

UNCLASSIFIED

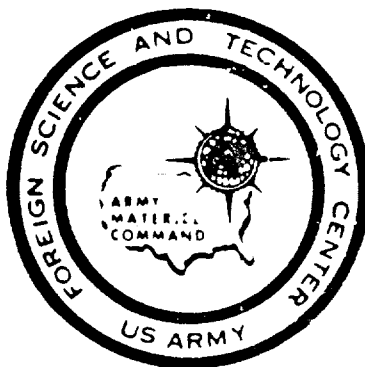
AD NUMBER
AD835456
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 1967. Other requests shall be referred to Army Foreign Science and Technology Center, Washington, DC.
AUTHORITY
USAFSTC ltr, 8 May 1969

THIS PAGE IS UNCLASSIFIED

FSTC-HT-23-67-66

66
45
53
8D

U.S. ARMY FOREIGN SCIENCE AND TECHNOLOGY CENTER



Combined Action and Potentiation of Clostridium Toxins

Country: USSR

TECHNICAL TRANSLATION

The translation rights for this document have not been obtained. This document is not in the public domain.

Each transmittal of this document outside the agencies of the U. S. Government must have prior approval of the U. S. Army Foreign Science and Technology Center.

Wash., D. C. 20314

TECHNICAL TRANSLATION

FSTC-HT-23- 67-68

COMBINED ACTION AND POTENTIATION
OF CLOSTRIDIUM TOXINS

by

A. I. Mitskevich

Source: TEKHNOLOGICHESKY ZHURNAL,
(Ukrainian), 1967, No. 5,
pp 8-9

Translated by: Joint Publications Research Service

This translation is a rendition of the original foreign text without any analytical or editorial comment. Statements or theories advocated or implied are those of the source and do not necessarily reflect the position or opinion of the US Army Foreign Science and Technology Center. This translation is published with a minimum of copy editing and graphics preparation in order to expedite the dissemination of information. Requests for additional copies of this document should be addressed to the Defense Documentation Center, Cameron Station, Alexandria, Virginia, ATTN: OSR-2

Product Identification: WTC-HT-23-67-68

Product Type: Human Translation

Product Classification: Unclassified

Publication Title: Combined Action and Potentiation of Clostridium Toxins

Publication Date:

FSIC Task Number: 7223628 23.01

DIA Task Number: Not applicable

Topic Tags: Clostridium perfringens, Cl. histolyticum, Cl. septicum,
Cl. oedematiens, potentiation, reticuloendothelial system,
gas infection, liver, spleen

Abstract: If previously mixed and held at 37°C for 45 min Clostridium

perfringens and Cl. histolyticum become more toxic. This shows up
as depression of the absorptive function of the liver and spleen reticulo-
endothelial system and increase in hemolytic and lethal effect.

This may explain the high incidence of fatalities in gas infections
from these bacteria.

Pages: 7

Tables: 3

Illustrations: None

COMBINED ACTION AND POTENTIATION OF CLOSTRIDIUM TOXINS

Mikrobiologichnyy zhurnal
(Journal of Microbiology),
No 2, 1967 Pages 125-129

A. I. Mitskevych

Gas infection as a rule is a polymicrobial disease in which the combination of the two causative agents, Clostridium perfringens and Clostridium histolyticum, substantially complicates the clinical picture and raises the percentage of fatalities [1-6].

Until recently research on microbe associations in gas infection was conducted mainly in the direction of studying the effect of the associating microbes on each other and the requisite attention was not devoted to the interaction of their toxins.

It has been established in the literature [7-15 etc.] that when the toxins of certain species of anaerobes, as well as of the toxins of these excitants and of particular species of aerobes, operate together they are seen to become potentiated.

Among the microbe associations which cause gas gangrene the combined action of the toxins of Cl. perfringens and of Cl. histolyticum is the least studied. In view of this we set ourselves the task of studying potentiation of these toxins during their combined action on the animal organism and under in vitro experimental conditions.

To clarify this phenomenon experiments were conducted on the effect of Cl. perfringens and Cl. histolyticum toxins on the function of the reticuloendothelial system, as well as on the change in the hemolytic and lethal activity of these toxins when they act together.

