2. E. V. Montsevichyute-Éringene, Pat. Fiziol., No. 4, 71 (1964).
3. V. N. Nikitin, in: Problems in Gerontology and Geriatrics [in Russian], Leningrad (1962), pp. 13-20.
4. Yu. S. Nikolaev, "Weight-reducing diatetic treatment of schizophrenia and its physiological basis," Author's Abstract of Doctoral Dissertation, Moscow (1960).
5. I. A. Oivin, Pat. Fiziol., No. 4, 76 (1960).
6. A. A. Pokrovskii, Yu. S. Nikolaev, L. I. Lando, et al., Sov. Med., No. 2, 70 (1969).
7. É. P. Tsyganov and V. A. Shaternikov, Vopr. Pitan., No. 6, 16 (1973).
8. D. F. Chebotarev and V. V. Frol'kis, The Cardiovascular System in Aging [in Russian], Leningrad (1967).
9. J. Corvilain, H. Loeb, A. Champenois, et al., Lancet, 1, 534 (1961).
10. D. Foster, J. Clin. Invest., 46, 1283 (1967).
11. B. Person, B. Hood, and G. Angervall, Acta Med. Scand., 188, 225 (1970).

CHANGES IN CORTICAL ELECTRICAL ACTIVITY IN POISONING BY Clostridium perfringens TYPE A TOXIN

A. V. Tselukh, R. F. Makul'kin, and G. N. Kryzhanovskii*

UDC 612.822.3.014.46:576.851.555.097.29

In cats lightly anesthetized with pentobarbital (15-20 mg/kg) cortical electrical activity was recorded during the development of poisoning by the toxin of Clostridium perfringens type A, injected intramuscularly (100 MLD/kg). Changes in cortical electrical activity occurred in two phases. In the first phase desynchronization of activity, preservation of evoked potentials and changes in the rhythm structure in response to photic stimulation were observed. Desynchronization was not observed after preliminary mesencephalic section (mesencephalic preparation), indicating involvement of the reticular formation in the pathological process and its role in the desynchronization effect. In the second phase cortical electrical activity was deeply inhibited, evoked potentials depressed, and the rhythm reconstruction reaction was disturbed.
KEY WORDS: electrocorticogram; electromyogram; evoked potential; rhythm reconstruction reaction; electrocardiogram; Clostridium perfringens type A toxin.

There is clinical and experimental evidence [1-3, 7, 8, 12, 13, 17] of a lesion of the CNS in anaerobic gas gangrene infection. Many aspects of the nature and pathogenetic mechanisms of these lesions still remain unexplained.

The object of this investigation was to study cortical electrical activity in the course of development of poisoning caused by Clostridium perfringens type A toxin.

EXPERIMENTAL METHOD

Experiments were carried out on 32 cats. In the experiments of series I (17 animals) intact cats were used, and to synchronize their EEG potentials, pentobarbital was injected intraperitoneally in a dose of 15-20 mg/kg; in series II (15 animals) the experiments were carried out on cats with high mesencephalic section. The brain stem was divided between the superior colliculi and thalamus. A wedge-shaped section of the mesen-

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Departments of Microbiology and Pathological Physiology, N. I. Pirogov Odessa Medical Institute. Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1192-1198, October, 1976. Original article submitted May 21, 1976.

