MORPHOLOGICAL AND HISTOCHEMICAL FEATURES OF THE NEUROMUSCULAR SYNAPSIS IN GUINEA PIGS SUFFERING FROM DIPHTHERITIC POLYNEURITIS

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Diphtheritic polyneuritis, accompanied by pareses and paralyses is one of the severe complications of diphtheria.

The morphological substrate of diphtheritic paralyses has been for a long time the object of investigations, but the question is still unsolved. The firmly rooted conviction that diphtheritic paralyses [2,10,13] are caused by parenchymatous neuritis does not fully satisfy the clinicians, as it fails to explain the peculiar clinical picture of the motor disorders in question [1, 7, 8, 9, 15, 18].

Changes in the nerves can be observed in all cases of diphtheria; these changes, however, are not always accompanied by motor disorders [6]. Besides, it is hard to understand why the clinical manifestations of motor disorders appear only 25-35 days after the onset of the illness, whereas the typical picture of neuritis develops between the 10th and 14th day.

The existence of these unsolved questions, concerning the pathogenesis of diphtheritic paralyses, is possibly connected with the gaps in our knowledge regarding the state of the innervating apparatus, and in particular the neuromuscular synapsis, in cases of diphtheria.

On the basis of physiological and pharmacological studies it is, at present, generally accepted that the functional state of the nervous system is to a certain degree determined by the state of the synaptic apparatus.

All these facts show that it is impossible to study the pathogenesis of motor disorders without adequate investigation of the neuromuscular synapsis.

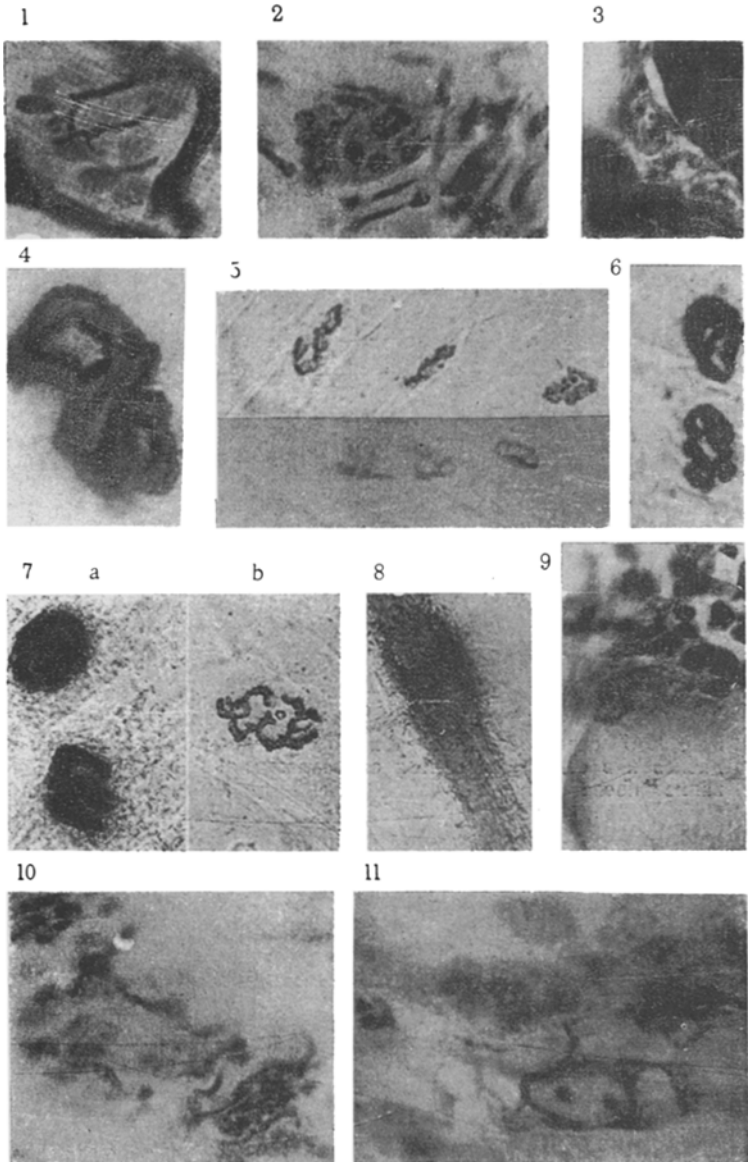
In the present paper we set ourselves the task of following up the state of the neuromuscular synapsis at various stages of diphtheritic intoxication in guinea pigs.

