

Fish Rats
Mice - Strychnine sulfate
 - Tetanus toxin

PROTECTING EFFECT OF ASCORBIC ACID IN STRYCHNINE POISONING
AND IN TETANUS (EXPERIMENTS IN FISH, MICE AND RATS)

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Summary: The median lethal dose (LD50) of strychnine sulfate
in fish (*Phallocerus caudimaculatus* H., *Lebistes reticulatus*
P.) and in mice is significantly raised by pretreatment of the
animals with high doses of vitamin C. In rats, pretreatment
with vitamin C affords a certain protection against the con-
vulsive and lethal effects of tetanus toxin (Dey).

Even in animals with established tetanic symptoms, vitamin
C treatment diminishes the death rate and/or postpones time
of death. High doses of ascorbic acid (0.1 g/kg i.v.) do not
cause neuromuscular blockade in rats.

The mechanisms which might be responsible for the "antitoxic"
effects of ascorbic acid are discussed.

Dey^{1,2,3} in 1965 and 1966 published two short notes on the
protecting effect of ascorbic acid, in mice and rats, against
the lethal and convulsive properties of strychnine sulfate
and tetanus toxin. The doses given by this author (1 to 2 mg/kg

of ascorbic acid i.p.) surpass by far those employed by a former author, Jungeblut⁴ who observed, in 1937, an inactivating action of vitamin C upon tetanus toxin. This inactivation, however, occurred only when the toxin was exposed, in vitro, to the direct action of ascorbic acid - a phenomenon not due to the acidity of the medium, since lowering of the pH by other means (pH 6) did not cause a similar inactivation. On the other hand, 60 guinea pigs injected with 2 MLD tetanus toxin were not protected even by repeated injections of vitamin C, in doses from 10 to 200 mg. Apart from the difference of species (rats, guinea pigs, respectively), employed by Dey and by Jungeblut, the experimental details given by either author are too aphoristic to permit any comparison between the two contradictory papers, or else an exact repetition of their experiments.

We became interested in this problem, some 6 years ago, and started an experimental study on the claimed protective effect of vitamin C upon the lethal and convulsive action of strychnine and tetanus toxin. Due to the lamentable, untimely death of our main collaborator Dr. Ariel de Oliveira Guedes, the investigation came to a stop, remaining unfinished, especially with regard to the vitamin C action upon tetanus toxin.

It was only now when we received, a few weeks ago, the honorable invitation for this symposium that we resumed some earlier experiments and revised the former protocols, buried for many years, whose results we are going to present you now, briefly.

We apologize for all shortcomings of our communication due to the mentioned circumstances. We regret especially that we are unable to give any definite data on the claimed detoxifying activity of vitamin C upon the lethal and convulsive effect of tetanus toxin, a phenomenon of definite clinical interest.

Methods

Our experiments on the "antitoxic"¹¹ effect of vitamin C were performed in fish and in mice treated with varying doses of strychnine sulfate. The tests with tetanus toxin were made in rats.

A. Strychnine

1. Fish

Following the technique used by Valle et al.⁵, who assayed the toxic effect of various psychotropic drugs in fish (Guaru = *Phallocerus caudimaculatus* Hensel, and Lebistes = *Lebistes reticulatus* Peters), we determined the median lethal dose (LD₅₀) of strychnine sulfate in a total of 1560 fishes of both species. In the test group, ascorbic acid (0.5 mg/ml) was added to the individual fish bowls, 12 hours before adding strychnine sulfate in doses varying from 10 to 80 µg/ml. In the control group, the water in the bowl was brought to the same pH as in test group (pH 6), by the addition of hydrochloric acid. Reading, i.e. counting of dead fish, was made at the end of the second hour following the addition of different strychnine doses to the individual fish bowls (Tab. 1, 2, Fig. 1).

Tab. 1. Composition of aquarium liquid.

NaCl	10.00 g
KCl	0.50 g
CaCl ₂	0.25 g
NaHCO ₃	0.50 g
H ₂ O	3.00 l

(pH = 7.8)

Tab. 2. Effect of ascorbic acid on medium lethal dose of strychnine sulfate in fish.

