CENTRAL NERVOUS CHANGES IN EXPERIMENTAL TETANUS AND THE MODE OF ACTION OF THE TETANUS TOXIN

COMMUNICATION I. IRRADIATION OF THE EXCITATION ON STIMULATING THE TETANIZED LIMB

G. N. Kryzhanovsky

From the Laboratory of Infectious Pathology (Head – Corresponding Member of the Acad. Med. Sci. USSR Professor A. Ya. Alymov) Section of General Pathology (Head – Academician A. D. Speransky) Institute of Normal and Pathologic Physiology Acad. Med. Sci. USSR, Moscow (Director – Active Member of Acad. Med. Sci. USSR V. N. Chernigovsky)

(Received July 25th, 1957. Presented by Active Member of the Acad. Med. Sci. USSR, V. N. Chernigovsky)

According to work done up to the present time, the symptoms of tetanus are due to changes in central nervous structures, and in particular the motor neurons [2-6,9-11,17,21,26,27]. According to one theory of the pathogenesis of tetanus, the Cl. tetani toxin enters the central nervous system along the motor nerve fibers and infects the motor neurons with which it first comes in contact [16,19]. Opponents of this theory, who maintain that there is a hematogenous spread of the toxin into the central nervous system agree that the motor nerve cells of the anterior horns of the spinal cord are first affected by the toxin [8]. Recently Eccles and his coworkers (Brooks, Curtis and Eccles, [10]), have concluded from electrophysiological investigations, that the tetanus toxin has a selective action on the synapses which terminate round the motor neurons and which normally mediate inhibitory effects. These experiments, as well as the earlier physiological experiments of other authors were carried out using a local tetanus.

Nevertheless a study of the symptoms of tetanus, particularly at the stage when the condition becomes generalized, gives reason to suppose that the pathogenic mechanism of this disease is more complex than has been supposed up to the present time.

The present work was undertaken with the object of studying certain functional nervous changes in experimental tetanus with a view to elucidating the mechanism of action of the tetanus toxin. In the present communication a study is made of the phenomena which we have found in investigating the clinical condition of ascending generalized tetanus in white rats.

EXPERIMENTAL METHOD

The experiments were carried out on white rats of weight 130-165 g. Dry tetanus toxin series 580 IEM AMN was used, and was diluted with glycerin (pH 6.9) and physiological saline; the necessary dilution (1 MLD for rats weighing 130 g. contained 0.05 ml of the solution) were made prior to the experiment. The toxin was given in two injections – into the muscles of the left lower leg and left thigh. At the same time, in order to block or attenuate any action of the tetanus through the bloodstream,* .025 units of the antiserum (diaferm 3

* Preliminary experiments showed that in these conditions the phenomena described below were shown more clearly.

of the IEM AMN was given intravenously into the lower right leg. The potentials were led off to a two channel amplifier system OKB of the AMN, the output of which was taken to a Siemen's string oscillograph. Muscle potentials were picked up with needle electrodes. An electronic stimulator was used to apply stimuli to the central ends of the nerves and to the spinal roots, in order to determine the levels of the convulsive reaction; the stimulator had a potential divider at the output and delivered square wave stimuli at a frequency of 50 cps and with a duration of 0.5 msec; the value of the stimuli was determined with a cathode ray oscillograph. Platinum stimulating electrodes were used. Dissection and division of the nerves and nerve roots was carried out under brief ether anesthesia. The stimuli were applied when the animal had recovered from the anesthetic. Where any trauma of the spinal cord was suspected, the animals were not used for the experiment. The spinal roots were exposed subdurally and were cut at their exit from the vertebral canal, and this gave a sufficiently long central end, so that the distance from the electrodes to the spinal cord was more than 1 cm length. The lumbar enlargement was exposed as little as possible. The sciatic nerves were cut in the region of the popliteal fossa before branching. Besides the usual section of the femoral and obturator nerves in the region of the groin, a high section was also made in the region of the lumbar plexus, so as to exclude the possibility of anastomoses with the sciatic. All branches of the latter from where it was sectioned, to the lumbar plexus were cut. When registering the potentials, a stimulus marker was included automatically when the stimuli were applied.

