CHANGES IN CORTICAL ELECTRICAL ACTIVITY FOLLOWING COMBINED ACTION OF *Clostridium perfringens* TOXIN AND METABOLIC PRODUCTS OF *Clostridium butyricum*

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A study of changes in the ECoG and ECG recorded during combined action of *Clostridium perfringens* type A toxin and filtrate of a broth culture of *Clostridium butyricum* showed that desynchronization and subsequent depression of cortical electrical activity developed later than after exposure to *Cl. perfringens* toxin alone. The general character of the changes in the cortical rhythm under these circumstances remained the same as after administration of *Cl. perfringens* toxin alone. ECG changes arose after shorter intervals. The *Cl. butyricum* filtrate caused changes in neither the ECoG nor the ECG. It is postulated that the effect of metabolic products of *Cl. butyricum* is to increase the permeability of the tissue barriers and, consequently, to promote penetration of *Cl. perfringens* toxin into the tissues, including into the CNS.

KEY WORDS: Clostridium perfringens; Clostridium butyricum; *desynchronization of rhythm; depression of cortical electrical activity; electrocorticogram; electrocardiogram.*

The clinical course of anaerobic gas gangrene is more severe in the case of wounds infected by microbial associations [1-4, 9, 10, 18]. This phenomenon has received little study. The combined action of toxins of tetanus [7, 8], gas gangrene [5, 6, 11, 12], and botulism [13] together with the metabolic products of commensal microorganisms is manifested as intensification of lethal, hemolytic, and other toxic effects. One of the associants of *Cl. perfringens* is *Cl. butyricum* [3, 17]. The writers showed previously [14, 15] that lethal and hemolytic effects are increased through the combined action of *Cl. perfringens* toxin and a filtrate of *Cl. butyricum*. A previous investigation [16] showed that brain electrical activity is significantly modified in poisoning caused by *Cl. perfringens* toxin.

The object of this investigation was to study changes in electrical activity of the cerebral cortex during the combined action of *Cl. perfringens* type A toxin and a filtrate of a broth culture of *Cl. butyricum*.

EXPERIMENTAL METHOD

Experiments were carried out on 53 cats under superficial pentobarbital anesthesia, maintained by periodic (every 6 h) injection of pentobarbital (15-20 mg/kg body weight intraperitoneally). The electrocorticogram (ECoG) was recorded on the 4-ÉÉG-1 electroencephalograph from electrodes implanted into the visual and sensomotor cortex; the reference electrode was fixed in the nasal bones. The rhythm reconstruction reaction (RRR) to flashes with a frequency of 5 and 10 Hz and a brightness of 0.3 J, the electrocardiogram (ECG), and evoked potentials (EP) to single flashes also were recorded. Superposed EP were photographed