Strychnine sulfate µg/ml (§)	Ascorbic acid mg/ml (§)	Number of fish	% Kill	% Empirical probit	Corrected probit	LD ₅₀ µg/ml
10	-	120	1.66	2.89	2.89	
20	-	120	17.50	4.06	4.00	36.93
30	-	120	33.33	4.57	4.66	=
40	-	120	45.00	4.87	5.13	10.02
50	-	120	73.33	5.62	5.49	
60	-	120	82.50	5.93	5.78	
20	0.5	120	2.50	3.04	3.15	
30	0.5	120	12.50	3.85	3.74	
40	0.5	120	20.83	4.19	4.16	71.29 (§§)
50	0.5	120	26.70	4.38	4.48	=
60	0.5	120	35.80	4.64	4.75	8.64
70	0.5	120	46.70	4.92	4.97	
80	0.5	120	62.50	5.32	5.15	

§ ml of aquarium liquid.

§§ p < 0.01.

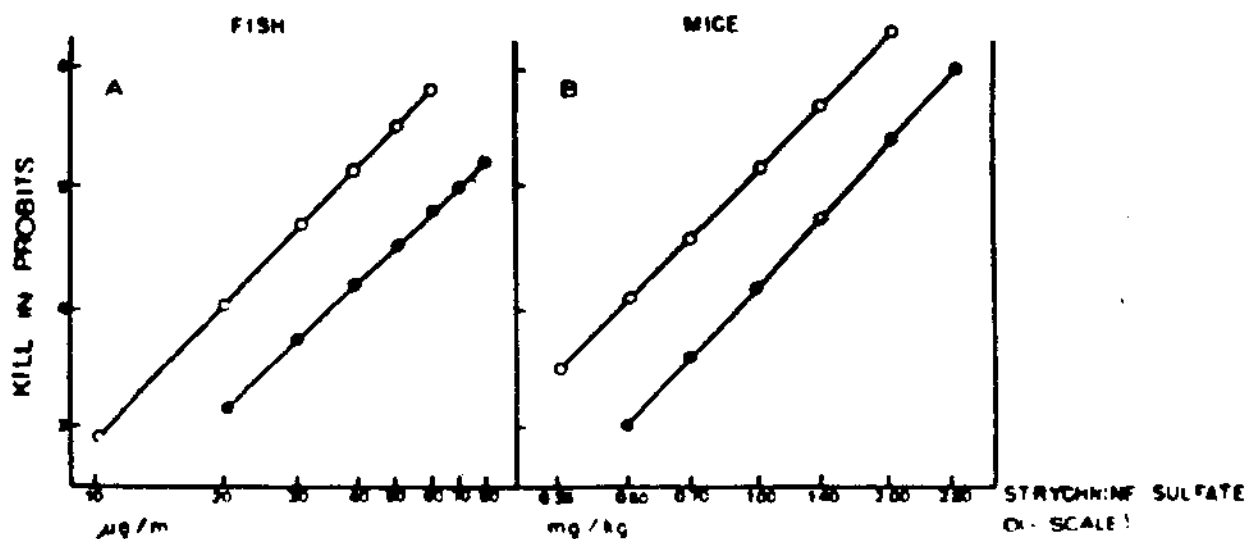


Fig. 1. Effect of vitamin C on DL₅₀ of strychnine in fish and in mice. ○—○ untreated controls, ●—● vitamin-C-treated animals.

Tab. 3. Effect of pretreatment of mice with sodium ascorbate on medium lethal dose of strychnine sulfate.

Strychnine sulfate mg/kg	Sodium ascorbate g/kg (§)	Number of mice	% Kill	% Empirical probits	Corrected probits	LD ₅₀ (mg/kg)
0.35	-	40	5.00	3.35	3.54	
0.50	-	25	16.00	4.00	4.10	
0.70	-	40	45.00	4.87	4.63	0.90
1.00	-	25	52.00	5.05	5.19	±
1.40	-	40	75.00	5.67	5.72	0.39
2.00	-	25	92.00	6.04	6.28	
2.80	-	40	100.00	-	-	
0.35	1.0	40	0.00	-	-	
0.50	1.0	25	4.00	3.24	3.02	
0.70	1.0	40	7.50	3.56	3.63	
1.00	1.0	25	16.00	4.00	4.28	1.60 (§§)
1.40	1.0	40	45.00	4.87	4.88	±
2.00	1.0	25	80.00	5.84	5.52	0.28
2.80	1.0	40	85.00	6.03	6.13	
4.00	1.0	25	100.00	-	-	

§) i.p. twice a day for 3 days.

§§) p < 0.05.

2. Mice

The experiments were performed on a total of 495 albino male mice weighing from 20 to 30 g. The test animals were injected intraperitoneally, on three subsequent days, twice daily, with an aqueous solution of sodium ascorbate (1 g/kg), the control group with 0.1 ml of deionized distilled water. On the fourth day, test animals and con-

trols were divided into 7 individual groups, receiving geometrically rising doses of strychnine sulfate, intraperitoneally. The number of deaths occurring in each group was checked at the end of the second hour, after intraperitoneal injection of strychnine sulfate (Tab. 3, Fig. 1).