EXPERIMENTAL RESULTS

The present work is devoted to the investigation of a phenomenon which we discovered: that during the development of a generalized tetanus of the ascending type, i.e., when there is at first a local tetanus of the limb which received the tetanus injection, and then a segmental and a general tetanus, at the stages when the condition has become generalized.convulsions of the whole body may be induced by applying the stimulus to the hind limb which received the toxin, (in future we shall refer to this as the tetanized limb). When the stimulus is applied to other parts of the body, including the opposite limb, either no convulsions, or very weak ones result.

This phenomenon is not shown in white rats only. Experiments have shown that it can be observed in other animals, e.g. in mice, rabbits, and in many cases in cats, after injection of corresponding doses of toxin. It may therefore be regarded as one of the characteristic signs of generalized ascending tetanus.

Investigation of this phenomenon has shown that provocation of convulsions by applying stimuli to the tetanized limb (e.g. by forced bending, or pressing on the foot or muscles of the leg etc.) is possible in the early stages, when stimuli applied to other places have no effect. As generalized tetanus advances the convulsions evoked by the stimulus to the tetanized limb become stronger and more prolonged: the limbs are extended, the tail raised, the muscles of the trunk contract, the head is thrown back in opisthotonus, the jaws are tightly clenched, the eyes half close, breathing is interrupted, and the animal frequently utters a groan. At the height of the generalized tetanus, a convulsive attack may be induced by even a mild bending of the tetanized limb or of one of its digits or by pressure on the pads of the feet with forceps. At the same time the convulsions evoked by similar stimuli applied to the other limbs, are less well shown, less prolonged and sometimes do not occur at all.

Electrical recordings made at various stages in the development of generalized tetanus confirmed what has been said above, and showed that both proprioceptor stimuli (stretching or compressing the muscles) and exteroceptor pain stimuli (pinching folds of skin or the pads of the feet with forceps) entering the central nervous system from the tetanized limb cause a very considerable increase in electrical activity with the generation of large potentials in various muscles of the trunk, neck and limbs (Fig. 1a and Fig. 2a, b). This activity coincides with the development of generalized convulsions. The electrical activity does not subside immediately the stimuli are withdrawn, but continues for a while, gradually decreasing, and the length of time for which the activity continues is greater the more severe the general picture of generalized tetanus.

When the same stimuli under the same experimental conditions are applied to the opposite limb, either there is no increase in electrical activity (Fig. 1,b and Fig. 2,b), or else there is a negligibly small increase.

To explain this phenomenon, it was at first supposed that the stimuli entering the central nervous system from the receptors of the tetanized limb were, from the onset, more powerful than those from the opposite side. Thus, for example, stretching the contracted muscles of the tetanized extremity should cause a more effective proprioceptor stimulation than stretching the less rigid muscles of the opposite side. Also, according to many others [20,25], tetanus toxin increases the excitability of the proprioceptors. Alternatively however, it is possible that the phenomenon is due to changes in the central and not in the peripheral nervous system.

To investigate this the femoral and obturator nerves were cut at a high level and the central ends of the sciatic nerve of the tetanized and of the opposite limb were stimulated. By this means any additonal proprioceptor stimulation was avoided. The results of these experiments are shown in Fig. 3 and it can be seen that the convulsion threshold for stimulation of the central end of the sciatic of the tetanized limb is several times lower than that for the opposite extremity, and that in the latter case the convulsions were less intense and sometimes poorly shown.

These results show that the facilitation of the induction of generalized convulsions from the tetanized side are due primarily to central nervous mechanisms. However, peripheral mechanisms may play a subsidiary part in this phenomenon.

To explain this effect, in view of the indication of the selective action of the toxin on the motor neuron region, it must be supposed that there is a considerable antidromic effect from the motor neurons supplying the tetanized limb, spreading into other reflex arcs.

