EFFECT OF MEDICATED SLEEP, PRODUCED BY BARABAMYL, ON THE COURSE OF EXPERIMENTAL DIPHTHERIA INTOXICATION

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Our preliminary observations showed that the subcutaneous administration of 1 MLD of diphtheria toxin to guinea pigs and of a double dose (2 MLD guinea pig) to rabbits causes lassitude, inhibition, limited mobility, and dyspnea in the animals after a period of 1-3 hours. At the same time, the fur rises, the body temperature reaches 40-41° (normally it is 38-39°). Beginning the next day, their condition worsens, their temperature falls progressively and at 33-32° the guines pigs die in 2-4 days, the rabbits in 3-7 days.

The therapeutic doses of barbamyl we found before for rabbits and guinea pigs were used for the therapy of diphtheria intoxication by sleep in the experiment. 3-4 days after toxin administration, i.e., at the height of the evident intoxication, 12 rabbits and 8 guinea pigs were given barbamyl internally in a warm water solution (in a dose of 0.15 ml per 1 kg of weight for the rabbits, of 0.1 ml per 1 kg of weight for the guinea pigs). From the next day, the soporific was administered twice a day by the interrupted medicated sleep method for 5-6 days.

We did not use a comparatively short period of time for the sleep therapy of diphtheria intoxication in the animals accidentally, but reached this decision on the basis of data from a preliminary study of the effect of barbamyl on the central nervous system and internal organs of healthy animals (rats, rabbits and guinea pigs). It was established by investigation that the prolonged administration of therapeutic doses of barbamyl leads to the development of profound changes in the animals' organs. These changes basically amount to an insufficient supply of blood to the tissues and to protein dystrophy of the parenchymatous organs [7]. Taking the above into account, in this experiment the duration of administering the soporific was set at 5-6 days. At the end of this time, regardless of the condition of the experimental animal, barbamyl administration was stopped.

As the first control, 12 rabbits and 4 guinea pigs were used which were administered the same doses of toxin subcutaneously, but without subsequent barbamyl administration.

Ten rabbits and 5 guinea pigs, which received barbamyl, as did the experimental animals, twice a day for 5-6 days, but without toxin administration, served as the second control. These animals were later decapitated at the same time that the experimental animals died or were killed.

Observations of the condition and behaviour of the experimental and control animals, their body temperature and survival served as the basic tests for judging the effect of the diphtheria toxin and the effectiveness of barbamyl sleep on the course of diphtheria intoxication. After the death or sacrifice of the animals, a detailed histological investigation of the brain and internal organs was carried out. The pieces of brain or internal organs taken from the animals were fixed in 10% formalin solution, and absolute alcohol, and were imbedded in celloidin. Ordinary

and special stains were used for the sections.

From observation of the animals it was established that the animals which were given only barbamyl (second control), behaved like the healthy ones after waking. Among animals in the first control group, which were poisoned by diphtheria toxin but without the administration of barbamyl, the phenomena of marked intoxication and prostration were observed, followed by death. The condition of the experimental animals was serious for 2-3 days and did not differ from the condition of the animals in the first control group. With the 3-4th day, the course of the diphtheritic process in the experimental animals began to differ, so we distinguished three subgroups, depending on the seriousness of the illness. In subgroup A (3 rabbits and 4 guinea pigs), the condition of the animals progressively grew worse and they died in the same length of time as did animals in the first control group, i.e., on the 3-8th day of the experiment.

A gradual improvement of the general condition, becoming especially noticeable on the 6-7th day of the experiment, but replaced by an unexpected deterioration on the 9-10th day of the experiment, was found among the animals in subgroup B (4 rabbits and 2 guinea pigs). Auscultation through a phonendoscope revealed a muffling of the heart sounds, indicating a picture of developing myocarditis. Barbamyl was again administered to three rabbits in this subgroup. However, its repeated administration did not produce improvement and all 6 animals died on the 12-15th day of the experiment.

In subgroup C (5 rabbits and 2 guinea pigs), from the 3-4th day of the experiment, a gradual improvement and then recovery of the animals was observed. In order to study the effect of barbamyl on the course of diphtheria intoxication, these animals were killed 25-40 days after toxin administration.