METHODS

Guinea pigs weighing between 250 and 300 g were given injections of diphtheria toxin in normal saline under the skin of the right foreleg, in doses which caused the animals' death on the 25th - 35th day. Between the 21st and 25th day paralyses and pareses of the right foreleg and of both hindlegs developed in the guinea pigs. In time, the paralysis spread further: respiratory disorders developed and the animals' voices became hoarse. The animals lost up to 1/3 of their original weight and perished under symptoms of marked adynamia.

Ninety-four guinea pigs were given injections of toxin; in 80 of them paralyses developed. In the remaining animals no respiratory disorders could be observed.

The control animals (38 guinea pigs) were given injections of normal saline.



Dynamics of changes in motor plate at different times during diphtheria intoxication in guinea pigs. 1) Control. No changes in neural apparatus; 2) control. Protoplasm of underside homogeneous; 3) control. Reaction to proteins very mild; 4) control. Appearance of specific cholinesterase; most intense hydrolysis in the outer portions of the subneural apparatus; 5) 3rd day of intoxication. Decrease in activity of non-specific cholinesterase in motor plates. Top – control, bottom – experiment; 6) 3rd day of intoxication. Increase in specific cholinesterase activity with diffuse distribution in subneural apparatus; 7) 32nd day of intoxication. High specific cholinesterase activity, outlines of subneural apparatus blurred, appearance of enzyme in muscle fiber (taken with a comparative microscope); a) experiment; b) control; 8) 35th day of intoxication. Appearance of specific cholinesterase in parts of muscle fiber adjoining plate; 9) 35th day of intoxication. Considerable proliferation of plate's nuclei; 10) 32nd day of intoxication. Motor plate, nuclei dead and protoplasm of underside destroyed; 11) 34th day of intoxication. Brashe's reaction, pyroninophilia of protoplasm of plate's underside, formation of two nucleoli in nucleus.

The crural muscles of the hindlegs and the intercostal muscles were investigated at different periods after the injection of toxin: on the 3-7th, 14-21st day and later, in step with the progression of the intoxication and the paralysis, until the 45th day.

Tissue sections fixed in formalin, in Carnoy's liquid, and also freshly frozen unfixed cryostat sections were investigated.

RNA was demonstrated by the method of Brachet, DNA by the method of Feulgen. Proteins containing the amino-acids: thyrosine, histidine and tryptophan were demonstrated by the method of Danielli and sulfhydryl groups of protein nature with p-nitrobromoacetophenone by the method of Yakovlev-Nistratova; specific and unspecific cholinesterase by the method of Koelle. Neurofibrillary structures were demonstrated by the method of Bielschowsky-Gross. Hematoxylin-eosin, picrofuchsin and thionin were used as general stains.

RESULTS

In the animals of the control group the terminal ramifications of the motor nerves showed a satisfactory degree of impregnation. The zone of the terminal ramifications contained elongated Schwann nuclei, rich in chromatin; the latter was densely packed and gave an intensive staining reaction for DNA. In the deeper layers the nuclei were of more spheroid shape and the chromatin was distributed more evenly in the form of fine lumps which gave a weak DNA reaction. The number of nuclei in the end-plates varied between 6 and 10 (See Fig. 1, and 2).

The protoplasm of the motor end-plates is of homogeneous or finely granular structure, stains weakly with thionin, contains RNA and is difficult to discriminate from sarcoplasm, as it has an equal degree of pyroninophilia. The sarcoplasm of the muscle fibers gives a strongly positive reaction for protein and for the sulfhydryl groups of proteins. This reaction is much weaker in the protoplasm of the motor end-plate and in sections looked at from the side, the end-plate appears as a light hillock with darker stained nuclei (See Fig. 3).

A high specific cholinesterase activity was found in the cytoplasm of the motor end-plates; it was mainly localized in the external layers of the end-plate, which in the sections seemed to be well-defined (See Fig. 4).

On the third day after the injection of the toxin an increase in the specific cholinesterase activity could be clearly seen; this became manifest in a more intensive reaction, producing greater quantities of precipitate; but no changes in the distribution of the precipitate could be observed within the sole of the end-plate (See Fig. 6). The unspecific cholinesterase activity, conversely, decreased (See Fig. 7).