One of the ways in which inhibition of the anterior horn cells is effected is through the interneurons being activated from stimuli passing along the recurrent motoneuron collaterals [12], as well as along the afferent fibers [15]. These cells enable the motoneurons to influence each other so that stimulation of one causes an inhibitory effect on its neighbors. Since Sherrington [7], on the basis of the observed changes in reciprocal innervation in tetanus advances the idea that tetanus may not only suppress the inhibitory action, but may change it into a stimulating one, we may suppose that such a system might mediate this transformation. There may be other mechanisms of the spread of the excitation from the motoneurons of the tetanized limb to other reflex arcs, and this may involve the system of interneurons [15].

To test this hypothesis we carried out experiments with antidromic stimulation of the motoneurons of the infected segments of the spinal cord by stimulating the central ends of the corresponding motor roots.

The results of these experiments are shown in the Table. Here it can be seen that in no case did the antidromic stimulation cause a convulsion. Even with powerful stimulation it was impossible to induce any reaction in the animal. Nevertheless, a relatively mild stimulus to the sensory roots of the same segments causes the typical convulsions. Thus in tetanus there are no abnormal antidromic effects resulting in stimulation of the motoneurons innervating the tetanized limb and consequently the induction of convulsion when a stimulus is applied to it cannot be due to any such effect. Our results agree with those of Eccles and his co-workers [10] who failed to find any transformation of inhibitory effects into excitatory stimuli in localized tetanus.

The results reported here lead us to suppose that the functional changes resulting in convulsions induced by the stimulation of the tetanized limb occur in the synapses formed by the afferent and interneurons of the reflex arcs which originate in the receptors of the tetanized limb. The destruction of some mechanism of restraint in these synapses allows the impulses originating in the periphery to spread and so to pass to other reflex arcs. The extent to which this excitation will spread depends on the extent to which reflex excitability is increased, i.e. the extent to which the inhibitory mechanisms of the various nervous pathways are suppressed, and on the number of such pathways involved.

Since at the onset of generalized ascending tetanus the functional changes occur chiefly on the side of injected toxin, and are absent or weak in other reflex arcs, stimuli entering the central nervous system from other receptor fields are to some extent suppressed in the synapses of the corresponding afferent neurons and interneurons and so do not attain the threshold value required for the induction of a generalized convulsion. To induce the latter the strength of the stimulus must be increased, i,e., the restraining effect of the synaptic mechanism must be overcome. As the condition spreads, inhibitory effects are reduced, the general reflex excitability is increased, and the threshold for the convulsive reaction evoked by stimulation of other receptor