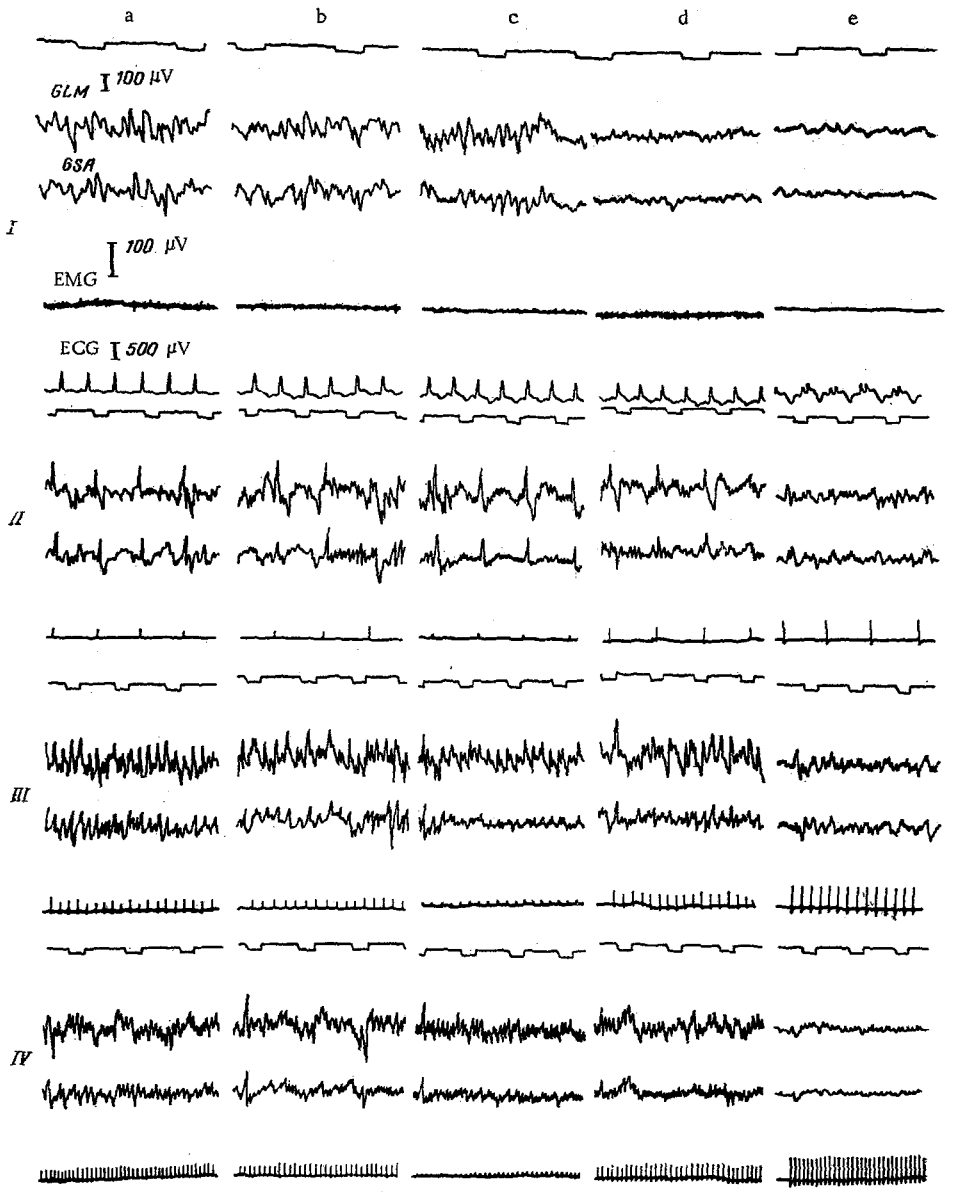

Fig. 1. ECoG, EMG, ECG, EP, and RRR during development of poisoning by Cl. perfringens type A toxin: a) before injection of toxin; b, c, d, e) 1, 3, 9, and 24 h respectively after injection of toxin. Here and in Fig. 3: I) ECoG, EMG of antigravity neck muscles, and ECG; II) EP; III and IV) RRR to repetitive flashes with frequencies of 5 and 10 Hz respectively. GLM) Middle part of lateral gyrus; GSA) anterior sigmoid gyrus; ECG in lead II. Time marker 1 sec. Signal calibration: 100 μV for ECoG and EMG, 500 μV for ECG.

cephalon was carried out by Villablanca's method [20]. The preparatory operations (tracheotomy, trephining of the skull, and insertion of the electrodes) were carried out under local anesthesia (1% procaine solution). The mesencephalon was divided by a thin spatula under brief intratracheal anesthesia, which was stopped immediately after completion of the section.

The spontaneous electrocorticogram (ECoG), evoked potentials (EP) to single flashes (energy 0.3 J, distance between source of light and cornea 40 cm), and the rhythm reconstruction response (RRR) were recorded in intact cats and in the mesencephalic preparations. The EP were recorded simultaneously on an ink-writing electroencephalograph and with the For-2 camera from the screen of an S-1-16 cathode-ray oscilloscope. From 10 to 15 EP were superposed. Values of the latent period, amplitude, and duration of the positive and negative components of EP were subjected to statistical analysis and the criteria of significance (P) of these values were determined. The F-1-0.2 flash generator was used as the source of photic stimulation.