Autopsy of the animals in the first control group with diphtheria intoxication without barbamyl administration, revealed hyperemia of the brain and internal organs, especially of the adrenals. Areas of emphysema and hemorrhages were found in the lungs. The skin at the place the toxin was injected was hyperemic, with areas of necrosis. On microscopic investigation of the brain, hyperemia, statis, hemorrhages into the white matter of the hemispheres, the subcortical area, stem and cervical segments of the spinal column were noted. In the nerve cells were observed paling of the cellular bodies, sometimes vacuolization. In the liver were hyperemia of the central veins and hepatic sinusoids, edema of the stroma, disintegration of the liver cells (Fig. 1) with small fatty drops and necrosis in them. In the kidneys were marked hyperemia of the cortical and medullary substance, stasis, thrombosis of the glomerular capillaries (Fig. 2), fibroid necrosis of the walls of individual loops and glomeruli. The endothelium of the capillaries was swollen, sometimes in a state of fatty degeneration. More or less distinct fatty dystrophy in the epithelium of the convoluted tubules. Hyperemia, as a rule, combined with fatty dystrophy in the myocardium. In the adrenals were hyperemia of both layers, edema and hemorrhage into the medullary substance and decreased secretory granulation in the cells were in the adrenals. In the cells of the cortical layer was a considerable decrease of lipoids, sometimes necrosis. In the lungs were areas of interstitial pneumonia, emphysema, atelectasis, hyperemia, hemorrhages. The follicles of the lumph nodes, spleen, tonsils and appendix - karyorrhexis. At the place of toxin injection - edema, hemorrhages, inflammatory infiltrations, necrosis of the skin and muscles.

Animals in the second control group, which received barbamyl without toxin administration, were killed at the time the experimental rabbits and guinea pigs died or were killed: after 3-8, 12-15 and 25-40 days. Histological examination of brain preparations of the animals revealed a loss of blood in the veins and capillaries. At the same time, the number of homogeneous cells in the cerebral cortex, stem, medulla oblongata and upper portions of the spinal column somewhat exceeded their usual number. In the weakly stained cells, dispersion of the tigroid was sometimes combined with vacuolization of the protoplasm. In the liver, kidneys, adrenals and myocardium emptying of the blood vessels was also observed, as a consequence of which the renal glomeruli sometimes were in an ischemic condition. Changes in the internal organs were combined with phenomena of protein dystrophy. However, it should be observed that the changes described in the brain and internal organs were chiefly observed in those animals which received barbamyl for 5-6 days, and the most evident changes were found in animals killed when the experimental animals died, in 12-15 days. Changes in the organs of rabbits and guinea pigs which were killed at the time of the early death of the experimental animals (from 3-5 days) were insigificant. Changes were not found in the organs of animals which received barbamyl for 5-6 days but were killed in 25-40 days. The investigations indicate that the changes which appear in the organs of animals (in this experiment of the second control group) with the short-term administration of barbamyl have a reversible nature. These observations are confirmed by the data of previous investigations of the effect of barbamyl on the central nervous system and internal organs

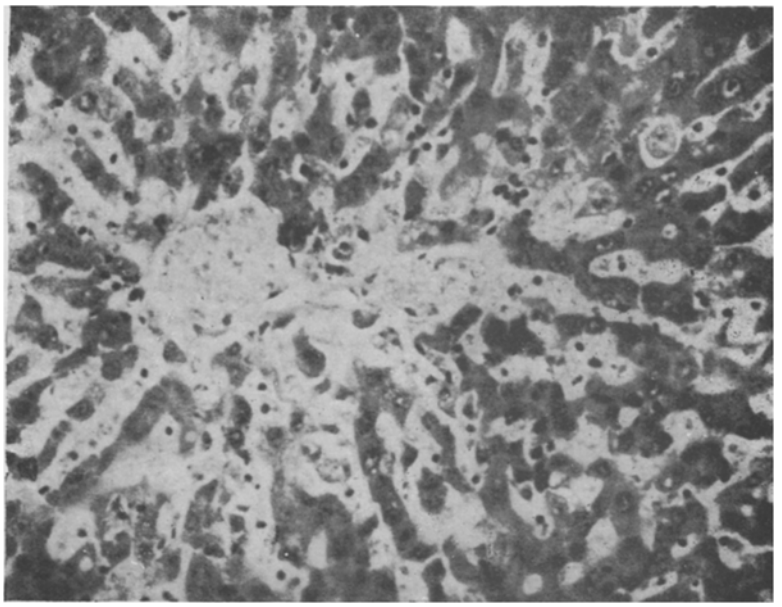

Fig. 1. Changes in the liver of a guinea pig which died on the 3rd day after diphtheria toxin administration. Hyperemia of the central veins and hepatic sinusoids. Edema of the stroma. Disintegration of the hepatic cells.

