EXPERIMENTAL DATA ON BIOCHEMICAL DISTURBANCES IN TETANUS

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UDC 616.981.551-092.9-008.9-092.18

The effect of hypoxia and also, evidently, of tetanus toxin, on many aspects of metabolism is extremely unfavorable. The creatine kinase and lactate dehydrogenase activity in the blood serum was increased as a result of release of the enzyme from skeletal muscle and also of an increase in the catalytic power of the enzyme. The hypoxia developing during tetanus led to nonrespiratory acidosis and to an increase in the potassium content. The blood sugar rose, evidently on account of a compensatory reaction to the sudden and considerable expenditure of energy during tetanic convulsions.

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Among the various pathological disturbances arising in tetanus an important place is occupied by biochemical changes in metabolism. However, the character and causes of these disturbances have not yet been explained.

The object of the present investigation was to study biochemical changes developing at various periods of tetanus in experiments on dogs.

EXPERIMENTAL MATERIAL AND METHOD

Healthy mongrel dogs of both sexes and of different weights were used in the experiments. Tetanus toxin was injected intramuscularly into the animals' right leg in a dose of 1 LD for dogs, after which a picture of general ascending tetanus developed in all the animals, terminating in death at the end of the 1st or beginning of the 2nd week.

The following biochemical indices were investigated: blood sugar [10], acid-base balance by Astrup's method [6], plasma electrolytes [12], activity of lactate dehydrogenase (LDH), creatine kinase, aldolace, and aspartate- and alanine-amino-transferase [11, 23, 24], activity of the iscenzymes of lactate dehydrogenase and creatine kinase in blood serum and tissue homogenates [5, 18], and also the water-soluble proteins of the heart, liver, skeletal muscle, and kidneys by electrophoresis in agar gel [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The results obtained revealed an increase in the blood sugar during tetanus (76 ± 4.4 mg% in healthy dogs, 144.2 ± 8.8 and 161 ± 7.2 mg% on the 4th and 7th days of development of tetanus respectively). These results agree with experimental data published in the literature [7, 8, 19, 20]. According to some workers hyperglycemia is a nonspecific response to a stress stimulus. It may be postulated that tetanic convulsions lead to a sudden and excessive expenditure of energy, giving rise to the compensatory response of elevation of the blood sugar.

It was therefore interesting to study changes in the ATP content in the rigid and healthy limbs. The published data on this matter are conflicting [7, 21]. An increase in ATPase activity in tetanus has been observed by several authors [14, 16]. It has also been shown that the compensatory formation of high-energy compounds in tetanus takes place on account of stimulation of lipid metabolism [2, 8]. However, such a compensatory mechanism can be assumed only at the time during tetanus before the increase in ketone bodies and the ketonemia have given rise to marked metabolic changes leading to acidosis. The ADP–creatine phosphate (CP) metabolism is considerably increased during hypoxia [3]. S. N. Lyalova and

Department of Normal Physiology and Pathophysiology and Clinic for Faculty Surgery, S. M. Kirov Military Medical Academy, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 66, No. 7, pp. 53-56, July, 1968. Original article submitted February 21, 1966.

TABLE 1. Changes in Activity of LDH Isoenzymes and Total Activity of Serum CK and LDH of Dogs during Tetanus, M ± m

Group	No. of observations	Total CK activity in μ/M ml/min)	Total DH activity (in units/ml)	Activity of isoenzymes (in units/ml)				
				LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Healthy animals	20	1,06±0,2	98,3±6,0	21,3±5,1	16,0±2,7	22,9±4,4	14,3±2,2	23,8±4,0
Tetanus, 4 days	8	3,27±0,8	133,9±7,8	26,3±3,6	21,2±2,2	27,8±2,9	19,2±1,0	39,4±5,1
days	8	8,6±1,3	195,9±9,2	31,1±4,2	29,2±3,9	31,1±2,2	28,3±4,0	76,2±8,9

TABLE 2. Indices of Acid-Base Balance in Dogs with Tetanus, M ± m

Group	No. of observations	pH	pCO_2	BE *
Healthy	20	7,30±0,2	36,0±0,3	—7,1±0,28
4 days	4	7,32±0,4	38,1±0,4	—7,6±0,4
7 »	7	7,27±0,4	37,4±0,3	—10,4±0,5

*BE denotes excess or deficiency of buffer bases.

N. S. Panteleeva [3] have demonstrated a speeding-up of the exchange between ADP and CP during tetanic contractions of the frog gastrocnemius muscle. This reaction is catalyzed by creatine kinase (CK). During experimental poisoning with tetanus toxin the serum CK activity in our investigations was increased 8 times on the 7th day (Table 1). This fact must be related both to hypoxic injury to the cell membrane and to release of enzyme from the tissues, and also to an increase in CK activity in the tissue. Skeletal muscle is known to contain about 50% of the isoenzyme LDH_5, with the slowest electrophoretic mobility [25]. In tissue hypoxia, LDH leaves the cells for the blood stream [17]. On the 7th day of experimental tetanus activity of LDH_5 (the isoenzyme with the slowest electrophoretic mobility) increased from 23.8 to 76.2 units/ml. Activity of LDH_4 and LDH_3 was increased at the same time. This relationship could be clearly seen as a result of isoenzyme studies of the blood serum of all the experimental animals. The results obtained for the LDH isoenzymes indicated release of LDH mainly from muscle tissue. Other evidence of this was given by investigation of the activity of CK isoenzymes. In this case, during the development of experimental tetanus the CK activity increased mainly on account of the muscle CK isoenzyme, CK_3. These results confirm those obtained during recently published investigations [18].