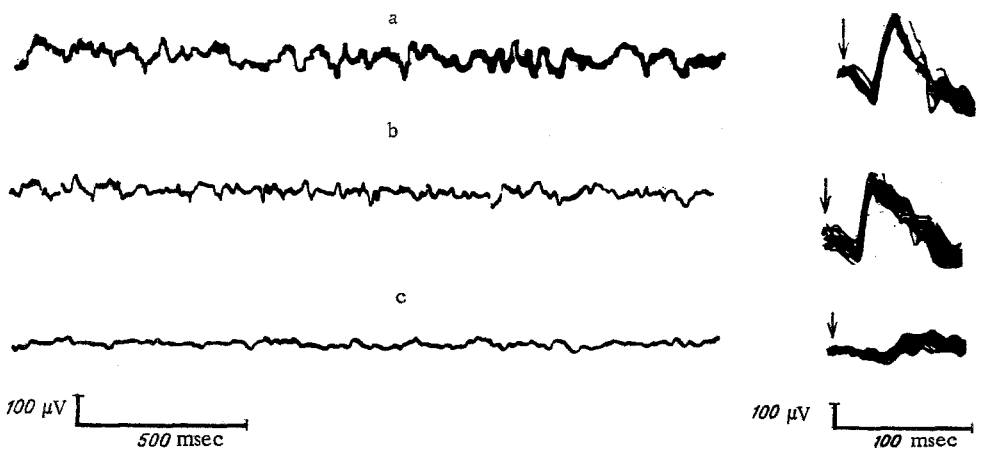

Fig. 2. ECoG and EP in cortex of cat at different stages of poisoning by Cl. perfringens type A toxin: a) ECoG and superposed EP before injection of toxin; b) during desynchronization; c) during depression. Time marker 100 and 500 msec. Signal calibration 100 μV.

To study changes in the functional state of the brain stem at various times after injection of the toxin (100 MLD/kg, intramuscularly), the electromyogram (EMG) of the antigravity muscles of the neck was recorded by needle electrodes. The ECG also was recorded.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I the effect of the toxin on the spontaneous ECoG, the EMG of the antigravity muscles of the neck, ECG, EP, and RRR in animals in a state of pentobarbital sleep was investigated. Before injection of the toxin (Fig. 1a. I) waves in the α and θ range predominated in the occipital and frontal regions, the antigravity muscles of the neck, according to the EMG, were in a state of marked tonic contraction, the rhythm of cardiac contractions was regular, and the main components of the ECG were clearly defined. In response to single flashes EP were recorded in the visual and sensomotor cortex after an identical latent period (Fig. 1a, II). During repetitive photic stimulation a generalized RRR was formed in the θ and Δ range (5-10 Hz; Fig. 1a, III, IV).

One hour after injection of the toxin EEG, EP, and RRR were substantially unchanged (Fig. 1b, I-IV). Inversion of the T wave was observed on the ECG but the cardiac rhythm was unchanged (Fig. 1b, I). The pattern of spontaneous and evoked cortical electrical activity 3 h after injection of the toxin remained the same (Fig. 1c, I-IV). The cardiac rhythm was regular and the negativity of the T-wave a little increased (Fig. 1c, I). Depression of slow-wave activity and enhancement of fast low-voltage activity in the β range were observed after 9 h on the EEG (Fig. 1b, I). The amplitude of the QRS complex of the ECG was reduced, and the T wave remained consistently negative during prolonged recording of the ECG (Fig. 1d, I). EP as before were clearly defined in the occipital and frontal leads, and RRR to repetitive flashes with frequencies of 5 and 10 Hz showed no

TABLE 1. Latent Period, Duration, and Amplitude of Positive and Negative Components of the EP to Photic Stimulation during Poisoning by Cl. perfringens Toxin

Phases of ECoG changes	Latent period of EP			Positive component of EP						Negative component of EP					
				duration, msec			amplitude, μV			duration, msec			amplitude, μV		
	$M \pm m$	t	P	$M \pm m$	t	P	$M \pm m$	t	P	$M \pm m$	t	P	$M \pm m$	t	P
Initial spontaneous EEG	13,9±0,12			10,85±0,33			62,1±3,2			46,9±0,5			247,8±4,1		
Phase of EEG desynchronization	14,1±0,2	0,90	>0,05	10,4±0,32	0,99	>0,05	61,4±4,3	0,15	>0,05	45,3±0,8	1,6	>0,05	238,0±9,1	0,98	>0,05
Phase of EEG depression	29,3±0,5	28,1	<0,001	20,9±0,58	14,9	<0,001	22,0±1,6	18,1	<0,001	38,8±1,9	4,5	<0,001	49,2±4,5	31,1	<0,001

