THE STATE OF THE LIMB TISSUES AFTER TRAUMA TO THE SCIATIC NERVE (AS DEMONSTRATED BY VITAL STAINING)

O. Ya. Ostryi and Ya. I. Azhipa

Academician A. D. Speranskii's Group at the Division of Biological Sciences of the AN SSSR, Moscow
(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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The problem of the nervous control of the physiological functions of the body and of its individual organs and systems has been generally applied by Soviet physiologists and pathologists in their teaching of the trophic influence of the nervous system and of the nervous dystrophies. The work of I. M. Sechenov [9], I. P. Pavolv [8], and V. M. Bekhterev [1] has revealed a number of important aspects of the trophic maintenance of the tissues.

This trophic influence of the nervous system, maintaining the normal state of the tissues, as A. D. Speranskii showed [10], is often revealed in its negative form, i.e., through processes of a neuro-dystrophic character. Various injuries to the nervous system, at the periphery or in the centers, are reflected in the tissues by a complex group of morphological, functional and metabolic disturbances, which at the same time bring into play the protective and compensatory reactions of the body. The analysis of these processes, their phasing in time and their localization in space are of great importance to physiology, pathology and clinical medicine. That is why, for many decades, there has been no end to the search for methods which would enable the discovery of the quantitative and qualitative characteristics of the development of neurodystrophic processes and the forms of their compensation.

When we studied the relevant material and sought means to ascertain the state of the tissues during life after certain types of injury to the nervous system, we directed our attention to the possibilities presented by the method of vital staining, as developed by D. N. Nasonov and V. Ya. Aleksandrov [6, 7] in connection with the study of the problem of paranecrosis. We know from the work of these authors that any change in the physicochemical state of the tissues or any slight reversible denaturation of the tissue proteins, caused by different stimuli, influence the sorptive power of the tissues in respect of vital dyes.

Having set out with aim of evaluating the changes arising in the tissues during the development of a nervous dystrophy, we used the relationship of the tissues to vital dyes as an index of their condition. For the purpose of our investigation, however, the method of vital staining had to be essentially modified and adapted to the study of the trend of the tissue changes in the intact animal.

METHOD

The work was undertaken on white rats weighing 130-150 g. In order to reproduce a neurodystrophic process in the animals, the left sciatic nerve was divided in aseptic conditions in the upper third of the thigh. The central end of the nerve was treated with 0.02-0.05 ml of a 2% solution of formalin. After various intervals of time (3, 7, 14 days), the animals were given an intravenous 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 per 30 g body weight, i.e., of 0.033 mg of the dry dye per g body weight.

In order to ascertain the degree of vital staining of the tissues, we used the method of elution of the dye, followed by colorimetry of the extracts. The dye was eluted with a 1% solution of hydrochloric acid in 96° ethyl alcohol. Elution of the dye from weighed samples of the respective tissues continued for 24 hours. The colorimetry was carried out by means of a FEK-M-3 photoelectric colorimeter. The quantity of dye was calculated per g weight of tissue and expressed in γ.

In order to determine the quantity of dye taken up by the tissues, the animals were decapitated one hour after intravenous injection of the dye. The animals in which the rate of excretion of neutral red from the tissues was determined were sacrificed 2 ½ hr after injection of the dye.

The basis of the technique which we employed consequently was: a)The principle of vital staining of the tissues of the body in vivo, the vital dye reaching the tissues via the blood stream; b) the principle of studying not only the sorptive power of the tissues, but also their power of excreting the dye in the course of a given period of time.

This approach enabled us to carry out the experiment in natural conditions and prevented possible artefacts due to the staining of tissues in vitro. The method enabled us to study the relationships which held good between the processes of accumulation and excretion of dye, so that a more complete picture could be obtained of biophysical and biochemical state of the tissues.

RESULTS

The degree of staining and the excretion of the dye by the muscles of the calf and thigh and also of the foot on the side of the injured nerve and on the contralateral side were studied in 50 white rats. In 30 animals the left sciatic nerve was divided and the central end of the nerve treated with formalin. In the animals of group 1 (15 rats) the content of vital dye in the tissues was determined one hour after its intravenous injection on the third, seventh and 14th days of development of the dystrophic process. In the animals of group 2 (15 rats), the content of dye was determined in the tissues $2\frac{1}{2}$ hr after injection at the same periods after trauma to the sciatic nerve. Five rats were investigated at each period. The remaining 20 rats acted as controls (ten for each group).

It can be seen from the details in Fig. 1 that on the third day after injury to the sciatic nerve a considerable accumulation of neutral red took place in the calf muscles of the injured limb, whereas in the opposite limb the level of accumulation of dye fell appreciably. Under these circumstances an increased content of dye was determined on the injured side until the 14th day. On the contralateral side at the 14th day the level of accumulation of dye by the calf muscles reached the value observed in the control animals. A different relationship was shown by the accumulation of dye by the thigh muscles. On the injured side no essential changes in accumulation were observed, whereas on the contralateral side the mus- also behaved like the calf muscles of the same side in respect of accumulation of dye (Fig. 2).