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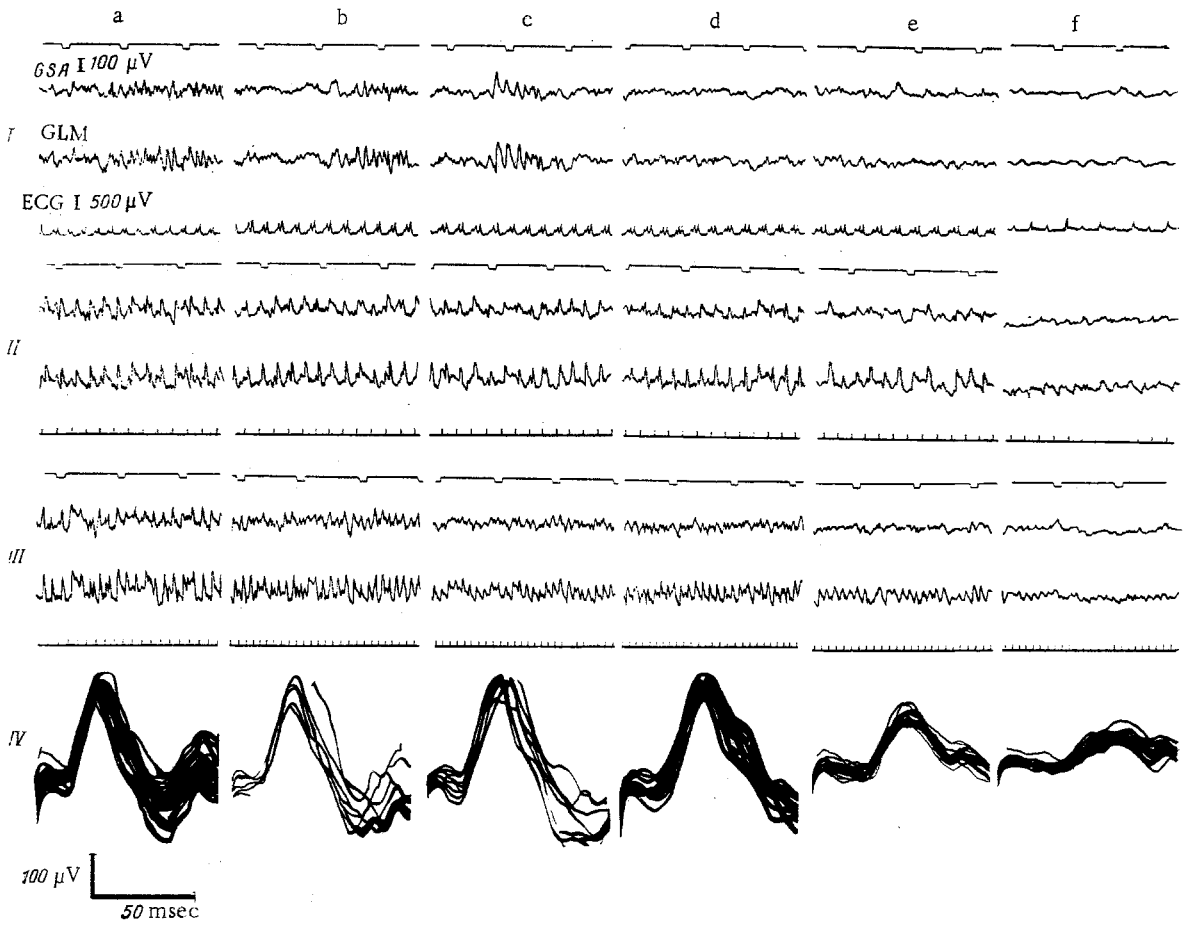

Fig. 1. ECoG, RRR, EP, and ECG at different stages of poisoning caused by *Cl. perfringens* type A toxin. Here and in Fig. 2: a) before injection of toxin; b-f) 3, 6, 9, 12, and 20 h after injection of toxin. ECoG and ECG (I), RRR to regular flashes with a frequency of 5 Hz (II) and 10 Hz (III), superposed EP (IV). Derivations: GSA) anterior sigmoid gyrus; GLM) middle part of lateral gyrus; ECG) lead (II). Time marker 1 sec. Stimulus calibration: 100 μV for ECoG and EP, 500 μV for ECG.

from the screen of an S-1-16 cathode-ray oscilloscope by means of a type FOR-2V camera. Flashes were generated by an FS-1-02 photostimulator. Spontaneous activity was recorded 1 h after the end of the preliminary operation. Purified *Cl. perfringens* type A toxin, diluted with physiological saline (100 MLD/kg body weight) and also *Cl. perfringens* toxin combined with filtrate of a broth culture of *Cl. butyricum* strain 237 (20-60 ml) were then injected into the thigh muscles and the test parameters again recorded. Control investigations also were carried out in which heat-inactivated (100°C, 20 min) *Cl. perfringens* toxin and native filtrate of the broth culture of *Cl. butyricum* in the same doses were injected.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I the dynamics of changes in ECoG, RRR, EP, and ECG after injection of *Cl. perfringens* toxin was studied. Before injection of the toxin, bursts of barbiturate spindles and irregular slow waves were clearly visible on the ECoG (Fig. 1a, I). The cardiac rhythm was regular, according to the ECG. Repetitive photic stimulation evoked an RRR in the theta and delta bands. Potentials of the RRR were generalized and were recorded not only from the visual projection area, but also from the sensomotor cortex (Fig. 1a, II, III). In response to single flashes typical EP were generated in the visual cortex (Fig. 1a, IV). Bursts of barbiturate spindles were recorded 3 h after injection of the toxin in the frontal and occipital cortex just as before, but the intervals between individual bursts were lengthened (Fig. 1b, I). RRR and EP were substantially unchanged (Fig. 1b, II-IV, Table 1). Spindle-shaped bursts were well defined on the ECoG after 6 h (Fig. 1c, I). The RRR to 5 and 10 flashes, 1 sec in duration, were generalized as before and clearly defined. No significant changes were observed in EP (Fig. 1c, I-IV). The cardiac rhythm