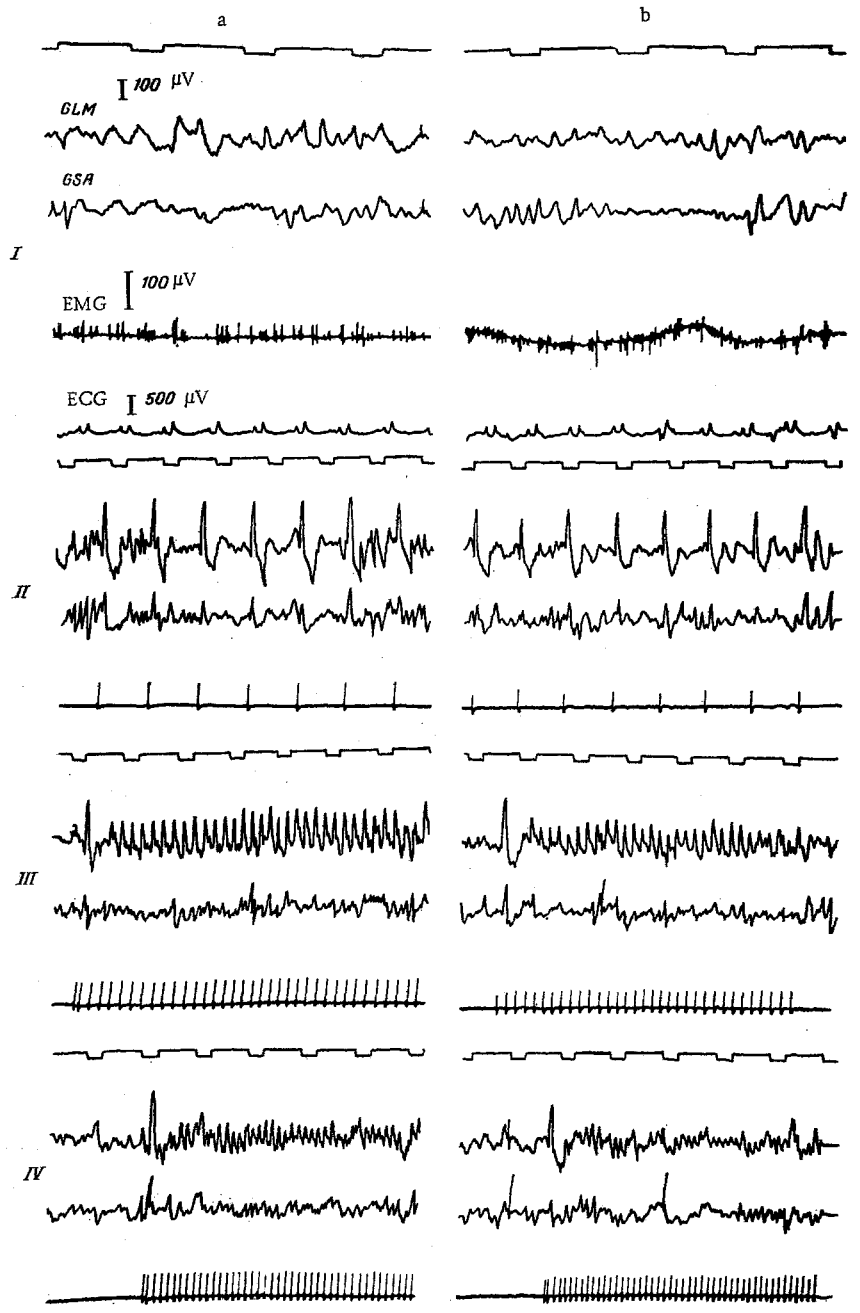

Fig. 3. ECoG, EMG, ECG, EP, and RRR during development of poisoning by Cl. perfringens type A toxin, in cat cerveau isolé preparation: a) before injection of toxin; b, c, d, e) 1, 6, 24, and 30 h respectively after injection of toxin.