Since we wished to ascertain to what extent the power of accumulation of vital dye not by individual tissues but by their natural groups changed under these conditions, we selected as a suitable object the complete foot. It can be seen from Fig. 3 that on both the injured the contralateral sides a fall in the accumulation of dye by the tissues of the foot took place at all periods.

In the process of development of dystrophy after trauma to one sciatic nerve, regular changes in the accumulation of vital dye by the tissues of both limbs were thus observed. Under these conditions the changes on the injured side could take the form of an increase in the level of fixation of dye or a decrease in the degree of staining of the tissues, depending on the object of investigation. In the contralateral limb a fall in the degree of fixation of dye was observed in all cases, with a tendency for it to normalize on the 14th day of the experiment to the control level. In accordance with the views of D. N. Nasonov and V. Ya. Aleksandrov [5, 6, 7], the changes observed in the tissues were regarded by us as reactions of a paranecrotic character, associated with a reversible denaturation of the tissue proteins.

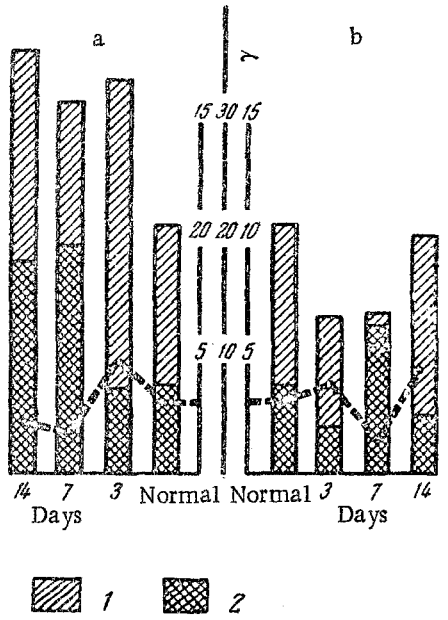

Fig. 1. Trend of the changes in vital staining of the calf muscles after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs: Whole column (1, 2) – content of dye in the tissues one hour after its injection (sorption); lower part of the column (2) – content of dye in the tissues $2\frac{1}{2}$ hr after its injection (residue); broken line – ratio of sorption of dye to residue (trophic potential).

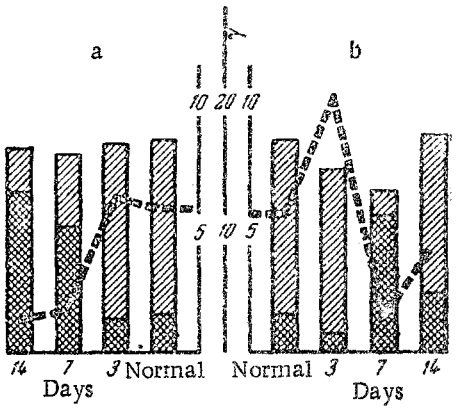

Fig. 2. Trend of the changes in vital staining of the thigh muscles after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs as in Fig. 1.

Different variants of the paranecrotic reaction were observed in our experiments. A noteworthy fact in this case was the decrease in the fixation of dye by the tissues in the contralateral limb, which may be regarded as a

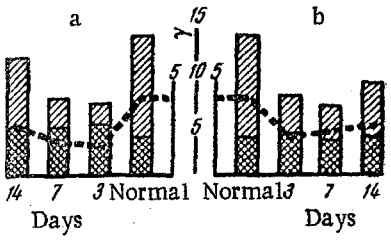

Fig. 3. Trend of the changes in vital staining of the foot after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs as in Fig. 1.

manifestation of a compensatory reaction of the animal in response to pathogenic stimulation. The asymmetry of the reactions of the limb tissues indicated their reflex origin.

D. N. Nasonov and V. Ya. Aleksandrov [5, 6, 7], on the subject of changes of a similar type in the state of tissues, point out that in processes of denaturation of proteins, reactions of a compensatory character should be distinguished, for they tend in the opposite direction. These reactions also lie at the basis of reversible paranecrosis, and of the fact that this process is biphasic in its course. This biphasic character of the paranecrotic reactions enabled A. A. Ukhtomskii [12], D. N. Nasonov and V. Ya. Aleksandrov [6, 7], L. L. Vasil'ev [2], N. V. Golikov [4], and their coworkers to associate the reactions of paranecrosis with the reactions of parabiosis of N. E. Vvedenskii [3].

Analysis of the results obtained with regard to the problem of the relationship between the trophic state of the tissues and their reactions of a paranecrotic character showed that it was difficult by one index of the accumulation of vital dye by the tissues to judge the changes occurring therein. A quantitative estimation of the vital dye accumulating in the tissues tells nothing of the ability of the tissues to rid themselves of vital dye, which represents ballast to them. Meanwhile, the function of normalization of the tissues, indissolubly connected with the function of compensation of disturbances of function, is a more important aspect for consideration in the evaluation of their physiological state. Besides the work described above, we therefore carried out at the same time an investigation of the power of the tissues to excrete vital dye.

