

HISTOPATHOLOGICAL CHANGES IN ANIMALS IN THE PRESENCE OF ANAEROBIC INFECTION AND WHEN SUBJECTED TO WEAK STIMULATION

E. A. Skvirskaya

From the Laboratory of Nerve Nutrition (Head — Prof. M. L. Borovskii) of the Department
of General Pathology (Head — Academician A. D. Speranskii) of the Institute of
Normal and Pathological Physiology (Director — Active Member AMN SSSR
V. N. Chernigovskii) AMN SSSR, Moscow

(Received November 27, 1957. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In previous investigations [4, 5] were demonstrated certain characteristics of the neurodystrophic process in guinea pigs infected with *B. perfringens* when the nerve supplying the infected muscle is affected.

In the current project carried out on white rats, the neuroreceptor apparatus of the infected muscle was subjected to additional action.

EXPERIMENTAL METHOD

A minimal lethal dose of vibron septicum mixed with a 2.5% solution of calcium chloride was administered into the gastrocnemius muscle of experimental rats. Twenty-four — 3 hours prior to infection or 1-3 hours following it, 0.1 ml of 0.25% solution of a stable preparation of novocain in apricot oil was injected into the same muscle or 0.1 ml of sterile apricot oil was injected 24 hours prior to infection. Control animals not subjected to supplementary intervention * were infected at the same time as the experimental animals. Three series of experiments were carried out on 118 rats.

EXPERIMENTAL RESULTS

The experimental results are presented in Tables 1 and 2.

In order to establish the effect of apricot oil on the pathogenicity of microbes, a third series of experiments was carried out the results of which are presented in Table 3.

Thus, injection of novocain or apricot oil in the same muscle that was infected, especially when administered 24 hours prior to the infection, prevents death of some of the animals and at times of the majority. Direct contact of the microbes with the apricot oil does not influence their pathogenicity significantly.

Observations indicated that the paw into which the microbes were injected showed involvement of a lesser degree in animals given apricot oil or novocain 24 hours prior to infection than in others. Histopathological examination of both gastrocnemius muscles and both sciatic nerves was performed in 14 rats of the second series of experiments sacrificed on the 1st, 7th and 45th day after infection. Sections were stained with scarlet red for fat and counterstained with hematoxylin.

Microscopic examination of the preparations showed the following. In one day after infection of rats of the fourth group (control) the right gastrocnemius muscle was found to be overrun by microbes.

*Infection of the animals was carried out by co-worker Z. I. Sobleva of the Laboratory of Infectious Pathology.

TABLE 1

The First Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished
		total	perished	survived	
First	Injection of novocain into the right gastrocnemius muscle 24 hours prior to infection	10	1	9	27 hours - 1 rat
Second	The same 3 hours prior to infection	10	7	3	20 " 1 " 22 " 4 rats 24 " 1 " 43 " 1 "
Third	The same 3 hours after infection	10	6	4	19 hours 2 rats 22 " 2 " 27 " 1 rat 43 " 1 "
Fourth	Infection without supplementary intervention (controls)	10	8	2	19 hours 2 rats 22 " 5 " 43 " 1 rat

TABLE 2

The Second Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished
		total	perished	survived	
First	Injection of novocain into the right gastrocnemius muscle 24 hours prior to infection	10	6	4	12 hours - 1 rat 24 " 3 rats 4 days 2 "
Second	The same 1 hour after infection	10	5	5	12 hours 2 rats 22 " 1 rat 24 hours 2 rats
Third	Injection of apricot oil into the right gastrocnemius muscle 24 hours prior to infection	10	3	7	12 hours 2 " 4 days 1 rat
Fourth	Infection without supplementary intervention (controls)	10	7	3	12 hours 4 rats 24 " 3 "