TABLE 1. Latent Period, Duration, and Amplitude of Positive and Negative Components of EP to Photic Stimulation after Injection of *Cl. perfringens* Toxin

Time of investigation	Latent period, msec			Positive components of EP			
				duration, msec			amplitude, μV
	$M \pm m$	t	P	$M \pm m$	t	P	$M \pm m$
Before injection of toxin	10,7±0,3	—	—	10,0±0,45	—	—	43,0±3,2
After injection of toxin:							
3 h	10,9±0,28	0,5	>0,05	9,2±0,36	0,8	>0,05	44,5±2,6
6 h	11,0±0,3	0,7	>0,05	9,9±0,38	0,16	>0,05	50,0±3,9
9 h	11,3±0,36	1,2	>0,05	13,2±0,39	5,8	<0,001	42,0±3,7
12 h	18,8±0,4	16	<0,001	15,7±0,39	11,4	<0,001	30,0±1,8
20 h	27,6±0,26	42,2	<0,001	17,9±0,45	13,1	<0,001	22,5±0,84

Time of investigation	Positive components of EP		Negative components of EP					
	amplitude, μV		duration, msec			amplitude, μV		
	t	P	$M \pm m$	t	P	$M \pm m$	t	P
Before injection of toxin	—	—	29,8±0,81	—	—	227±5,5	—	—
After injection of toxin:								
3 h	0,36	>0,05	31,0±0,68	0,85	>0,05	239±6,0	2,08	>0,05
6 h	1,7	>0,05	34,4±0,94	3,8	<0,01	243±6,5	1,8	>0,05
9 h	0,2	>0,05	38,0±0,91	6,8	<0,001	224±4,9	0,33	>0,05
12 h	3,6	<0,01	52,4±0,75	20,5	<0,001	143±1,6	15,0	<0,001
20 h	6,8	<0,001	60,7±0,45	34,3	<0,001	76±5,6	21,4	<0,001

TABLE 2. Latent Period, Duration, and Amplitude of Positive and Negative Components of EP to Photic Stimulation after Combined Injection of *Cl. perfringens* Toxin and Filtrate of a Culture of *Cl. butyricum*

Time of investigation	Latent period, msec			Positive components of EP			
				duration, msec			amplitude, μV
	$M \pm m$	t	P	$M \pm m$	t	P	$M \pm m$
Before injection of toxin	12,6±0,26	—	—	14,0±0,26	—	—	42,5±3,4
After injection of toxin:							
3 h	13,2±0,3	1,6	>0,05	13,2±1,05	0,8	>0,05	49,2±2,5
6 h	15,7±0,33	4,4	<0,001	13,0±0,7	2,5	<0,05	63,6±4,0
9 h	24,0±0,21	3,4	<0,001	16,3±0,4	5,1	<0,001	43,5±2,9
12 h	27,9±0,32	3,6	<0,001	16,4±0,5	5,4	<0,001	22,4±1,4

Time of investigation	Positive components of EP		Negative components of EP					
	amplitude, μV		duration, msec			amplitude, μV		
	t	P	$M \pm m$	t	P	$M \pm m$	t	P
Before injection of toxin	—	—	26,2±0,7	—	—	184±7,8	—	—
After injection of toxin:								
3 h	1,5	>0,05	28,6±0,5	1,4	>0,05	168±4,8	1,7	>0,05
6 h	4,0	<0,001	32,4±2,1	2,8	<0,05	138±5,4	4,8	<0,001
9 h	0,25	>0,05	41,2±0,6	16,6	<0,001	91,5±5,7	8,9	<0,001
12 h	5,4	<0,001	42,7±0,87	13,3	<0,001	61,0±5,0	13	<0,001

was regular and the T wave negative (Fig. 1c, I). Bursts of spindles were absent from the ECoG after 9 h and it was dominated by a fast low-voltage activity and irregular slow waves of low amplitude in the delta band (Fig. 1d, I). No significant change was observed in RRR and EP (Fig. 1d, II-IV). The cardiac rhythm was regular and the T wave negative (Fig. 1d, I). Irregular low-amplitude waves in the delta band were recorded after 12 h in the ECoG and the fast low-voltage rhythms were poorly defined (Fig. 1e, I). The RRR to 5 and 10 flashes per second was recorded from the visual projection area, but no regular rhythms of RRR were recorded in the sensomotor cortex (Fig. 1e, II, III). The latent period, amplitude, and duration of EP differed statistically significantly from those in the spontaneous recording and during desynchronization (Fig. 1e, IV; Table 1). The cardiac rhythm was reg-

