THE PROGRESS OF DYSTROPHIC CHANGES WITHIN THE NERVOUS SYSTEM (AS SHOWN BY VITAL STAINING)

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The study of the mechanisms of development of a nervous dystrophy in association with various forms of injury to the nervous system is of great importance for the analysis of the pathogenesis of many diseases and manifestations of compensatory reactions of the body, aimed at restoration of the deranged functions.

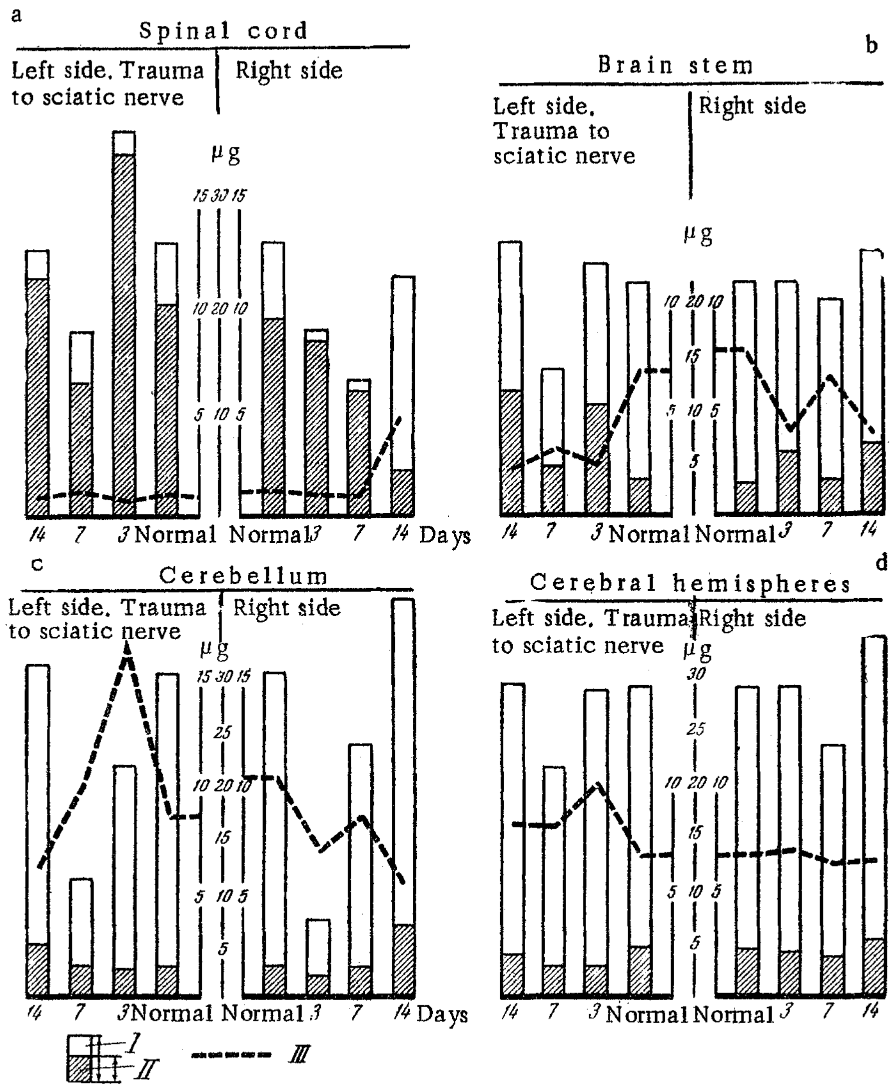
From a large volume of factual evidence, A. D. Speranskii [5] discovered a series of laws governing the character and the stages of development of a nervous dystrophic process in the peripheral tissues; at the same time he directed attention to the changes arising under these conditions in the various divisions of the nervous system itself.