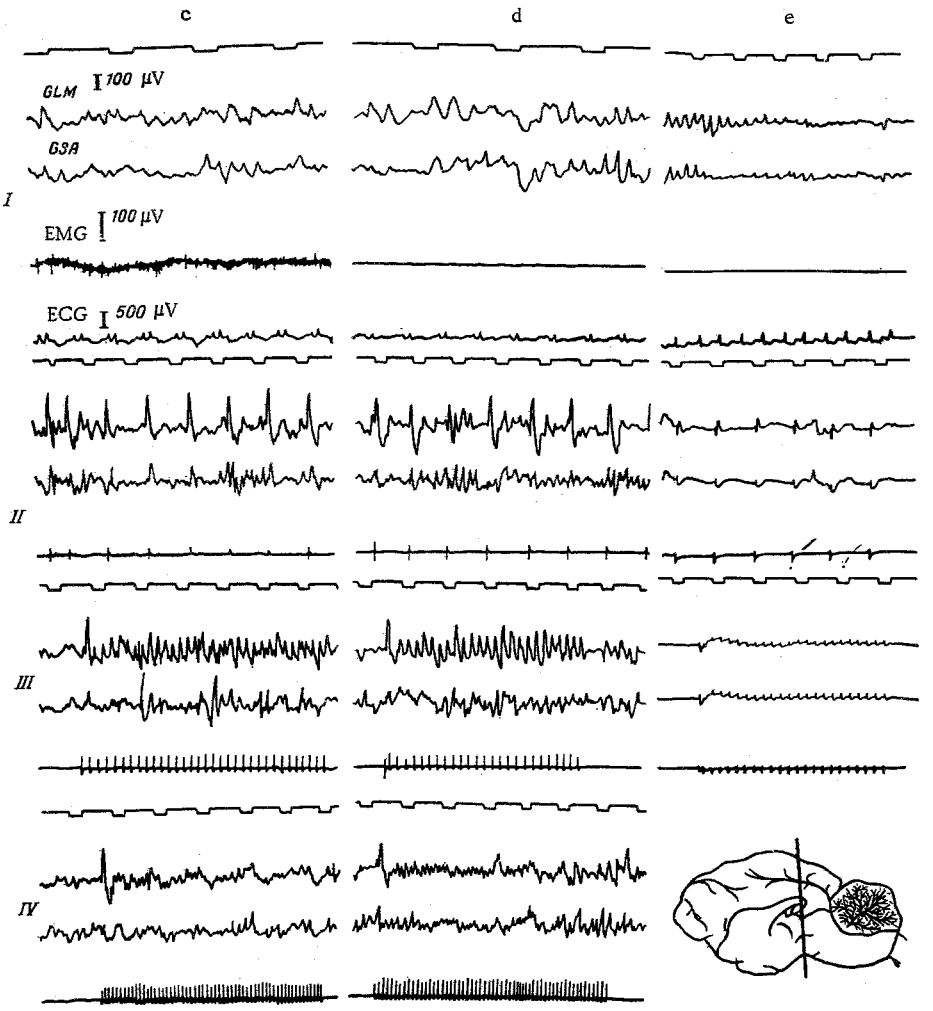

Fig. 3 continued

appreciable change (Fig. 1d, II-IV). The ECoG was very much flatter after 24 h and no EMG potentials were recorded from the neck muscles; the ECG showed sharp changes: a decrease in amplitude of the R wave, a dome-shaped ST segment, and negative T wave; low-amplitude EP developed in response to single flashes. The RRR in response to repetitive photic stimulation with a frequency of 5 Hz were poorly defined, and at a frequency of 10 Hz they were absent (Fig. 1e, I-IV).

Since it is difficult to obtain the precise characteristics of EP when recorded on an ink-writing electroencephalograph, in some experiments they were recorded on a cathode-ray oscilloscope with a high scanning speed by the superposition method (Fig. 2). The numerical data are given in Table 1. Before injection of the toxin waves were recorded on the ECoG in the α and Δ range, and the EP were clearly defined and had the ordinary configuration (Fig. 2a). The numerical characteristics of the superposed EP are given in Table 1. On repeated recording of the ECoG, desynchronization remained stable and low-voltage waves with frequencies of 17-21 Hz were dominant, although irregular slow waves in the Δ range also were recorded (Fig. 2b). Statistical analysis showed that the latent period, amplitude, and duration of the positive and negative components of the EP, recorded against this background of the EEG, were essentially indistinguishable from those observed before injection of the toxin. During flattening of the EEG (Fig. 2c) the latent period was considerably increased but the amplitude of the positive and negative components was reduced (Table 1). In the animals in the early stages of poisoning by Cl. perfringens type A toxin desynchronization of the ECoG thus developed and, as the EP showed, the functional state of the cortex remained good. Later the ECoG became depressed and this was accompanied by marked worsening of the functional state of the cortex.