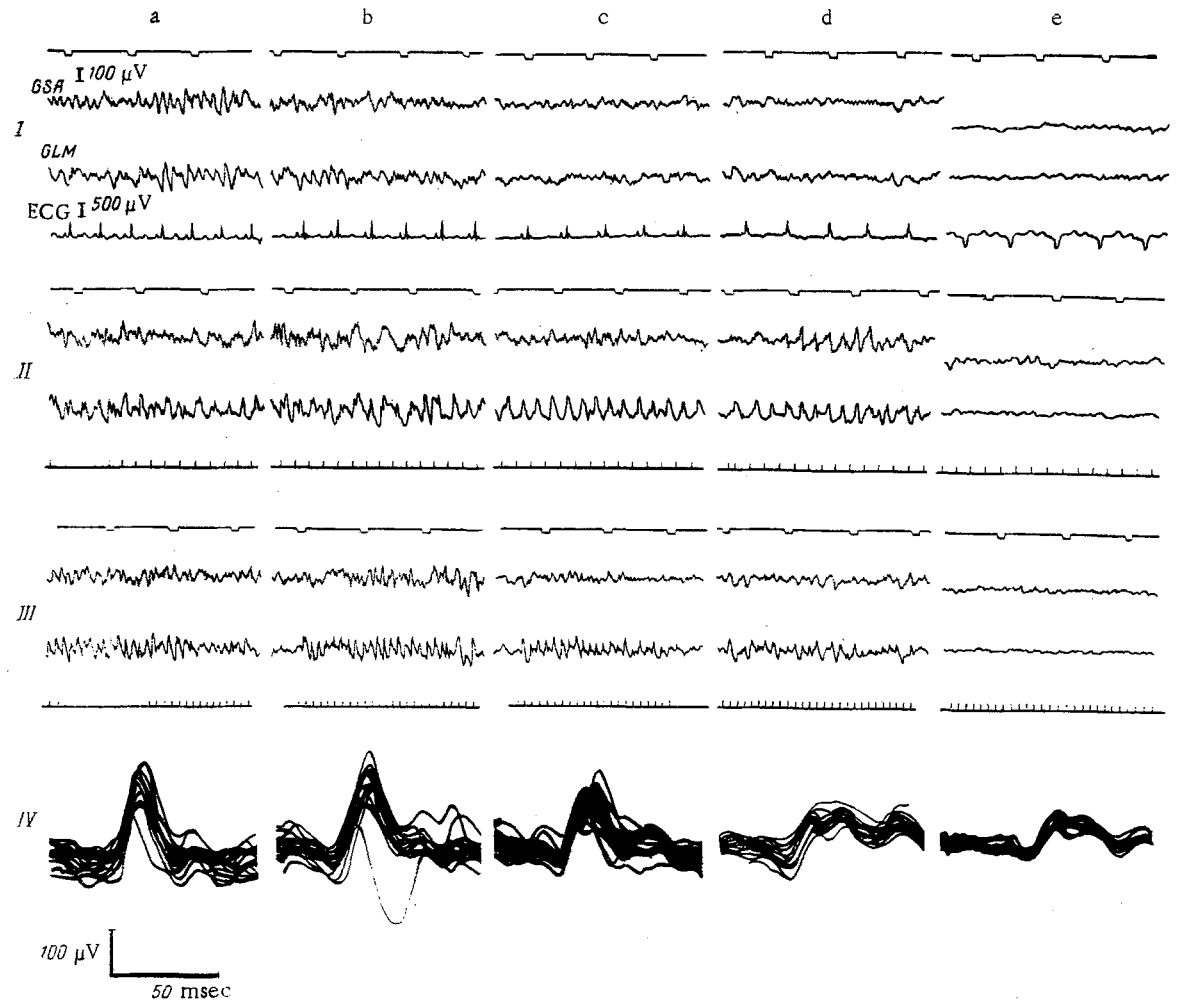

Fig. 2. ECoG, RRR, EP, and ECG at different stages of poisoning caused by combined action of *Cl. perfringens* type A toxin and filtrate of broth culture of *Cl. butyricum*.