The progress of dystrophic changes within the nervous system [5] has been the subject of detailed neurohistological investigations of the peripheral and central division of the nervous system. Work by B. S. Doinikov [3], M. L. Borovskii [2] and their co-workers has revealed both specialized and regular forms of lesion in the various nerve formations.

In summing up these findings, A. D. Speranskii [5] concluded that specialized and standard forms of dystrophic changes could take place in the nervous system. He emphasized the necessity of taking into consideration not only the properties of the pathogenic stimulus, but also the properties of the pathogenic stimulus in changing the state of the tissue by a reflex means. This is shown by the fact that the histopathological changes developing in the nervous system itself are not continuous in character and may be localized beyond the bounds of the segment in which the nervous trauma has been applied. In Speranskii's words [5], the pathological process "leap-frogged" over extensive and entire regions of the nervous system.

Of particular importance were experimental findings showing the asymmetrical character of the lesions in the various nerve formations, both in the same segment and in the more remote areas of the nervous system. In some cases the lesions of the nerve cells were more severe not on the injured but on the contralateral side. These and other facts of a similar character, and also the analysis of the qualitative and quantitative forms and variants of development of dystrophic changes within the nervous system, demonstrated the necessity of taking into consideration here not only the direct action of the pathogenic stimuli, but also the action of pathogenic stimuli operating through the nervous system.

Because the special features of the development and localization of dystrophic changes in the nervous system may shed light on certain aspects of the pathogenesis of many diseases and on the forms of the compensatory mechanisms of a protective and adaptive character brought into play under those circumstances, many authorities have sought suitable approaches to the investigation of these processes, paying great attention to the technique of their detection and study.



Changes in the vital staining power of the spinal cord (a), the brain stem (b), the cerebellum (c) and the cerebral hemispheres (d) of white rats after trauma to the left sciatic nerve. I) Content of dye in the tissues 1 hr after injection (sorption); II) Content of dye in the tissues 2.5 hr after injection (residue); III) ratio of sorption to residue (trophic potential).

From our previous work [1, 4] devoted to the investigation of the state of muscle tissue after trauma to the sciatic nerve, as shown by vital staining, we came to the conclusion that a close connection exists between processes of paranecrosis and processes of a neurodystrophic character. The phenomena of paranecrosis, expressing changes in the physico-chemical state of the protein substrate of the tissues in response to the action of the most widely differing stimuli in the environment, also reflect those changes in the tissues which are the result of the developing neurodystrophic process, underlying which are metabolic changes of varying severity.

In the present work we set out to use the method of vital staining to detect physico-chemical changes in the various divisions of the nervous system in connection with the development of dystrophic changes.

METHOD

The investigations were carried out on white rats weighing 130-150 g. In order to study the development of dystrophic changes within the nervous system, in these animals the sciatic nerve was divided and its central end was fixed with 0.02-0.05 ml of a 2% formalin solution. After 3, 7, and 14 days the animals were given an intra-

Changes in the Values of the Ratio of the Content of Neutral Red in the Tissues of the Left Side to its Content in the Tissues of the Right Side as a Result of Trauma to the Left Sciatic Nerve

Divisions of the central nervous system	Normal		3rd day		7th day		14th day	
	a*	b	a	b	a	b	a	b
Cerebral hemispheres	1	1	0,97	0,67	0,91	0,73	0,88	0,73
Crebellum	1	1,02	2,92	1,18	0,48	0,4	0,83	0,76
Brain stem	1,01	1,03	1,07	1,77	0,68	1,37	1,05	1,79
Spinal cord	1,0	1,08	2,06	2,06	1,36	1,48	1,11	3,95

* a) Ratio of dye content in tissues of left side to dye content in tissues of right side according to accumulation indices; b) ratio of dye content in tissues of left side to dye content in tissues of right side according to dye residue indices.

venous injection (into the saphenous vein) of a 1% solution of neutral red in Ringer's solution (without $NaHCO_3$) in a dose of 0.1 ml/30 g body weight, i.e., of 0.033 mg/g body weight of the dry stain. So that the degree of vital staining of the various divisions of the central nervous system (spinal cord, brain stem with the mesencephalon and diencephalon, cerebellum and cerebral hemispheres) could be estimated, on these days the animals were decapitated one hour after injection of the dye (in order to ascertain its degree of fixation) and 2.5 hours after injection (in order to ascertain the degree of excretion of the dye). A 1% solution of HCl in 96° ethyl alcohol was used for elution of the dye.