At this stage the usual histological methods failed to reveal any differences compared to the control sections.

In the subsequent period until the 14th day, the specific cholinesterase activity remained on a high level, whereas the unspecific cholinesterase activity gradually decreased during that time.

On the 14th day the number of nuclei in the motor end-plates showed a marked increase and reached in a number of cases 15-20 (See Fig. 9). The end-plates increased in size. The pyroninophilic character of the cytoplasm in the sole of the end-plate increased. This stain disappeared after treatment of the sections with ribonuclease. Many nuclei within the end-plates contained several nucleoli, rich in RNA (See fig. 11). The structure of the cytoplasm in the sole of the end-plate remained homogeneous or finely granular. The tetrazonium reaction and the reaction for sulfhydryl groups was of the same intensity as in the control sections. The terminal nerve fibers appeared to be strongly argyrophilic and coarser than usual.

On the 21st day after the injection of the toxin the specific cholinesterase activity still persisted on a higher level than in the control animals; but the distribution of the enzyme within the motor end-plate showed certain changes: the higher activity in the peripheral parts was now replaced by a more diffuse distribution in all parts of the end-plate and in consequence the outlines of the end-plate became less distinct (See Fig. 7a); besides, the enzyme could be demonstrated in parts of the muscle fibers, adjacent to the synapsis, where, formerly, no enzyme had been found (See Fig. 8).

At this stage, only traces of unspecific cholinesterase activity could be found in the zone of the neuromuscular synapsis.

In addition to the proliferation of the nuclei within the end-plates destruction of the nuclei could be observed. Nuclear detritus could be seen in the zone of the motor end-plates. The cytoplasm of the end-plates was of strongly pyroninophilic character and contained coarse lumps of a basophilic substance (See Fig. 10), which substance disap-

peared after treatment with ribonuclease. The more deeply situated nuclei in the end-plates, which contained less chromatin and gave a weaker DNA reaction, were swollen and had indistinct outlines; their nucleoli, however, preserved their pyroninophilic character.

Between the 21st and the 45th day the degenerative processes in the motor end-plates became more intensive, keeping in step with the progression of the paralyses. The specific cholinesterase activity decreased in some parts of the end-plates in almost all synapses. This was apparently caused by the focal development of necrobiotic processes within the end-plates.

Specific cholinesterase activity could be demonstrated at a considerable distance from the end-plates in the sarcoplasm of those muscle fibers in which the synaptic apparatus had undergone changes.

At this stage no more unspecific cholinesterase activity could be demonstrated.

Our findings thus showed that, during the development of diphtheritic intoxication changes take place in the specific and unspecific cholinesterase activity, i.e. in the activity of those enzymes which play an important part in the transmission of stimuli from the nerve to the muscle. These changes develop in the initial stages of the intoxication, at a time when the usual histological methods fail to reveal any structural change.

At later stages proliferative processes accompanied by a further increase in the specific cholinesterase activity develop in the motor end-plates. Simultaneously the RNA-content in the cytoplasm, and in the nucleoli, and the DNA-content in the nuclei of the end-plate increase. By the time the motor disorders in the form of pareses and paralyses are fully developed, the changes in the cholinesterase activity have reached their maximum. At the same time destructive processes develop in the motor end-plates, affecting both the nuclei and the cytoplasm.

The specific cholinesterase activity shows a particularly marked increase between the 21st and 25th day, i.e. at the time when the proliferative processes in the motor end-plates are most intensive and when the first clinical symptoms of motor disorders begin to appear.

Later, the enzyme distribution changes and the activity decreases in some parts of the end-plates simultaneously with the development of degenerative processes in the nuclei and the cytoplasm in the sole of the end-plate.

This period is characterized, above all, by the presence of a demonstrable specific cholinesterase activity in a considerable stretch of the muscle fibers.

Experiments in which sections from sick and healthy animals were simultaneously incubated, showed that the presence of specific cholinesterase activity in the muscle fibers was no artefact. In all experiments of that type we invariably obtained identical results: in healthy animals specific cholinesterase was present in the motor end-plates only, whereas in animals at the stage of pareses and paralyses the enzyme was distributed diffusely over both the motor end-plates and the muscle fibers.