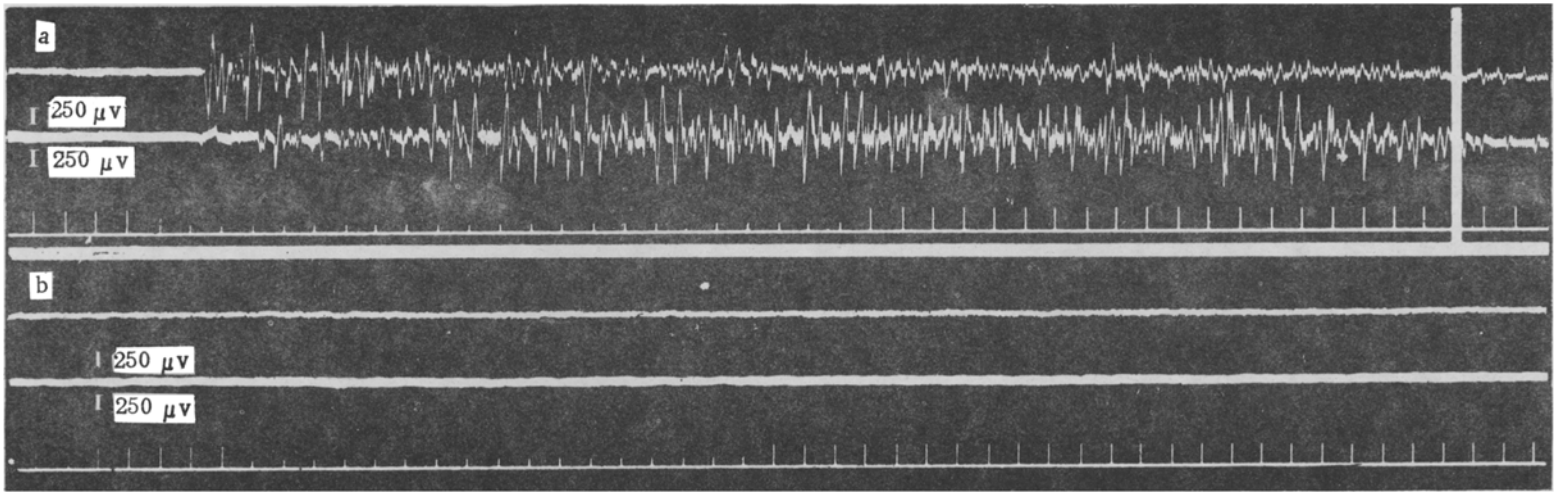

Fig. 1. Electrical activity in the triceps muscle of the left (upper oscillograph tracing) of the anterior roots induced by gentle flexion of the left (tetanized, a) and right (b) foot.
Upper oscillogram restricted to 6.9 cm (0.3 seconds). End of selected portion shown by white line across tracing. Time marking–20 msec. Reduction in size of time markings indicates period of pressure on foot. Experiment of 1/3/1955. Rat No. 5. Clearly shown segmental tetanus, increase of general excitability.