All the nerve formations were divided by a longitudinal incision into right and left halves. Weighed samples were taken and placed in the above solution for 24 hours, after which the quantity of vital dye was determined by colorimetry (with the FEK-M colorimeter). The dye content was calculated per gram body weight and expressed in μg. The results obtained were compared with those obtained in control animals.

The values of the fixation and excretion of the dye and the ratio between these values were used as indices of the degree of change in the nerve formations.

By this approach it is possible to determine not only the degree of damage to each particular nerve formation, but also their capacity to restore their original condition. These indices thus make it possible to ascertain the trophic state of the tissue, which may be defined quantitatively by the ratio between the values of the excretion and accumulation of the dye, expressed as a percentage. We also took into account the coefficients of asymmetry, i.e., the ratios between the values of accumulation and excretion of dye by the tissues of the injured side and the corresponding indices on the contralateral side. This coefficient enabled us to determine the degree of variation in the staining of the tissues of anatomically symmetrical areas at different levels of the central nervous system.

The values of accumulation and excretion of neutral red by the tissues of the control animals were characterized by insignificant individual variations (within limits of a few micrograms). An even smaller difference was observed between these values in the symmetrical areas of different divisions of the central nervous system of the same animal. In the experimental animals we took into consideration only those differences which were outside the limits of the variations found in the control animals.

RESULTS

In 15 operated animals (5 for each period) we studied the index of fixation of the vital dye, and in another 15 operated animals (also 5 for each period) – the index of excretion of the dye. Twenty rats (10 in each group) were used for determination of these indices in normal animals.

The results obtained are shown in the diagrams (see Figure, a, b, c, d). Analysis of the diagrams shows that along the path of development of the dystrophic changes in the central nervous system, significant changes were observed in the capacity to accumulate and to excrete the vital dye. The progress of the neurodystrophic process was characterized above all by asymmetry in the accumulation and excretion of the dye, and hence by asymmetry in the trophic potential of the divisions of the central nervous system that were investigated (by the term "trophic potential" is understood the capacity of the tissue to normalize its physico-chemical state, which may be expressed by the ratio between the value of the accumulation of dye and the value of its excretion). It may be seen from the diagrams that in every case there was asymmetry in the accumulation of excretion of dye. The degree of asymmetry and its trend (towards increase or decrease) were different on the injured and contralateral sides. Asymmetry of these indices could also be observed at different periods of development of the dystrophic process. The actual values of this asymmetry are given in the Table.

The obvious asymmetry in the indices of accumulation and excretion of the dye (in all the investigated divisions of the central nervous system) is evidence of a reflex mechanism in its production, and the magnitudes of the corresponding changes express the quantitative aspect of the modified reactive power of the substrate under study.

After the application of trauma to the nerve, asymmetry of the trophic potential was also observed in nearly all cases (the ratio of the accumulation of the dye to its residual content in the tissues), which indicates that relationships are being formed between the processes of alteration and compensatory, restorative processes along the path of spread of the dystrophic changes within the nervous system.

It may be seen from the diagram (see Figure, a) that in spite of considerable changes in the accumulation and excretion of the vital dye, at nearly all periods the spinal cord maintained its trophic potential on the side of injury at the same level, whereas on the contralateral side this index was obviously increased on the 14th day, to several times its normal value. The tendency of the nerve tissue of the spinal cord to rid itself rapidly of the unwanted dye suggests the bringing into play of protective, compensatory mechanisms.