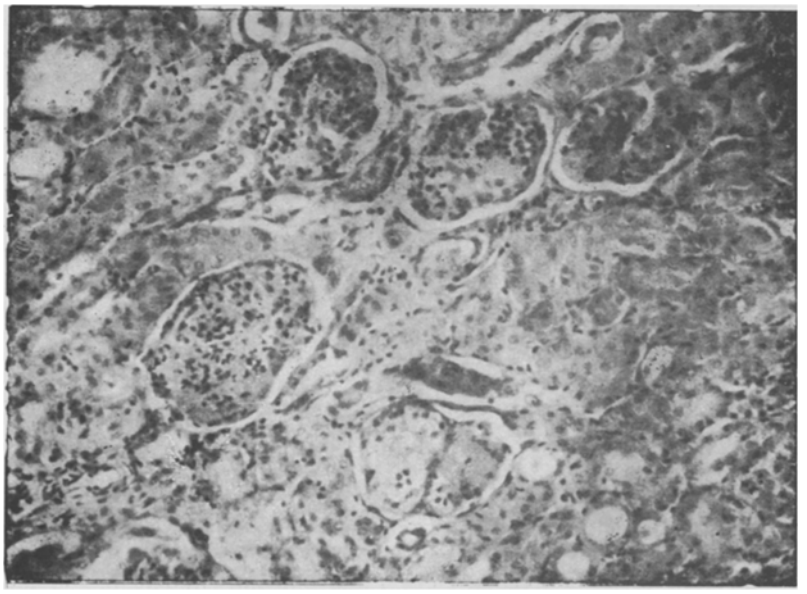

Fig. 2. Changes in the kidney of a rabbit which died on the 5th day after diphtheria toxin administration. Stasis and thrombosis of the glomerular capillaries.

of healthy animals: the length of time free of barbamyl administration (25-30 days) after its short-term administration (5-6 days) is sufficient to normalize the described changes [7].

At autopsy of the experimental animals in subgroup A (death on the 3-8th day), the changes in the organs were completely analagous to the changes which were found in the animals of the first control group (with diphtheria intoxication without the administration of barbamyl). However, on histological investigation it was noted that the disturbance of the blood circulation in the brain and internal organs of these animals had an acute nature (hyperemia, hemorrhages), while the phenomena in the animals of the first control group were prodominately

those of a slowed movement of the blood (stasis, thrombosis, plasmorrhagy). In the cortical cells of the hemispheres, in the stem of the medulla oblongata and in the spinal column of the animals in subgroup A there was a noticeable increase in the number of homogeneous cells, together with the changes typical of intoxication (pallor, tigrolysis).

Study of preparations from the parenchymatous organs of experimental and control animals also established a difference in their morphological manifestitations.

While circulatory disturbances combined with phenomena of fatty dystrophy in animals with diphtheria intoxication without barbamyl administration, in the experimental animals protein dystrophy in the liver, kdineys and myocardium predominated over the fatty, so characteristic of diphtheria in man and animals.

On autopsy of rabbits and guinea pigs of subgroup B (which died in 12-15 days) signs of cardiac decompensation were grossly evident: an increase in the heart volume, edema of the serosas. On microscopic examination, the usual hyperemia of the blood vessels of the brain and internal organs was found. The changes in the nerve cells of the brain were the same as those in the animals of subgroup A. In preparations stained by Marchi's and Spielmeyer's methods, swelling of the myelin fibers, their fragmentation, and sometimes areas of demyelination in the myeloid layer of the cortex of the hemispheres, in the hippocampus, pons and medulla oblongata could be seen.

In the parenchymatous organs the changes were of one type and were in essence phenomena of protein dystrophy. The heart deserved the greatest attention during this period of death of the experimental animals. In it, in addition to dystrophic changes, was found a picture of evident productive myocarditis with necrosis and muscular breakdown, but without a vascular reaction (Fig. 3).