The first series of experiments utilized dry Cl. perfringens toxins of the series 16 and 1:10-52 and dry Cl. histolyticum toxin of series 16 obtained from the Institute of Epidemiology and Microbiology imeni M.F. Gamaley with which 18 rabbits and 120 white mice were infected. The toxins and their mixtures were injected in subtotal doses

both directly after preparation thereof and after having been kept in a thermostat at 37°C for 45 min. Inactivated toxins were injected into the control animals.

Blocking action from subtotal doses of toxins and their mixtures on cells of the reticuloendothelial system of the liver and spleen was detected in rabbits and white mice to an almost identical degree (Table I). At the same time a difference was observed in the poisonous properties of the toxins injected. It was most pronounced in the Cl. histolyticum toxin and exceeded the analogous action of the rest of the toxins by a factor of two or three. This is particularly apparent when the toxin is kept in the thermostat at 37°C for 45 min. Mixing the toxins of Cl. perfringens and Cl. histolyticum and then holding them in the thermostat for 45 min causes a four to sixfold rise in their poisonous properties over those of other toxins, with the exception of Cl. perfringens and Cl. septicum.

Besides a study of the effect of Cl. perfringens and Cl. histolyticum toxins on the reticuloendothelial system, research was carried out on the hemolytic and lethal properties of these toxins. Potentiation of the toxins of Cl. perfringens and Cl. histolyticum was studied in in vitro experiments -- 21 series -- and in experiments on 8 rabbits, 10 guinea pigs, and 267 white mice. The experiments made use both of the indicated series of dry toxins and the natural toxins of Cl. perfringens strain 235 and Cl. histolyticum strain 5. It has been established that the hemolytic action of the Cl. perfringens and Cl. histolyticum toxins increases substantially if, after mixing, they are kept in a thermostat for 45 min at 37°C (Table II). This is corroborated by experiments with a background dose of each of these two toxins. The hemolysis reactions were set up in a volume of 1.5 cc, of which 1 cc comprised the toxins in a 0.85% solution of sodium chloride and 0.5%, a 5% suspension of leporine erythrocytes. Holding the toxin of Cl. perfringens at 37°C for 45 min strengthened its hemolytic properties. This is especially apparent when potentiating the toxins of Cl. perfringens and Cl. histolyticum, where a four to eightfold intensification was observed.

A rise in hemolytic activity in the potentiation of toxins of Cl. perfringens and Cl. histolyticum became evident as increased lethal action in experiments on animals. This applies in equal measure to rabbits, guinea pigs, and white mice (Table III). Analysis of the data indicates that the toxins mentioned intensify their lethal properties when they are kept at a temperature of 37°C for 45 min. This intensification becomes considerably greater (by a factor of four to eight) if they have been previously mixed and so held before being injected into the animals. Similar results were obtained in experiments on rabbits and guinea pigs.

TABLE 1. Comparative Data on Effect of Toxins of Causative Agents of Gas Infection on Reticuloendothelial Cells of the Liver and Spleen