The desynchronizing effect thus observed could depend on activation of the brain-stem reticular formation or on depression of the thalamocortical synchronizing system. To decide this issue experiments were carried out on "ecéphale isolé" preparations with total mesencephalic section.

The results of this series of experiments showed that the dynamics of changes in spontaneous and evoked cortical electrical activity differed significantly from that in the intact animals. From 1 to 3h after mesencephalic section the EEG was dominated by waves with a frequency of 7-8 Hz and by irregular waves in the Δ band. The antigravity neck muscles, as the EMG showed, were in a state of tonic contraction. No signs of cardiac arrhythmia were found on recording the ECG. The EP were well defined in the visual and projection cortex and they were also recorded in the sensomotor cortex, although their amplitude in this area was much lower. Under the influence of regular flashes (5-10 Hz) an RRR was formed mainly in the visual cortex (Fig. 3a, I-IV). One hour after injection of the toxin the ECoG as before was dominated by waves in the α range, the tone of the antigravity neck muscles remained high, and some changes were found in the ECG (in individual complexes the T wave was negative and a small increase was found in the P and R waves). Evoked potentials and RRR to repetitive flashes with frequencies of 5 and 10 Hz showed no significant change (Fig. 3b, I-IV). A similar pattern of the spontaneous ECoG, EP, RRR, and EMG also was observed 6 h after injection of the toxin (Fig. 3c, I-IV). An increase in amplitude of the negative T wave was observed on the ECG. Later, 24 h after injection of the toxin (Fig. 3d, I-IV), α-like waves with a frequency of 7-8 Hz and slow waves in the α range were recorded in the ECoG. The EMG was strongly inhibited. The amplitude of the P waves and of the QRS ventricular complex was reduced, and the ST segment was now convex in shape. Evoked potentials and RRR were substantially unchanged during repetitive photic stimulation with a frequency of 5 Hz. In response to regular flashes at 10-Hz rhythm binding to the flashes was complete during the first 4 sec, after which the reproduction of these frequencies was disturbed. After 30 h the ECoG was flattened, no EMG potentials were recorded, and the ECG was considerably modified: The amplitude of the R wave was increased and became irregular, and in some complexes the cardiac rhythm was irregular. In response to single flashes low-amplitude EP were recorded in the occipital leads only. The RRR was not reproduced in response to repetitive flashes (Fig. 3e, I-III).

During poisoning caused by Cl. perfringens type A toxin, under conditions of anatomical separation of the brain stem and forebrain, no marked changes were thus observed in the functional state of the cerebral cortex, unlike in intact animals. Since cortical activity in the θ and α rhythms was unchanged, it can be concluded that the toxin had no action on the thalamocortical synchronizing system, which is known to be the morphological and functional substrate for the generation of these cortical rhythms [18, 19]; consequently, the ECoG desynchronization effect observed in intact animals is attributable to involvement of the brain-stem activating reticular formation in the process.

Hypoxia in the initial stages of oxygen deprivation is known to produce a desynchronization effect [5]. It is possible that the hypoxic components may play a substantial role in the mechanism of the changes in brain electrical activity observed. Evidence in support of this hypothesis is given by changes in the ECG. However, these changes can also be interpreted differently. It has been shown [15] that after intravenous injection of Cl. perfringens toxin into rabbits, morphological features reflecting lesions of the inferior and anterior subendocardium and anterior pericardium are observed within 30-90 min, and they coincide with flattening or inversion of the T wave and ST segment and also with lengthening of the QRS complex, findings which indicate a disturbance of intraventricular conduction. Consequently, the changes observed in the ECG may be connected with the direct action of the toxin on the myocardium. It can accordingly be considered that the direct action of the toxin on the brain stem evidently plays a pathogenetic role in the origin of the desynchronization.