In our opinion this phenomenon can be explained either with the diffusion of products of hydrolysis of acetylcholine due to changes in the physicochemical properties of the muscle fiber, caused by the diphtheritic toxin, or – what seems to be more probable – with changes in the pH optimum for cholinesterase in the muscle fibers, which optimum is normally different from the pH optimum for cholinesterase in the motor end-plates [19]; consequently, in cases of intoxication the cholinesterase activity in the muscle fibers can be demonstrated by Koelle's method simultaneously with the cholinesterase activity in the nerve endings.

It could also be assumed that due to disorders in the innervating mechanism the muscle fibers acquire tonic properties in the course of diphtheritic intoxication, as it has been shown by A. G. Ginetsinskii and co-workers [2, 17] in experiments concerning the denervation of skeletal muscles.

Our investigations showed that in cases of diphtheritic intoxication, metabolic disorders develop in the initial stages of the process in the region of the neuromuscular synapses.

Later proliferative changes develop against a background of biochemical disorders, changes which become manifest in an increase in the number of nuclei in the Schwann glia of the motor end-plates.

By the time the motor disorders are fully developed the nuclei and the cytoplasm in the sole of the motor end-plates suffer progressive necrobiotic changes in addition to the metabolic disorders; certain changes can also be observed in the muscle fibers innervated by the end-plates in question.

Our findings warrant the assumption that functional disorders in the neuromuscular synapses play an important part in the development of motor disorders in cases of diphtheritic intoxication.

SUMMARY

Polyneuritis was induced in guinea pigs by subcutaneous administration of diphtheria toxin. By the 25th-35th day there appeared a typical picture of diphtheria paralyses, followed by the death of the animals. The muscles of the shin (posterior extremities) were examined at various periods after the toxin administration. Evidence is presented to the effect that diphtheria intoxication provoked metabolic disturbances in the area of the neuro-muscular synapses with the change of the cholinesterasic activity; later, there occurred proliferation of the Schwann nuclei in the motor nodes. The changes became especially marked by the time the development of motor disturbances had become evident. The data obtained lead to a suggestion of an important role played in diphtheria intoxication by the functional derangement of the neuro-muscular synapsis.

LITERATURE CITED

1. N. K. Belaya, Pediatriya, No. 1, p. 41 (1957).
2. O. P. Vishnevskaya, Problems of the Pathology of the Nervous System [in Russian], Moscow-Leningrad, p. 185 1940.
3. A. G. Ginetsinskii, Fiziol. zhurn. SSSR, Vol. 33, No. 4, p. 413 (1947).
4. A. G. Ginetsinskii and N. M. Shamarina, Trudy Fiziologicheskogo in-ta im. Pavlova., Moscow-Leningrad, Vol. 4, p. 139 (1949).
5. V. V. Portugalov, (Dissertation)., The Histophysiology of Nerve Endings [in Russian], Moscow, 1953.
6. M. A. Skvortsov, Morbid Anatomy of the Most Important Diseases of Childhood [in Russian], Moscow, 1946.
7. E. I. Yampol'skaya, Pediatriya, No. 2, p. 19 (1958).
8. A. Beer, Ergebn. inn. Med. Kinderheilk., Bd. 60, S. 657 (1941).
9. W. Behr, Dtsch. med. Wschr. Bd. 62, S. 771 (1936).
10. M. R. Brown, Bull. Hist. Med., v. 31, p. 375 (1957).
11. R. B. Couteaux, in: International Review of Cytology. New York, v. 4, p. 335 (1955).
12. F. A. Denz, Brit. J. exp. Path., v. 34, p. 329 (1953).
13. C. M. Fischer and R. D. Adams, J. neuropath. exp. Neurol., v. 15, p. 243 (1956).
14. M. A. Gerebtzoff, C. R. Soc. Biol., v. 149, p. 823 (1955).
15. F. Hansen, Klin. Wschr., Bd. 18, S. 877 (1939).
16. G. B. Koelle, Am. J. Med., v. 19, p. 661 (1955).
17. W. Krucke, Handbuch der Speziellen pathologischen Anatomic and Histologic, O. Lubarsh, F. Henke, and R. Rossle, Berlin 1955, Bd. 13, 5, S. 183.
18. A. B. Potter, Zbl. ges. Neurol. Psychiat., Bd. 56, S. 827 (1930).
19. E. Varga, A. Kover, and T. Kovacs, 4 Internationaler Kongress fur Biochem. Wien, 1-6 September, 1958, S. 87.

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