The fact that the trophic potential was maintained at the same level, despite the changes in the absolute values of accumulation and excretion of the dye, demonstrates the plastic functions of the nervous system, permitting the initial functional state to be maintained notwithstanding the action of the pathogenic stimulus.

In the second diagram (see Figure, b) it may be seen that the trophic potential of the brain stem fell much lower on the side of injury than on the contralateral side, and that this was due mainly to disturbance of the function of excretion of the dye.

As regards the cerebellum (see Figure, c) the opposite behavior was found – the trophic potential fell sharply on the contralateral side but increased considerably on the side of injury, and fell below normal only on the 14th day after division and fixation of the left sciatic nerve. This increase in the potential took place as a result of both a diminution in accumulation of the dye and an increase in its excretion.

The study of the dynamics of the trophic potential of the cerebral hemispheres showed (see Figure, d) that on the contralateral side (in relation to the injury to the sciatic nerve) this integral index was relatively stable, whereas on the injured side it increased considerably. In other words, the cerebral hemispheres not only compensated for the developing pathological process until the 14th day after injury to the nerve, but also put into action additional protective functions of a plastic character. It must be pointed out that the initial level of the trophic potential of the divisions of the central nervous system described above in normal conditions is far higher than the level of the trophic potential of the spinal cord.

As shown by the values of the trophic potential of the individual divisions of the central nervous system, it is thus possible to estimate not only the movement of the dystrophic changes within the nervous system but also the briging into play of the accompanying processes of a compensatory or restorative character [1]. In our experiments these processes were followed until the 14th day after injury to the nerve, and in no case did trophic ulcers develop. Corresponding to clinical manifestations of compensatory mechanisms, the manifestation of these mechanisms could be observed in the various divisions of the nervous system, and, in particular, in the cerebral hemispheres. It is important to ascertain in the future how these indices change in the various stages of development of tissue dystrophies at the periphery.

The method which we have developed makes it possible to trace the relationships existing between the changes in the central nervous system and processes arising in the corresponding tissues and organs. Analysis of these correlated processes may be important for the understanding of the mechanisms of development of systemic pathological processes and individual nosological forms when pathegenic stimuli of differing nature act upon the body.

SUMMARY

Rat tissues were stained with neutral red by introducing the dye into the blood. Both the sorption indices, and the ability of the tissues to get rid of the stain were taken into consideration. The ratio of the sorption of the dye to excretion was also studied. This ratio was the "trophic potential" of the tissues, since it characterized the degree of their compensatory ability. During development of the neurodystrophic process (caused by division of the sciatic nerve and its treatment with 2% formalin solution) asymmetrical changes of the staining indices were observed in various portions of the central nervous system (spinal cord, cerebellum, brain stem, cerebral hemispheres). The asymmetrical development of paranecrotic reactions in the central nervous system points to their reflex origin. The results obtained show that the higher poritions of the nervous system possess greater plasticity than the lower, as manifested by the maintenance of the initial level of the "trophic potential" and the ability to increase it notwithstanding the development of dystrophic processess in the tissues. An close connection is inferred to exist between the processes of parabiosis and paranecrosis and trophical disturbances of the nervous system.

LITERATURE CITED

1. Ya.I. Azhipa, A. N. Aliev, M. A. Askerov, et al., Theses and Abstracts of Proceedings of an Extended Scientific Session of the Institute of Normal and Pathological Physiology [in Russian] (Moscow, 1958) p. 8.
2. M. L. Borovskii, Nerve Regeneration and Trophism [in Russian] (Moscow, 1952).
3. B. S. Doinikov, Selected Works on Neuromorphology and Neuropathology [in Russian] (Moscow, 1955).
4. O. Ya. Ostryi and Ya. I. Azhipa, Byull. Éksp. Biol. Med 49, 4, 44 (1960).
5. A. D. Speranskii, Elements in the Construction of a Theory of Medicine [in Russian] (Moscow-Leningrad, 1935).