ular and the T wave negative (Fig. 1e, I). Irregular low-voltage slow waves were visible after 20 h on the ECoG but no waves in the beta band were recorded (Fig. 1f, I). An RRR to 5 and 10 flashes per second was low in amplitude and was recorded from the visual projection area (Fig. 1f, II, III). EP were sharply depressed and their latent period considerably lengthened (Fig. 1f, IV). The ECG showed a sharply reduced heart rate, the amplitude of the R wave varied in different cardiac cycles, and the T wave was negative (Fig. 1f, I).

In the experiments of series II the character of changes in the same parameters was studied at different times after injection of a mixture of *Cl. perfringens* toxin and filtrate of a broth culture of *Cl. butyricum*. The spontaneous ECoG (Fig. 2a, I) was dominated by bursts of barbiturate spindles and slow waves in the delta and theta bands. The cardiac rhythm was regular (Fig. 2a, I). The RRR to 5 and 10 flashes per second was clearly defined. In response to single flashes EP were generated in the visual cortex (Fig. 2a, II-IV). Barbiturate spindles were still present after 3 h (Fig. 2b, I). The RRR also persisted, but some change could be observed in the regularity of reproduction and shape of the responses (Fig. 2b, II, III). The EP were not significantly changed (Fig. 2b, IV). The heart rate was slowed (Fig. 2b, I). However, bursts of barbiturate spindles could not be recorded after 6 h (Fig. 2c, I) and the ECoG consisted basically of alpha-like waves with a well-marked beta rhythm superposed on them. The RRR to flashes at 5 Hz was clearly defined in the visual cortex but irregularly reproduced in the sensomotor cortex; to 10 Hz reproduction was relatively complete in the visual cortex but disturbed in the sensomotor. The amplitude of the negative component of EP was reduced and the latent period lengthened (Fig. 2c, II-IV; Table 2). The amplitude of the R and T waves was reduced and the heart rate even slower than in the preceding period (Fig. 2c, I). An irregular low-amplitude slow activity was recorded after 9 h on the ECoG and the beta rhythm was ill-defined (Fig. 2d, I). The