(a) Назва токсину	(b) Кількість токсину, що вводиться тваринам	(c) Тип тварин	(d) Кількість	(e) Досліджувані органи	Результати дослідження (f) кількість фарби в клітинах РЕС		
					(g) Контроль	Токсини	
						не оброблені (h)	оброблені (i)
<i>Cl. perfringens</i>	30	(h) Кролики	3	Печінка (m)	32,2	14,7	8,7
		(i) Кролики	3	Селезінка (m)	41,9	13,8	9,8
	0,9	(h) Миші	12	Печінка (m)	35,4	5,9	3,9
		(i) Миші	12	Селезінка (m)	39,2	6,1	4,7
<i>Cl. septicum</i>	30	(h) Кролики	3	Печінка (m)	51,5	12,3	12,1
		(i) Кролики	3	Селезінка (m)	50,4	14,2	13,8
	0,9	(h) Миші	12	Печінка (m)	39,0	15,4	15,0
		(i) Миші	12	Селезінка (m)	39,6	15,7	15,4
<i>Cl. oedematiens</i>	30	(h) Кролики	3	Печінка (m)	52,7	19,1	19,0
		(i) Кролики	3	Селезінка (m)	50,6	18,6	18,1
	0,9	(h) Миші	12	Печінка (m)	31,1	14,8	14,0
		(i) Миші	12	Селезінка (m)	31,1	14,8	14,0
<i>Cl. histolyticum</i>	30	(h) Кролики	3	Печінка (m)	49,6	7,5	4,7
		(i) Кролики	3	Селезінка (m)	51,5	7,1	5,2
	0,9	(h) Миші	12	Печінка (m)	32,7	9,7	5,5
		(i) Миші	12	Селезінка (m)	36,4	9,9	6,1
(b) Суміш токсинів							
<i>Cl. perfringens</i> i <i>septicum</i>	15	(h) Кролики	33	Печінка (m)	47,9	4,8	1,4
	15	(i) Кролики	33	Селезінка (m)	54,2	5,2	1,7
	0,45	(h) Миші	12	Печінка (m)	39,5	5,7	2,3
	0,45	(i) Миші	12	Селезінка (m)	38,4	5,4	2,7
<i>Cl. perfringens</i> i <i>histolyticum</i>	15	(h) Кролики	3	Печінка (m)	40,4	5,8	1,9
	15	(i) Кролики	3	Селезінка (m)	49,6	6,1	2,3
	0,45	(h) Миші	12	Печінка (m)	37,5	6,8	3,9
	0,45	(i) Миші	12	Селезінка (m)	38,5	6,9	3,4
<i>Cl. perfringens</i> i <i>oedematiens</i>	0,45	"	12	Печінка (m)	40,7	7,9	5,4
	0,45	"	12	Селезінка (m)	41,4	8,3	5,8
<i>Cl. septicum</i> i <i>oedematiens</i>	0,45	"	12	Печінка (m)	37,6	18,5	18,1
	0,45	"	12	Селезінка (m)	37,7	20,4	20,0
<i>Cl. oedematiens</i> i <i>histolyticum</i>	0,45	"	12	Печінка (m)	40,4	19,7	16,1
	0,45	"	12	Селезінка (m)	39,7	20,1	16,4
<i>Cl. septicum</i> i <i>histolyticum</i>	0,45	"	12	Печінка (m)	37,9	15,4	12,7
	0,45	"	12	Селезінка (m)	37,4	16,5	12,9

Key: (a) Toxin name, (b) MLD (for mice) injected in animals, (c) Experimental animals, (d) Number, (e) Organs studied, (f) Number of ink in reticuloendothelial cells, (g) Control, (h) Toxins, (i) Not heat-treated, (j) Heat-treated, (k) Rabbits, (l) Mice, (m) Liver, (n) Spleen, (o) Toxin mixes

TABLE II. Intensification in Hemolytic Properties of
Toxins of Cl. perfringens and Cl. histolyticum When
Kept in Thermostat for 45 min

(a) Токсин (в мл)		(b) не выдержаны		(c) выдержаны		(f) Оценка реакции гемолиза через 2 год
<u>Cl. perfringens</u> (d) серия 1:10	<u>Cl. histolyticum</u> (e) серия 16	<u>Cl. perfringens</u> (d) серия 1:10	<u>Cl. histolyticum</u> (e) серия 16	<u>Cl. perfringens</u> (d) серия 1:10	<u>Cl. histolyticum</u> (e) серия 16	
0,4						++
0,3						+
0,2						++
0,1						++
0,05						+
	0,4					++
	0,3					+
	0,2					++
	0,1					++
		0,4				++
		0,3				++
		0,2				+
		0,1				+
		0,05				++
		0,025				+
			0,4			++
			0,3			+
			0,2			++
			0,1			+
		0,2	0,2			++++
		0,1	0,1			++++
		0,05	0,05			++
		0,025	0,025			+
		0,012	0,012			+

Key: (a) Toxins in cc, (b) Not heat-treated,
(c) Heat-treated, (d) Series 1:10, (e) Series 16,
(f) Estimate of hemolytic reaction
in 2 hr

Conclusions

1. In the combined action of the toxins of Cl. perfringens and Cl. histolyticum is observed a potentiation of their poisonous effect, whose strength rises if the toxin mixture is previously exposed at 37°C for 45 min.
2. The potentiation of the toxins, particularly of Cl. perfringens and Cl. histolyticum, makes itself manifest in drastic depression of the absorptive function of the cells of the reticulo-endothelial system of the liver and spleen and in rise in the hemolytic and lethal activity.
3. Potentiation of the toxins and intensification of their poisonous properties may explain the severity of the clinical picture and high percentage of fatalities in gas infection caused by Clostridium