TABLE 3

The Third Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished following infection
		total	perished	survived	
First	Infection of apricot oil into the left gastrocnemius muscle 24 hours prior to infection of the same muscle	10	5	5	22 hours — 1 rat 24 " 2 rats 27 " 1 rat 4 days 2 rats
Second	Injection of apricot oil into the left gastrocnemius muscle 24 hours prior to infection of the muscle of the left thigh	10	5	5	22 " 5 rats
Third	Injection of a mixture of microbial suspension and apricot oil into the left gastrocnemius muscle	9	7	2	22 " 2 rats 24 " 2 " 27 " 2 " 42 " 1 rat
Fourth	Infection without supplementary intervention	9	9	0	22 " 8 rats 72 " 1 rat

Edema of the intermuscular connective tissue, fragmentation, swelling and fatty degeneration in many muscle fibers and hemorrhage are very much in evidence (Fig. 1). The numerous foci of round cell infiltrates, consisting of lymphocytes and to a lesser extent of polymorphonuclear leucocytes, contain numerous degenerated cells. No mitoses were seen in the infiltrates or uninvolved tissue. Nuclear proliferation, fatty degeneration of individual muscle fibers, and infrequently — small foci of round cell infiltrates, were observed in the left gastrocnemius muscle. The right sciatic nerve showed that the epineurium, was thickened, hyperemic and contained large foci of round cell infiltrates. There were no changes in the left sciatic nerve.

In the right gastrocnemius muscle of the experimental animals were seen the same destructive changes as seen in the control rat, but they were observed in a few muscle fibers. In the uninvolved muscle tissue of these animals, proliferation of muscle and connective tissue nuclei was less marked than in control rats and mitoses were encountered. At the site of injection of novocain or oil there was a cavity containing a considerable quantity of oil. In this cavity as well as in the surrounding area there were collections of round cells which were bound with cellular matrix to round cell infiltrates in other portions of the sections (Fig. 2). In distinction from findings at examination of analogous preparations from control animals, these infiltrates of lymphocytes and polymorphonuclear leucocytes also contained many polyblasts and macrophages among which not infrequently one could find cells with mitotic figures. Nuclear proliferation and at times mitoses could be seen in the left gastrocnemius muscle. In this muscle, areas of fatty degeneration along the course of muscle fibers as well as vascular hyperemia and proliferation of perivascular connective tissue are seen on occasion. Only in the rat from the second group could foci of infiltrates within the substance of the nerve be seen in the right sciatic nerve; hyperemia and fresh hemorrhages in the epineurium were found in the left sciatic nerve.

Seven days following infection (Fig. 2) of the right gastrocnemius muscle of the control rat, hyperemia with thickening of the vessel walls and proliferation of connective tissue replacing involved muscle tissue could

be found. Fatty degeneration of muscle fibers was rather mild. The right sciatic nerve was hyperemic and adherent to the adjacent tissues. In the left gastrocnemius muscle were found hyperemia and proliferation of perivascular connective tissue; in the left sciatic nerve — hyperemia and proliferation of connective tissue elements in its coats.

In experimental rats of the first and third groups, also 7 days following infection, there was replacement of part of the muscle tissue of the right gastrocnemius muscle with granulation tissue and numerous foci of round cell infiltrates. Here were present fatty degeneration of muscle coats and myelin coats of the nerve fibers as well as macrophages loaded with fat and pigment. At the site of injection of novocain and oil there is a slit-like space filled with fat and surrounded by a layer of lymphoid, histiocytic and other connective tissue elements. In the preserved muscle tissue one can observe in some fields of vision proliferation of muscle nuclei and connective tissue elements among which occasional cells with mitotic figures are present. Proliferation of adipose cells is marked, especially along the course of vessels and nerves. In the left gastrocnemius muscle one can see now and then fatty degeneration of muscle fibers and hyperemia. Proliferation of adipose cells, of muscle nuclei in chain-like arrangements and of connective tissue cells which in places obscure portions of muscle fibers are conspicuous. In portions of the right sciatic nerve the epineurium is thickened and adherent to the surrounding tissues; now and then focal collections of endoneural nuclei are encountered. In the left sciatic nerve only an insignificant thickening of the epineurium is observed.