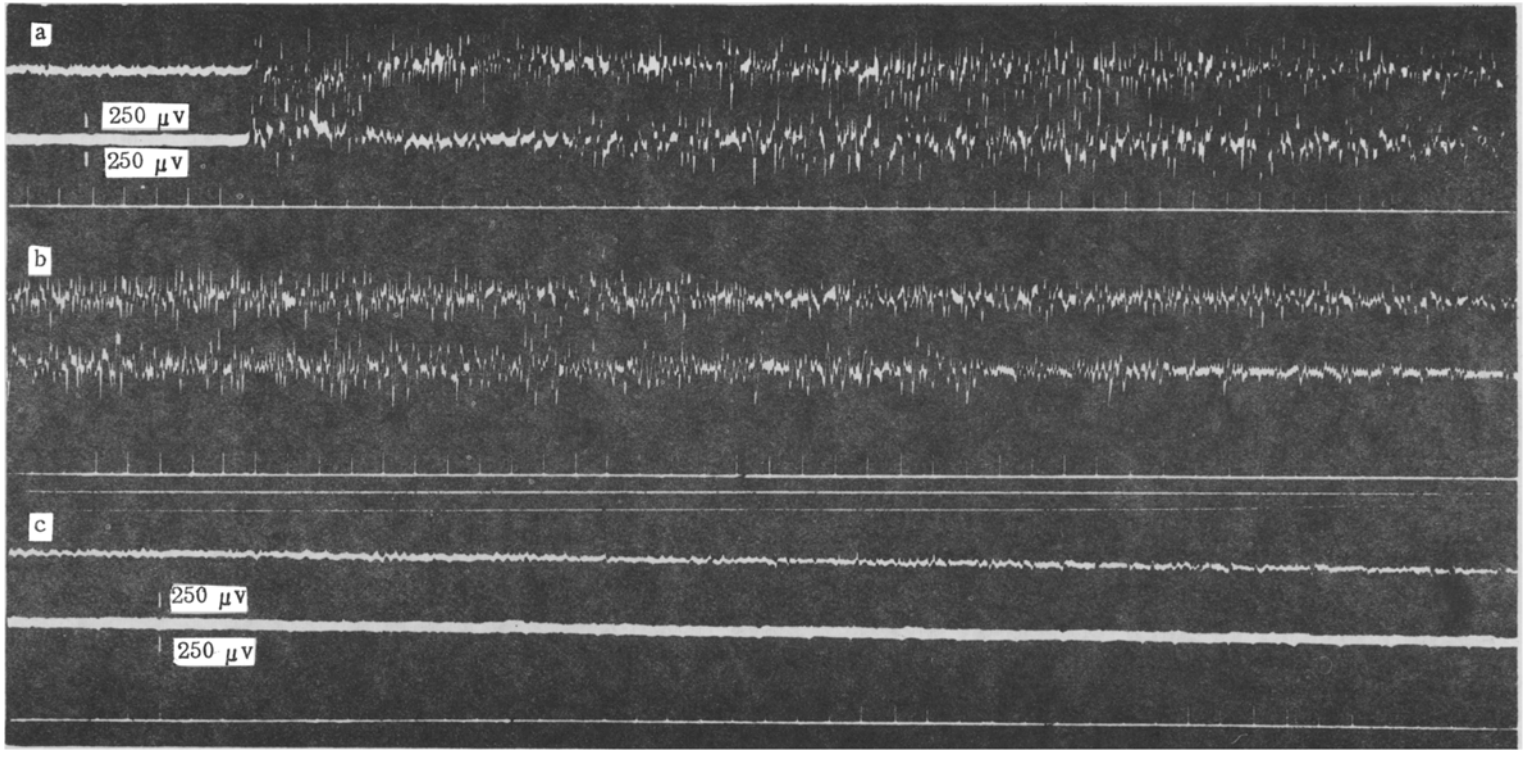

Fig. 2. Electrical activity in the dorsal neck muscles (upper tracing of each pair) and in the posterior group of muscles of the right thigh (lower tracing of each pair) on gentle flexion of the left (a and b) and right (c) foot.
Oscillogram b is the continuation of a, and is shortened to 3.5 cm (0.17 seconds). Other curves as in Fig. 1. Same experiment.

fields (e.g. that of the opposite limb) are reduced (see Fig. 3). At the peak of the tetanus even the weakest stimulus may induce a convulsive attack.

It was pointed out above that the duration of the convulsions induced by stimulation of the tetanized limb increases according to the severity of the tetanus. The electrical activity in the various muscle groups, which develops during the convulsions and indicates stimulation of the motoneurons, does not cease at the end of stimulation, but continues for a certain time and gradually fades away (see Fig. 1, a and Fig. 2, a and b). Experiments have shown that the after discharge is not caused by additional stimuli originating from the periphery, as similar effects are found in experiments where the central ends of the sciatic nerve are stimulated after high section of the other nerve trunks. It follows from the work of Eccles and his co-workers [10] that the synapses on the motoneuron cells do not initiate this after discharge. When it is realized that the length of the after discharge of the motoneurons depends chiefly on the number of interneurons which are involved and which form closed self-excitatory loops [1,13,14,15,18,22], it follows that the number and the degree of involvement of the interneurons increases according to the severity of the tetanus.

The above results give reason to suppose that the action of the tetanus toxin is not confined to the motoneurons, but spreads through the synapses to affect other reflex arcs. In this respect, the mechanism of action of tetanus toxin closely resembles that of strychnine poisoning.