It is apparent from the figures shown that the power of the tissues of the injured and uninjured limbs to excrete vital dye did not follow a parallel course to their power to fix the dye. In some cases an increase in the fixation of neutral red was accompanied by its increased excretion (on the third day – the calf muscles of the injured limb), and in others the rates of excretion of the dye fell behind the rates of fixation. In these conditions different variants of the relationships between the values of the accumulation and excretion of dye were observed. A decrease in the degree of excretion of the dye might be observed: with an increased level of accumulation of dye (seventh day – calf muscles on the injured side); with a normal level of accumulation (seventh day – thigh muscles on the injured side) and with a simultaneous fall in the level of accumulation (seventh day – calf and thigh muscles in the uninjured limb). Finally, in some cases a fall in the level of accumulation of dye was accompanied by an increase in its excretion (third day – thigh muscles on the uninjured side).

The relationships thus revealed between the accumulation and excretion of vital dye by the tissues gave a more complete definition of the biophysical and biochemical state of the tissues. Advantage is taken here of the fact that the index of clearance of ballast dye from the tissues reflects the incorporation of compensatory mechanisms directed towards the normalization of their functional state.

In order to obtain a quantitative picture of the trophic potential of the tissues a coefficient was adopted which expressed the ratio between the amount of dye adsorbed and the amount remaining in the tissues. When determined in this way, the trophic potential of the tissues is a numerical index, not only of changes in the original resistance of the tissues in relation to corresponding stimuli, but also of their ability to restore and maintain their biophysical and biochemical state at the optimal level in accordance with the environmental conditions.

An analysis of the findings shows the value of this numerical index in the evaluation of the state of the tissues.

If, for example, consideration is taken only of the accumulation of neutral red by the thigh muscles on the side of the injured nerve, the conclusion may be reached that the muscles had undergone no changes, whereas, in fact, on the seventh and 14th days there had been sharp falls in the ability of these muscles to excrete the dye. The results obtained point to the prospects of analysis of the mutual relationships of nervous dystrophic processes, paranecrosis and processes of a parabiotic character.

All these processes ultimately reflect the changes undergone by the functional mobility or lability of the tissue substrates in normal and pathological conditions. In this connection we must remember the statement by A. A. Ukhtomskii, that: "... any tissue which more resiliently resists alteration more readily modifies it to suit its own ends and more rapidly returns to its original preparedness for work and will at the same time be more labile".

The definition of the trophic potential of a tissue by means of the paranecrotic test coincides fully with A. A. Ukhtomskii's statement regarding tissue lability. An increase in the coefficient of trophic potential coincided in our experiments with a more rapid clearance of dye from the tissue, and conversely, a decrease indicated a diminution of this power. Our test may thus be used to give a more profound insight into the condition of a tissue, which is of great importance in the analysis of a wide range of problems connected with nervous trophicity and nervous dystrophies and with the problem of compensation of physiological functional derangements in the body.

SUMMARY

The authors modified the method of supravital staining of the tissues developed by D. N. Nasonov and V. Ya. Alexandrov. The staining was conducted in an intact animal by injecting neutral red into the blood stream. The study of the sorption of the stain and the process of its liberation by the tissues made it possible to put forward a definition of trophic potential of the tissues. Unilateral trauma of the sciatic nerve (section and treatment of its central end by a 2% formalin solution) asymmetrically changes the coordination between the acculation and liberation of the stain by the muscles of the shin and thigh, as well as by the tissues of the foot. This reveals phases characterized by the rise and fall of the trophic potential, pointing to the inclusion of the compensatory mechanisms paralleling the development of the neurodystrophic process.

The processes of paranecrosis, parabiosis, and those of a trophic condition of the tissues are compared theoretically.

LITERATURE CITED

[1] V. M. Bekhterev, Fundamental Knowledge of the Functions of the Brain 3 [in Russian] (St. Petersburg, 1905), pp. 672-685.

[2] L. L. Vasil'ev, The Importance of N. E. Vvedenskii's Physiological Teaching to Neuropathology [in Russian] (Leningrad, 1953).

[3] N. E. Vvedenskii, Excitation, Inhibition and Narcosis [in Russian] (St. Petersburg, 1901).

[4] N. V. Golikov, Physiological Lability and its Changes in Fundamental Nervous Processes [in Russian] (Leningrad, 1950).

[5] D. N. Nasonov, Abstracts of Proceedings of the Sixth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian] (Khar'kov, 1958) p. 73.

[6] D. N. Nasonov and V. A. Aleksandrov, Arkh. Biol. Nauk SSSR, Seriya A 36, 95 (1934).

[7] D. N. Nasonov and Y. YA. Aleksandrov, The Reaction of Living Matter to External Interference [in Russian] (Moscow-Leningrad, 1940).

[8] I. P. Pavlov, Complete Collected Works 1 [in Russian] (Moscow-Leningrad, 1940) p. 331.

[9] I. M. Sechenov, Physiology of the Nervous System [in Russian] (St. Petersburg, 1866) p. 77.

[10] A. D. Speranskii, Selected Works [in Russian] (Moscow, 1955).

[11] A. A. Ukhtomskii, in: Problems of Biology and Medicine [in Russian] (Moscow-Leningrad, 1935) p. 239.

[12] A. A. Ukhtomskii, Priroda 10, 1 (1935).