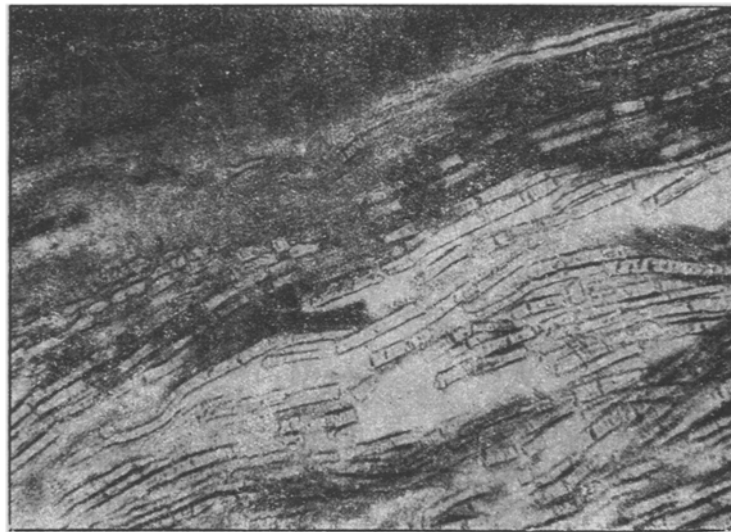


Fig. 1. Condition of muscle tissue (right gastrocnemius muscle) of a control rat sacrificed 24 hours after infection. Destructive changes in muscle tissue are evident. Fat stain. Microphotogram 67.2 x.

In the rat of the second group the histopathological picture of the muscles and nerves resembles the one just described. The difference is in the greater intensity of the destructive process in the right gastrocnemius muscle where structureless fragments of muscle fibers are surrounded by giant cells, histiocytes, macrophages and fibroblasts. The entire area of involvement is delimited from the remaining muscle tissue by a roller of round cells.

On the 45th day after infection, proliferation of connective tissue cells (including massive proliferation of connective tissue) of muscle nuclei and adipose cells in both muscle and coats of both nerves is more pronounced in experimental than in control rats.

It is obvious from the cited microscopic findings that during the first day after infection the less striking destructive changes in the muscles and nerves characteristic of the pathological process being studied were observed in rats of the first and third groups; they increased in the second group and reached the maximum degree in animals of the fourth group (controls). This agrees with findings observed in the involved extremity which were made while the animals in this series of experiments were still alive.

The preparations indicate that the pathological process in the region of infection differed in the experimental and control animals in the degree of involvement of muscle tissue and in the character of reaction on the part of tissue. The latter, apparently, determined the outcome of the infection and the subsequent course of the processes of restoration. As compared to controls, the experimental animals exhibited a lesser degree of destructive changes in the muscles and a greater degree of activity on the part of mesenchymal elements including adipose cells.