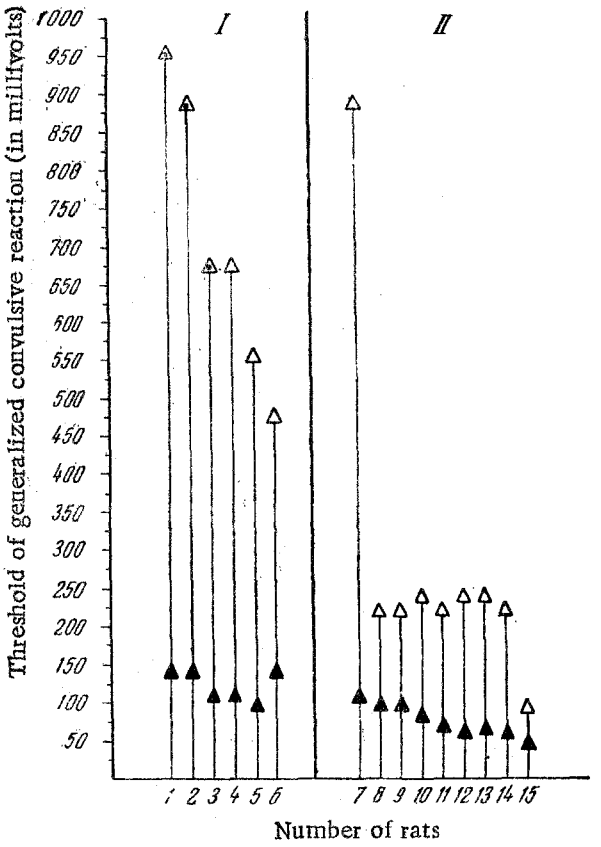

Fig. 3. Thresholds of generalized convulsive reaction caused by stimulating the central ends of the left (tetanized limb) and the right sciatic nerves.

▲) left sciatic nerve; △) right sciatic nerve. The sign ◬ indicates that stimulation of the right sciatic caused no definitive convulsions at the indicated threshold or at higher levels of excitation. I and II) stages in the development of generalized tetanus. I) first signs of segmental tetanus, increased general excitability; II) clearly shown or completely developed segmental tetanus, great increase in general excitability, spontaneous convulsions in some of the rats. In Rat No. 7 there was a clearly shown segmental tetanus, but a relatively small increase in general reflex excitability.

Reactions of Rats Infected with Tetanus Toxin to Stimulation of the Sensory and Motor Spinal Roots Innervating the Tetanized Limb

Number of rats	Weight in grams	Dose of toxin in ml	Day of illness	Description of condition	Roots stimulated		Strength of stimulus (in mv)	Reaction
1	165	0.2	3	Signs of segmental tetanus. Increase of general excitability.	Sensory	L_5+L_6	100	++
					Motor	L_6	950	—
2	165	0.2	3	The same	Sensory	L_5+L_6	145	++
				" "	Motor	L_6	950	—
3	160	0.2	4		Sensory	L_5+L_6	70	++
				" "	Motor	L_5+L_6	950	—
4	165	0.2	5		Sensory	L_6	70	++
					Motor	L_6	950	—
5	165	0.2	3	Signs of segmental tetanus. Considerable increase in general excitability, spontaneous convulsions.	Sensory	L_5+L_6	45	+++
					Motor	L_5+L_6	950	—
6	165	0.2	3	The same	Sensory	L_5+L_6	45	+++
					Motor	L_6	950	—
7	165	0.2	4	Segmental tetanus. Increase in general excitability.	Sensory	L_5+L_6	60	++
					Motor	L_5+L_6	950	—
8	165	0.2	4	The same	Sensory	L_6	60	+++
					Motor	L_6	950	—
9	160	0.2	3	Segmental tetanus. Considerable increase in general excitability, spontaneous convulsions.	Sensory	L_5+L_6	50	+++
					Motor	L_5+L_6	950	—
10	165	0.2	3	The same	Sensory	L_5, L_6	50	++
					Motor	L_5+L_6	950	—
11	165	0.2	3	" "	Sensory	L_5+L_6	45	++
					Motor	L_5+L_6	950	—
12	165	0.2	3	" "	Sensory	L_5, L_6	50	++
					Motor	L_5+L_6	950	—
13	165	0.2	3	" "	Sensory	L_5, L_6	50	+++
					Motor	L_5+L_6	950	—
14	165	0.2	4	" "	Sensory	L_5+L_6	45	+++
					Motor	L_6	950	—
15	165	0.2	6	" "	Sensory	L_5, L_6	40	+++
					Motor	L_5+L_6	950	—

Note: $L_5 + L_6$ indicates simultaneous stimulation of the two roots; L_5, L_6 – separate stimulation of each; ++ strong generalized convulsions; +++ very strong generalized convulsion; — no convulsions.

