infrequent and mild.

In brain preparations stained by the Bielschowsky method the heavily damaged cells (mainly of the stem) show edema, agglutination, and falling apart and dissolution of the neurofibrils.

The oligodendroglia – the dense as well as the drainage cells, stained by the method of Aleksandrov – are characterized by edema and vacuolization, and sometimes by an increasing number of branches in the drainage cells.

The astrocytes of the glia, stained accordingly to Cajal, exhibit the so-called "ameboid disease", manifested by the uneven edema of the body of the astrocytes and their branches. Among them can be seen wrinkled astrocytes with shortened and curved branches; separate groups undergo destruction (Fig. 2).

In preparations impregnated by the method of Beletsky there appears a proliferation of macroglial cells, sometimes with the formation of neurophagic knots (see above).

Four rats, sacrificed a month after a single dose of tolerated-toxic barbamyl (0.4, 0.8, 1.5, and 2 g per 100 g wt. of rat), showed in cerebral hemispheres in the subcortical nuclei, brain stem and cerebellar nuclei a marked wrinkling of the nerve cells. The damage was especially heavy near the aqueduct of Sylvius and also in the nuclei of the VII, X and XII cranial nerves. The wrinkled cells and their branches acquired a variable

form (Fig. 3). On the backgrounds of these described structures can be seen tigrolysis, vacuolization, lysis, falling apart, and the dropping out of cells and also "ghost-cells". In the surviving cells the protoplasm is thick, and the tigroid substance and the nucleus are readily distinguished.

In preparations stained with hematoxylin-eosin in this period, there was noted a severe edema of the myelin sheaths, especially frequent near the aqueduct of Sylvius. The swollen sheaths acquired a bright basophilic coloration. The myelin substance swelled so much that in it a fine basophilic granulation was apparent. When stained by the method of Spielmeyer in those regions could be seen foci of demyelinization.

In brain preparations stained by the method of Snesarev the reticular sheaths do not show marked changes. There is only noted some edema of these sheaths in separate vessels and a definite diapedesis of erythrocytes.[1]

There were almost no changes in the peripheral nervous system.

Microscopic examination of the myocardium of rats which had received tolerated-toxic doses of barbamyl and had been killed on the 6th day of the experiment, revealed a marked engorgement of the veins and capillaries and hemorrhages into the stroma; in the muscles – albuminous dystrophy and necrosis. Among animals sacrificed after a month there was noted proliferation in clumps of histiocytes.

The dystrophic changes in the liver, kidneys, and adrenals of rats sacrificed on the 6th day of the experiment are the symptoms of albuminous dystrophy combined with vacuolization and necrosis. In animals sacrificed a month after the indicated doses of the hypnotic, the changes in the cells of these organs were somewhat less pronounced and merely were manifestations associated with proliferative changes.

As can be seen from the presented material, the expression of pathological changes in the central nervous system and in the internal organs of the animals depends on the dosages of the somniferous drug; the higher the dose of barbamyl, the more severe is the anatomical picture of the experiment.

Therapeutic doses of barbamyl produce only reversible changes in the brain and internal organs. With tolerated-toxic doses there are severe irreversible dystrophic changes in the cortex of the hemispheres, the subcortical regions, and the brain stem.

The therapeutic dose of barbamyl even in relatively insensitive creatures, such as the rat, and with the use of a single daily dose, after a definite time interval (in our experiments after 18 days) is capable of producing dystrophic changes in the brain and internal organs; this should be taken into consideration when using this preparation clinically.

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