TABLE III. Effect of Potentiation of Cl. perfringens and Cl. histolyticum Toxins on Their Lethal Properties

(a) Токсини (в ДЛМ)				(d) Результати дослідів		
(b) не витримані		(c) витримані		(e)	(f)	(g)
<u>Cl. perfringens</u>	<u>Cl. histolyticum</u>	<u>Cl. perfringens</u>	<u>Cl. histolyticum</u>	Заражено	Загинуло	Живі
1				12	12	0
0,75				12	0	12
	1			12	12	0
	0,75			12	0	12
		1		12	12	0
		0,75		12	7	5
		0,5		12	4	8
		0,25		12	0	12
			1	12	12	0
			0,75	12	3	9
			0,5	12	0	12
0,5	0,5			12	12	0
0,25	0,25			12	0	12
		0,5	0,5	12	12	0
		0,25	0,25	12	11	1
		0,12	0,12	12	9	3
		0,06	0,06	12	2	10
		0,03	0,03	12	2	10
		0,015	0,015	12	0	12

Key: (a) Toxins in MLD, (b) Not heat-treated, (c) Heat-treated, (d) Experimental results, (e) Infected, (f) Died, (g) Lived

perfringens and Clostridium histolyticum.

Bibliography

1. Arapov, D.A. Gazovaya gangrena (Gas Gangrene). Medgiz (Medical Publishing House), Moscow, 1942.
2. Akhutin, M.N. Khirurgiya (Surgery), No 9, 1939.
3. Mel'nikov, A.V. Klinika gazovoy infektsii ognestrel'nykh ran (Clinical Treatment of Gas Infection of Gunshot Wounds). Medgiz (Medical Publishing House), Moscow, 1945.
4. Gnilyov, T.B. Sbornik nauchnykh trudov (Collection of Scientific Papers). Vol 2, Dnepropetrovsk, 1956.
5. Tsakhnovits, M.M. Zhurnal mikrobiologii, epidemiologii i immunologii (Journal of Microbiology, Epidemiology, and Immunology), 1944.
6. Chernaya, L.A., and Kovtunovich, G.P. Gazovaya infektsiya (Gas Infection). Kiev, 1950.
7. Zhak, S.P. Zhurnal mikrobiologii, epidemiologii i immunologii (Journal of Microbiology, Epidemiology, and Immunology), 1956.
8. Zhak, S.P. Ibid., 1957.
9. Donets, Yu.I. Ibid., 1962.
10. Dumkina, N.V. Sbornik trudov Odesskogo medin-ta (Collection of Transactions of the Odessa Medical Institute), Vol 13, 1960.
11. Levenshtam, M.A. Zhurnal mikrobiologii, epidemiologii i immunologii (Journal of Microbiology, Epidemiology, and Immunology), 1956.

12. Minervin, S.M. Sbornik trudov Odesskogo medin-ta (Collection of Transactions of the Odessa Medical Institute), Vol 13, 1960.
13. Minervin, S.M. Trudy Vsesoyuznogo s"yezda gigen., epid., mikr. i inf. (Proceedings of the All-Union Congress on Hygiene, Epidemics, Microbes, and Infection). Medgiz (Medical Publishing House), Moscow, 1965.
14. Mitskevych, A.I. Simposium po voprosam toksinogeneza i antitoksino-obrazovaniya (Symposium on Problems in Toxinogenesis and Antitoxin Formation). Publishing House of the Academy of Medical Sciences USSR, Moscow, 1963.
15. Tselukh, A.V. Sbornik trudov Odesskogo medin-ta (Collection of Transactions of the Odessa Medical Institute), Vol 13, 1960.

Received 12 July 1965.

DISTRIBUTION AND AVAILABILITY CHANGES

IDENTIFICATION	FORMER STATEMENT	NEW STATEMENT	AUTHORITY
AD-835 456L Army Foreign Science and Technology Center, Washington, D. C. Rept. no. FSTC-HT- 23-67-68 1967	USGO: others to Army Foreign Science and Technology Center, Washington, D. C.	No limitation	USAFSTC ltr, 8 May 69