Investigation of aldolase and aspartate- and alanine-aminotransferase activity showed that the activity of these enzymes rose very slightly as the disease progressed, in general agreement with published findings [22].

Evidence of tissue hypoxia in tetanus is given by results obtained during investigation of the acid-base balance by Astrup's micromethod (Table 2).

It is considered that the initial alkalosis arising during tetanus as a result of hyperventilation changes into nonrespiratory acidosis [13], due according to some investigators both to hemodynamic disturbances and to the action of the toxin [1]. In our investigations a tendency for the pH to change slightly toward the alkaline side appeared by the 4th day of the experiment, and by the 7th day a definite nonrespiratory acidosis was present (pH 7.27; pCO_2 37.4; BE = −10.4). It is evident that in tetanus the prevention of changes in acid-base balance is a matter of definite importance. Sodium lactate [2] is suitable for this purpose, and for the treatment of tetanus under clinical conditions we use tris-buffer (trihydroxymethylaminomethane).

Because of the developing hypoxia in tetanus, it was decided to study changes in electrolytes, particularly in the serum potassium and sodium concentration. The potassium concentration was found to have risen on the 7th day to 4.3 ± 0.26 meq/liter, and this was accompanied by a slight fall in the sodium level.

Our study of the content of water-soluble proteins in the skeletal muscle, heart, liver, and kidneys by electrophoresis in agar gel revealed no significant changes in these proteins.

The results obtained indicate that tetanus toxin and hypoxia have an extremely unfavorable effect on many aspects of metabolism. This is particularly true of changes in energy and carbohydrate metabolism. Permeability of the cell membranes is disturbed and the activity of CK and LDH in the blood serum is in-

creased mainly on account of release of the enzymes from the skeletal muscle (however, some increase in the catalytic power of the enzymes cannot be ruled out in addition).

The hypoxia developing during tetanus leads to nonrespiratory acidosis and to an increase in the potassium concentration; the blood sugar also rises, evidently because of a compensatory reaction to the sudden and considerable expenditure of energy during tetanic spasms.

Because of the great variety of changes taking place in metabolism, it is difficult to organize pathogenic therapy, which must take into account not only the action of the toxin, but also the hypoxic consequences of tetanus.

LITERATURE CITED

1. V. F. Duboskaya, Sov. Med., No. 8, 60 (1963).
2. G. A. Ivashkevich and A. S. Luchko, Abstracts of Proceedings of the 1st Conference of Anesthesiologists of the Ukrainian SSR [in Russian], Kiev (1964).
3. S. N. Lyzlova and N. S. Panteleeva, in: Physiology and Biochemistry of the Nervous System and Muscles [in Russian], Leningrad (1957).
4. I. M. Markelov, Changes in the Fractional Composition of the Heart Proteins in Experimental Myocardial Infarction and Radiation Sickness [in Russian], Candidate dissertation, Leningrad (1964).
5. I. M. Markelov, Lab. Delo, No. 12, 707 (1966).
6. R. A. Meitina, E. I. Mironova, É. D. Nisnevich, et al., Éksper. Khir., No. 5, 29 (1962).
7. N. P. Meshkova, R. A. Matveeva, and L. S. Shkarenkova, Vopr. Med. Khimii., No. 1, 85 (1961).
8. N. P. Meshkova, T. Yu. Lipskaya, and N. A. Oreshnikova, Vopr. Med Khimii, No. 1, 78 (1962).
9. M. E. Raiskina, Vopr. Med. Khimii, No. 2, 83 (1959).
10. A. B. Raitsis and O. A. Usinova, Lab. Delo, No. 1, 29 (1965).
11. V. I. Tovarnitskii and E. N. Voluiskaya, Lab. Delo, No. 6, 7 (1955).
12. I. Todorov, Clinical and Laboratory Investigations in Pediatrics [in Russian], Sofia (1963).
13. P. I. Shelestyuk, Abstracts of Proceedings of the 1st Conference of Anesthesiologists of the Ukranian SSR [in Russian], Kiev (1964).
14. S. Asakura, T. Soki, S. Oki, et al., Med. J. Osaka Univ., 8, 289 (1958).
15. S. Asakura, T. Soki, S. Oki, et al., Med. J. Osaka Univ., 8, 279 (1958).
16. S. Asakura, T. Soki, S. Oki, et al., Med. J. Osaka Univ., 8, 653 (1958).
17. F. H. Bruns, in: Europlan Symposium of Medical Enzymology, Proceedings, Basel (1961), p. 71.
18. I. A. Brody and M. A. Hatcher, Arch. Neurol. (Chicago), 16, 89 (1967).
19. I. A. Brody and M. A. Hatcher, J. Infect. Dis., 114, 157 (1964).
20. B. Mach and Z. Skawikska, Przegl. Epidem., 14, 137 (1960).
21. L. Michelazzi, M. Mor, and M. Dianzani, Experientia (Basel), 11, 73 (1955).
22. D. Mullan and V. Dubowitz, Lancet, 2, 505 (1964).
23. H. Muller, A. Wollenberger, and A. Kovarikowa, Dtsch. Gesund-Wes., 17, 1055 (1962).
24. S. Reitman and S. Frankel, Am. J. Clin. Path., 28, 56 (1957).
25. T. Wieland and G. Pfleiderer, Biochem. Z., 329, 112 (1957).