B. Tetanus toxin

Rats

As to the antitoxic effect of ascorbic acid against tetanus toxin, we must limit ourselves to quoting the results reported formerly by Dey² in 1966 and to a short summary of our own recent, however still incomplete observations (Tab. 4).

Results

It results from these experiments that pretreatment with ascorbic acid or with sodium ascorbate, in fish and in mice, respectively, is followed by a statistically significant increase of the LD₅₀ of strychnine sulfate. It must be stressed here that all mice dying from strychnine poisoning, in the control as well as in the vitamin C treated group, suffered the same mode of death with generalized, bilateral tonic convulsions.

Our own, limited experiments on the antitoxic effect of vitamin C in rats injected with tetanus toxin do not permit definite conclusions yet: in fully established tetanus, on the fourth day after the toxin injection, the intravenous injection of 0.1 g/kg of vitamin C into anaesthetized rats seemed to protect

Tab. 4. Effect of ascorbic acid (i.v.) on established tetanic symptoms in rats.

No of rats	09/08/76			13/08			14/08			15/08		
	Local tetanus	Con-vulsions	Death	Treatment	Local tetanus	Con-vulsions	Death	Treatment	Local tetanus	Con-vulsions	Death	
Control	+	-	-	0.6 mM	+	+	-	0.6 mM	-	-	+	
	+	-	-	HCl	-	-	+	HCl	-	-	+	
	+	-	-	(pH=4.0)	+	+	-	(pH=4.0)	-	-	+	
	+	+	-	0.25 ml	-	-	+	0.25 ml	-	-	+	
	+	-	-	i.v.	+	+	-	i.p.	-	-	+	
Tetanus	+	+	-	Ascorbic acid	+	-	-	Ascorbic acid	-	-	+	
	+	-	-	acid	+	-	-	acid	-	-	+	
	+	-	-	0.1 g/kg	+	-	-	0.1 g/kg	-	-	+	
	+	-	-	0.25 ml	+	-	-	0.25 ml	+	-	-	
	+	-	-	i.v.	+	-	-	i.p.	+	-	-	

⁵ Anesthesia with chloral hydrate.

them against generalized convulsions and to reduce death rate and/or postpone time of death. Further experiments, on a broader statistical base, are indispensable.

As a supplement to our studies on the antitoxic effect of vitamin C, in strychnine poisoning and in tetanus, we also assayed the LD₅₀ of intraperitoneally applied pentylenetetrazol in a vitamin-C-treated test group of mice, which had been injected with 1.0 g/kg of ascorbic acid (10% solution i.p., 15 minutes before the application of the convulsant drug. As controls served an equal number of mice which had been injected with diluted hydrochloric acid of the same pH as the ascorbic acid solution (pH 4.0).

There was no statistically significant difference between the LD₅₀ of the control and vitamin C group of mice.

Discussion

As to the mechanisms which might be operative in the antitoxic effect of vitamin C against strychnine and tetanus toxin, the following possibilities are at issue:

1. Chemical inactivation in the animal body;
 2. Neuromuscular blockade and/or interference with intramedullar synaptic transmission.
-
1. A chemical in vivo inactivation of strychnine, by the direct contact of this drug with sodium ascorbate, apparently, can be excluded, since incubation of both substances (with an excess of Na ascorbate), in vitro, at 37°C, during 2 hours, did not diminish the toxic effect of strychnine in either fish or mice. On the other hand, Dey (personal communication) believes "that it is the rise in plasma ascorbic acid con-

centration which annuls the strychnine action¹¹. He also found that cysteine as well as "a number of pentose sugars" give a good protection against strychnine. It remains a point at issue, how far ascorbic acid owes this "antitoxic" effect to its unspecific activity as a potent redox catalyist.

2. A blocking effect of vitamin C at the neuromuscular junction does not come into play, here: doses of 0.1 g/kg of vitamin C, given by intravenous route, did not diminish neuromuscular transmission at the rat gastrocnemius preparation, during a 30 minutes' observation time (F. Eichbaum: unpublished experiments).

As to the influence of vitamin C on medullar synaptic transmission, there do not exist yet any pertinent investigations.

3. Finally, before extrapolating the reported results to other species, it must be stressed that these experiments were performed in species (mouse and rat) which, in contraposition to fish, guinea pig and primates, including man, are capable of synthetizing vitamin C in their own organism.

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