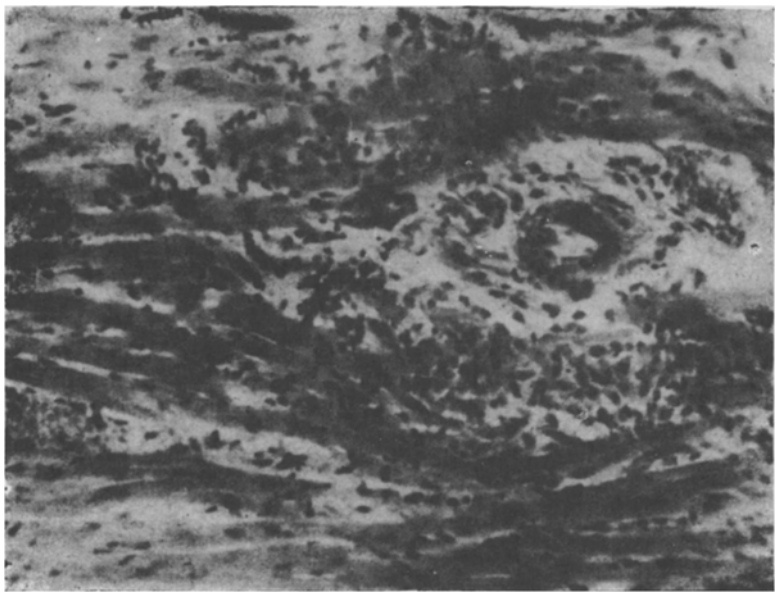

Fig. 3. Changes in the myocardium of a rabbit which died on the 15th day after diphtheria toxin administration. Productive myocarditis, necrosis of the muscles and their disintegration (experiment with administration of barbamyl).

Such a form of myocarditis is unusual in diphtheria, since the interstitial-parenchymatous form of myocarditis with clear manifestations of vascular reaction is typical of diphtheria in man [1, 3, 8, etc.] and animals [2, 4, etc.]. The absence of exudative inflammation from the picture of myocarditis in the experiment should, apparently, be connected with the application of barbamyl sleep. In the lungs, the picture of areas of interstitial pneumonia was observed; in the follicles of the lymph nodes, spleen, tonsils – hyperplasia of the reticular cells; in the skin, subcutaneous tissue, and muscles - leucocytic infiltration with widespread necrosis, often occupying a considerable portion of the body and abdomen.

Among animals in subgroup C, killed 25-40 days after the administration of toxin, on autopsy were found scars only at the place the toxin was administered and necrosis of the underlying tissues. On histological investigation of preparations of the brain and internal organs, a noticeable lessening of the above-described changes or their complete absence, was observed and only in the myocardium could residual signs of productive inflammation be seen after 40 days. Basically, proliferation of the cells of the parenchymatous organs was noted during the period.

Thus, observations showed that barbamyl, administered by the method of interrupted medicated sleep for a comparatively short period of time (5-6 days), has a favorable effect on the course of diphtheria intoxication in animals, since circulatory disturbances, typical of collapse, play a large role in the pathogenesis of this disease. Barbamyl is like an antagonist in this experiment since, as the present and preceding works [7] showed, the decrease of blood in the organs is typical of it; a decrease which occurs, apparently, either as a consequence of redistribution of the blood in the system or of its thickening because of dehydration; it is known that the water metabolism is sharply disturbed during medicated sleep. However, this view remains hypothetical as yet.

Further special study is required for the solution of this question.

SUMMARY

The beneficial effect of Barbamyl induced sleep on the development of diphtheria intoxication was shown in rabbits and guinea-pigs. Applying interrupted medicated sleep during 5-6 days we succeeded in an experiment to double the longevity of animals; in a number of cases a complete recovery of the animals was observed.

Microscopic analysis of the organs of the experimental animals showed that Barbamyl protects against drastic circulatory disturbances characteristic of diphtheria intoxication and decreases fatty degeneration in the parenchymatous organs.

Barbamyl induced sleep does not affect the development of pathological processes in the place of the diphtheria toxin injection.

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* In Russian.