LITERATURE CITED

1. D. A. Arapov, Anaerobic Gas Gangrene Infection [in Russian], Moscow (1972).
2. N. N. Berezovskaya and L. I. Il'ina, Byull. Éksp. Biol. Med., No. 2, 44 (1954).
3. A. N. Berkutov, "Anaerobic Injection of gunshot wounds," Doctoral Dissertation, Leningrad (1951).
4. V. B. Votyakova, Abstracts of Proceedings of a Scientific Session of Minsk Medical Institute [in Russian], Minsk (1954), pp. 31-32.
5. A. M. Gurvich, Electrical Activity of the Dying and Reviving Brain [in Russian], Leningrad (1966).
6. R. Yu. Ilyuchenok, Neuro-Humoral Mechanisms of the Brain-Stem Reticular Formation [in Russian], Moscow (1965).
7. S. Z. Kostyukova, Klin. Med., No. 3, 49-54 (1945).
8. K. A. Kuz'mina, "Pathophysiological analysis of the mechanisms of action of toxins of gas gangrene on the nervous system," Doctoral Dissertation, Saratov (1968).
9. S. V. Magaeva and O. Ya. Ostryi, in: Neurotrophic Problems in the Theory and Practice of Medicine [in Russian], Moscow (1963), p. 18.

10. A. F. Popov, "The state of the autonomic innervation of the heart and blood vessels in experimental anerobic poisoning," Author's Abstract of Doctoral Dissertation, Kazan' (1973).
11. M. Sh. Promyslov, Dokl. Akad. Nauk SSSR, 80, No. 4, 653 (1951).
12. E. A. Skvirskaya, in: Problems in Immunopathology [in Russian], Moscow (1963), pp. 88-92.
13. A. V. Smol'yannikov, in: Textbook of Pathological Anatomy in Several Volumes [in Russian], Vol. 9, Moscow (1964), pp. 365-385.
14. E. M. Dempsey and R. S. Morison, Am. J. Physiol., 135, 301 (1942).
15. P. D. Ellner, J. Bact., 82, 275 (1961).
16. W. Feldberg, J. Physiol. (London), 193, 367 (1945).
17. E. Habermann, Arch. Pharmak. Exp. Path., 338, 502 (1960).
18. H. Jasper, Electroenceph. Clin. Neurophysiol., 1, 405 (1949).
19. G. Moruzzi and H. W. Magoun, Electroenceph. Clin. Neurophysiol., 1, 455 (1949).
20. J. Villablanca, Electroenceph. Clin. Neurophysiol., 19, 576 (1965).

ACETYLCHOLINESTERASE DURING AGING OF HUMAN ERYTHROCYTES

M. V. Kamyshentsev and M. N. Blinov

UDC 612.671.11.015.1:577.152.311

Acetylcholinesterase activity differs in the membranes of young, mature, and old human erythrocytes: It is highest in the mature and lowest in the old cells. The enzymes in young and mature erythrocytes is in the form of three, but in the old cells in the form of two molecular components. The results suggest that changes in the structural organization of acetylcholinesterase in the erythrocyte membrane have a direct bearing on the aging of red blood cells.
KEY WORDS: acetylcholinesterase; aging of erythrocytes.

Considerable progress has recently been made in the study of the structural chemical organization of the acetylcholinesterase (ACE) of erythrocytes, but the functional role of this enzyme, localized on the outer surface of the cell membrane, still remains uncertain [5, 6, 9, 12]. At the same time, it is known that during aging of the erythrocyte population the activity of this enzyme changes appreciably and becomes minimal in the osmotically most fragile old cells [8, 10].

The object of this investigation was to study whether a connection exists between the weakening of the osmotic resistance of the cell membrane (leading to aging and to subsequent death of the erythrocytes) and a disturbance of the molecular organization of ACE.

EXPERIMENTAL METHOD

To separate erythrocytes on the basis of their maturity, stepwise hemolysis [10] with NaCl solutions of different concentrations (0.40, 0.38, and 0.36%) was used. Membranes of hemolyzed erythrocytes were washed with solutions of low ionic strength to remove hemoglobin [7]. Acetylcholinesterase was solubilized by incubation of the cell "ghosts" in 0.5% (final concentration) solution of Triton X-100 for 3 h at 37°C, after which it was fractionated by electrophoresis in a disk of polyacrylamide gel (PAG) [2, 12]. The PAG was prepared with the polymer and copolymer in a ratio of 41 : 1 and polymerized at 60°C for 40 min. After completion of electrophoresis in gel, the activity of the enzyme was revealed [3]. The approximate molecular weight of the enzyme component was determined by electrophoresis of marker proteins of known molecular weight: yeast catalase

Laboratory of Biochemistry, Institute of Hematology and Blood Transfusion, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Il'in.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1198-1200, October, 1976. Orignal article submitted April 6, 1976.