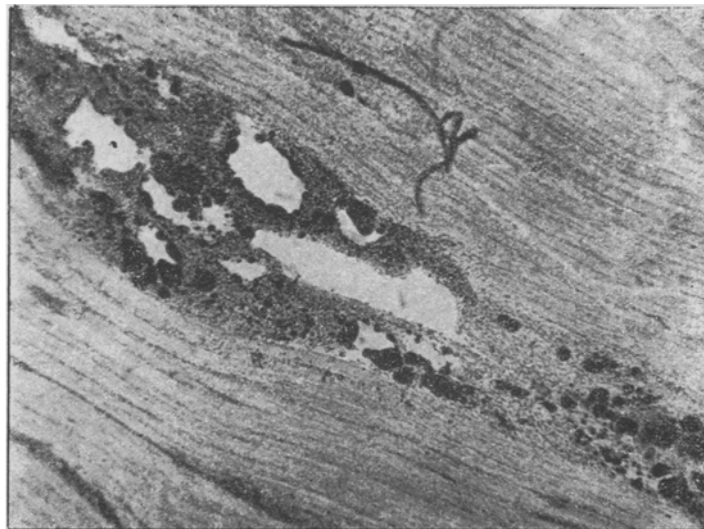


Fig. 2. Condition of muscle tissue (right gastrocnemius muscle) of a rat sacrificed one day following infection. Relative preservation of muscle tissue at the site of injection of apricot oil is evident. Fat stain. Microphotogram 67.2 X.

In the literature of recent years dealing with the problem of anaerobic infection, more and more attention is being paid to the reactivity of the organism which is associated with the functional condition of the nervous system. Academician A. D. Speranskii's position on the leading role of the nervous system in pathological and protective and adaptive processes was confirmed by the investigation of the mechanisms of development of gas gangrene [1, 2, 3, 6]. The same authors pointed out the favorable action of novocain as an anesthetic substance on the outcome of experimental infection with microbes and toxins of gas gangrene.

Reactions in the form of proliferation of muscle nuclei, appearance of adipose cells, and connective tissue and blood elements were observed by us not only in the muscle into which novocain, oil and microbes were injected, but in the contralateral — so-called healthy muscle and in the coats of its nerves. Consequently, it is possible to speak of general stimulation of proliferative processes in mesenchymal tissues by a nonspecific irritation. An organism prepared in such a manner may be found to be resistant to a lethal dose of vibron septique. Under such conditions the pathological process in the focal lesion loses in intensity, which, as our earlier observations indicated, favors a decrease in changes in the nervous system.

SUMMARY

A culture of vibron septique was injected into the gastrocnemius of white rats 24 or 3 hours previous to the infection or 1 to 3 hours following it a stable preparation of novocain in apricot oil or apricot oil alone was injected into the same muscle. Control rats were injected simultaneously, but without any additional interventions. The outcome of the infection was more favorable in the experimental rats. The injection of novocain or apricot oil, especially carried out 24 hours before the infection prevented the death of a number, at times of the majority of the animals. The extremity into which the microbe was injected was found to be affected less in animals in which novocain solution or apricot oil was injected 24 hours previous to the infection (in comparison with the rest of the animals). The microscopic examination of both gastrocnemius muscles and

both sciatic nerves demonstrated that novocain or apricot oil injection brought about a decrease in the destructive changes in the affected muscle and an intensification of the reactive processes in the mesenchymal elements. The latter, evidently, promoted an increase of the resistance of the animals to the lethal dose of vibrioseptique.

LITERATURE CITED

- [1] A. N. Aliev, "Experimental analysis of the mechanisms of intoxication in anaerobic infection (on the order of intoxication of white rats with toxin)" Candidate's thesis, Moscow, 1956.*
- [2] M. K. Karpov, "Nervous mechanisms in the pathogenesis of experimental gas gangrene," Author's essay thesis, Leningrad (1953); Zhurn. Mikrobiol. Epidemiol. i Immunobiol. No. 11, 7-25 (1952).*
- [3] O. Ia. Ostryi, Z. I. Sobieva and A. N. Aliev, Thesis reported at the 2nd All-Union Congress of Pathologophysiolgists, Kiev, 279-280 (1956).*
- [4] E. A. Skvirskaya, The Problem of Reactivity in Pathology, Moscow (1954) 150-158.*
- [5] E. A. Skvirskaya, Doklady Akad. Nauk SSSR 111, 6, 1388-1389 (1956).
- [6] Z. I. Sobieva, "Experimental analysis of the mechanisms of acquiring and anaerobic infection," Candidate's thesis, Moscow, 1953.*
- [7] A. D. Speranskii, Selected Works, Moscow, 458-465, 1955.*

*In Russian.