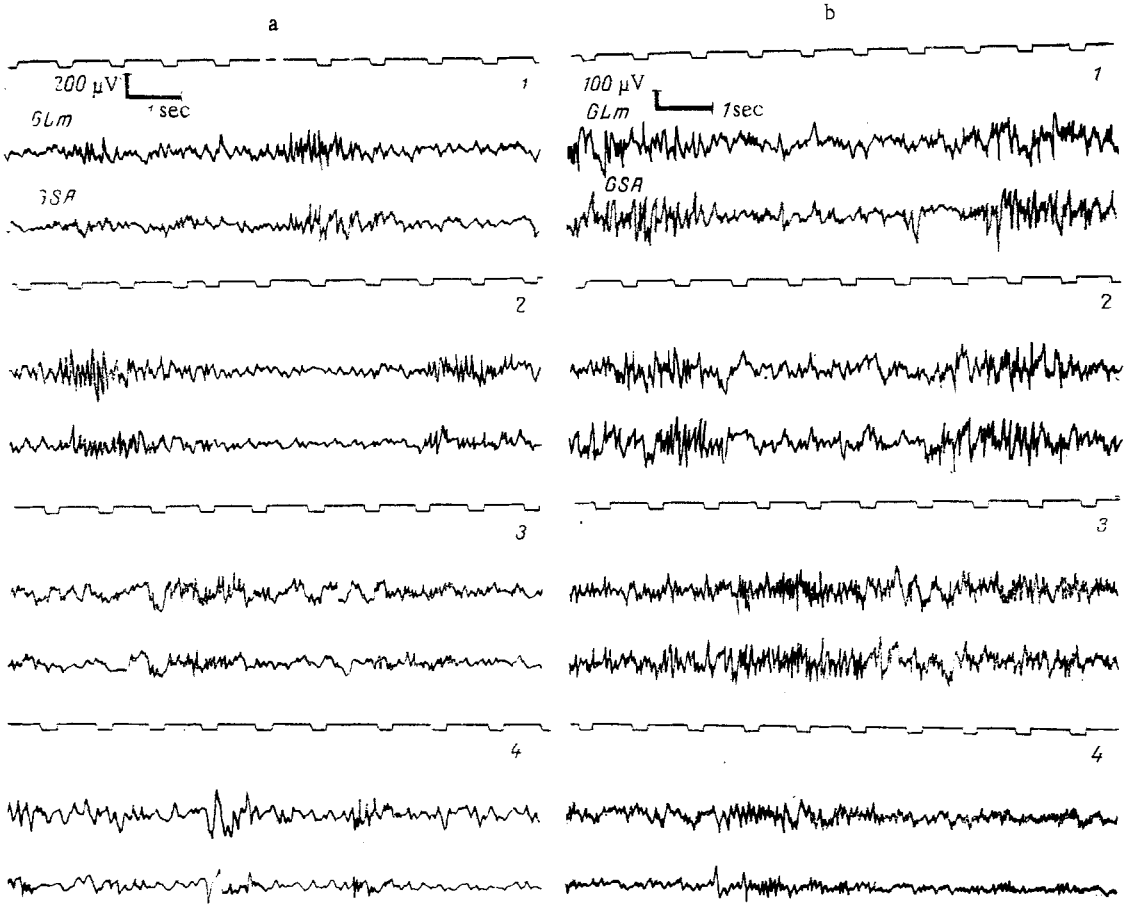

Fig. 3. ECoG at different times after injection of filtrate of broth culture of *Cl. butyricum* (a) and heat-inactivated *Cl. perfringens* type A toxin (b). a: 1) Before injection; 2-4) 1, 6, and 24 h after injection of filtrate; b: 1) before injection; 2-4) 1, 10, and 12 h after injection of heat-inactivated toxin. Derivations: GSA) anterior sigmoid gyrus; GLM) middle part of lateral gyrus. Time marker 1 sec. Stimulus calibration: 200 μV (a) and 100 μV (b) for ECoG.

RRR to 5 Hz still remained in the visual cortex, but in the sensomotor cortex it was unstable; to 10 Hz it was incomplete in the visual cortex and virtually absent in the sensomotor cortex (Fig. 2d, II, III). EP to single flashes were sharply altered (Fig. 2d, IV). The latent period and the duration of the positive and negative components of EP were lengthened and the amplitude of each component was considerably reduced (Table 2). The P wave on the ECG was poorly defined, the R wave reduplicated, and the T wave negative (Fig. 2d, I). After 12 h irregular low-amplitude waves in the delta and alpha bands were recorded on the ECoG but the fast low-voltage waves could not be distinguished (Fig. 2e, I). The RRR to flashes at 5 and 10 Hz was virtually not reproduced (Fig. 2e, II, III). EP to single flashes were low in amplitude and had a long latent period (Fig. 2e, IV; Table 2). Considerable deformation of individual components of the ECG was observed (Fig. 2e, I).

In the last two series of experiments the effects of the filtrate of the broth culture of *Cl. butyricum* (Fig. 3a) and of heat-inactivated *Cl. perfringens* toxin (Fig. 3b) on cortical electrical activity were studied. The experiments showed no significant change in brain electrical activity.

The results show that exposure to the combined action of *Cl. perfringens* type A toxin and filtrate of a broth culture of *Cl. butyricum* led to the earlier onset of desynchronization of cortical activity and its subsequent depression than the action of *Cl. perfringens* toxin alone. The direction and character of the changes in cortical rhythmic activity were the same as those produced by the action of one toxin alone. The chief difference was a reduction in the time of onset of desynchronization of the ECoG and a considerable shortening of the interval between this phase and the subsequent phase of depression. Parallel obser-

vations showed that after injection of toxin and filtrate the lifespan of the animals was reduced compared with that of animals receiving toxin alone. Filtrate of *Cl. butyricum*, while not itself giving a pathogenic effect, presumably quickens the penetration of *Cl. perfringens* toxin into the tissues, including into the CNS, thereby leading to a more severe course of poisoning produced by *Cl. perfringens* toxin. This effect is probably not unique and it can tentatively be suggested that one mechanism of the increased pathogenic action of neurotropic toxins of Clostridia resulting from their association with other microorganisms is increased permeability of the barriers produced by metabolic products of the commensal organisms.

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