SUMMARY

Experiments were performed on white rats, affected by ascending general tetanus. The general convulsions were provoked with great ease by applying stimulation to the extremity into which a lethal dose of tetanus toxin had been introduced. This phenomenon was not connected with increased excitability of extero- and proprioceptors and cannot be explained by the functional changes of the synapses, which have an inhibiting effect on the motor cells of the anterior horns of the spinal cord. The data which were obtained allow us to conclude that the effect of tetanus toxin is not limited to the area of motoneurons but that it, likewise, spreads to the synaptic formations of other portions of the multineuronic reflex arcs.

LITERATURE CITED

[1] M. Breize, Electrical Activity of the Nervous System.* IL, Moscow, (1955).

[2] N. V. Golikov, Leningrad State University Science Notes, biological series, 16, 99, 6-45 (1949).

[3] E. A. Gromova, Byull. Eksptl. Biol. i Med., 12, 29-34 (1953).

[4] E. A. Gromova, In the book: The Problem of Reactivity in Pathology,** Moscow, 66-73 (1954).

[5] G. N. Kryzhanovsky, Investigation of the experimental physiological analysis of the mechanism of tetanus. Candidate's dissertation, Moscow, (1952).

[6] G. N Kryzhanovsky, In the book: The Problem of Reactivity and Pathology,** Moscow, 52-60 (1954).

[7] C. Sherrington, in the book: The Reflex Activity of the Spinal Cord*, 212-215 (1935).

[8] I. I. Abel, W. M. Firor and W. Chalian, Bull. Johns Hopkins Hospital, 63, 373-403 (1938).

[9] G. H. Acheson, O. D. Ratnoff and E. B. Schoenbach, J. Exp. Med., 75, 5 465-480 (1942).

[10] V. B. Brooks, D. R. Curtis and J. C. Eccles, J. Physiol., 135, 655-672 (1957).

[11] J. R. Davies, R. S. Morgan, E. A. Wright and G. P. Wright, Arch. internat. Physiol., 62, 2, 248-263 (1954).

[12] J. C. Eccles, P. Fatt and K. Koketsu, J. Physiol., 126, 3, 524-562 (1954).

[13] A. Forbes, Amer. J. Physiol., 56, 1, 273-312 (1921).

[14] A. Forbes, Physiol. Rev.,2, 361 (1922).

[15] K. Frank and M.G. F. Fuortes, J. Physiol., 131, 2,424-435 (1956).

[16] U. Friedemann, A. Hollanber and G. M. Tarlov, J. Immunol., 40, 3, 325-364 (1941).

[17] G. Liljestrand and R. Magnus, Pfluger's Arch., 176, 1-2, 168-208 (1919).

[18] R. Lorente de No , Amer. J. Physiol., 113, 3, 505-523 (1935).

[19] H. Meyer and F. Ranson, Arch. exper. Pathol. u Pharmak., 49, 6, 369-416 (1903).

[20] A. Perdrup, Acta pharm.e toxicol, 2, 121-137 (1946).

[21] W. S. Ranson, Arch. Neurol. a. Psychiatr., 20, 663-701 (1928).

[22] S. W. Ranson and J. C. Hinsey, Amer. J. Physiol., 94, 2, 471-495 (1930).

[23] B. J. Renshaw, Neurophysiol., 4, 2, 167-183 (1941).

[24] B. J. Renshaw, Neurophysiol., 9, 3, 191-204 (1946).

[25] H. Schaefer, Arch exp. Path. u. Pharm. 203, 1, 59-84 (1944).

[26] E. A. Wright, J. Immunol., 71, 2, 41-44 (1953).

[27] E. A. Wright, R. S. Morgan and G. P. Wright, Lancet 11, 6729, 316-319,(1952).

* Russian translation.

** In Russian.