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Action of *l*-Ascorbic Acid upon the Isolated Frog Heart.*

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It was recently reported that hydrogen peroxide is the cause of the positive inotropic and of the irreversible systolic effect upon the frog heart of ascorbic acid solutions.[1] The evidence available in the literature indicates that the hydrogen peroxide formation in ascorbic acid solutions is related to the dehydrogenation of the molecule, and, more specifically, to the catalytic action of copper in this process.[2] It is known that the catalytic action of copper can be prevented by the formation of complexes between the metal and suitable substances like protein[3] or diethyldithiocarbamate.[4] Serum globulin and sodium diethyldithiocarbamate were therefore used to determine whether copper inhibitors were able to diminish or prevent the action of *l*-ascorbic acid solutions upon the frog heart.

Method. 50 to 100 mg of *l*-ascorbic acid were dissolved in one cc of distilled water; and this solution, after neutralization with sodium bicarbonate, was diluted to an initial concentration of approximately 1:10,000 with bicarbonate buffer solution (NaCl 85.4; KCl 1.9; $CaCl_2$ 0.99; $NaHCO_3$ 27.0 mM/L) saturated with 95% oxygen and 5% carbon dioxide. The pH of the solution was between 7.5 and 7.8. The room temperature varied between 20 and 25°C. In the experiments with inhibitor, this was dissolved in the bicarbonate buffer solution previous to the addition of the *l*-ascorbic acid. The serum globulin was prepared in the laboratory of Dr. E. J. Cohn and consisted of 90% gamma-globulin and 10% beta-globulin.

The frog hearts were isolated as previously described,[1] and the bicarbonate buffer solution was continuously perfused through the cannula

TABLE I.
(Isolated frog hearts.)
Inhibition by Serum Globulin and by Sodium Diethyldithiocarbamate of *l*-Ascorbic Acid Destruction and Corresponding Modification of Heart Action of *l*-Ascorbic Acid Solutions.

Inhibitor		*l*-Ascorbic acid: Concentration in mg %				*l*-Ascorbic acid: % destruction After			Systolic effect After	
			After							
Name	Conc. in mg %	Initial	1 hrs	2 hrs	3	1 hrs	2 hrs	3	2 hrs	3 hrs
Serum globulin	1.0	10.0	7.2	4.3		28	57		complete	
(consisting of	2.0	14.4	8.6	4.7		40	67		"	
90% γ-globulin	5.0	10.8	8.0	4.8	2.8	26	56	74	begins	complete
and 10%	10.0	10.5	9.0	8.7	8.0	14	17	24	none	doubtful
β globulin)	20.0	8.3	8.3	8.3	8.3	0	0	0	"	none
Sodium diethyl	0.1	10.0	9.8	9.0	8.7	2	10	13	"	"
dithiocarbamate	0.1	10.0	9.6	9.2	8.6	4	8	14	"	"
	1.0	10.9	10.3	10.1	9.6	5	7	12	"	"
	1.0	10.9	10.3	10.2	9.7	5	6	11	"	"

* This work was carried out under the auspices of the University Committee on Pharmacotherapy.

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at a rate of 2 to 2.5 cc per minute, with a gas mixture of 95% oxygen and 5% carbon dioxide bubbling through the fluid in the cannula.

The ascorbic acid determinations were made with the 2,6-dichlorindophenol method. The approximate concentration of hydrogen peroxide was determined by its luminescent reaction with 3-aminophthalhydrazide.[5,1]

Results. Without inhibitor, solutions of *l*-ascorbic acid with an initial concentration of 1:10,000 (in Ringer solution not specifically freed from copper[6]) caused systolic standstill within one to 2 hours, 80 to 90% of the *l*-ascorbic acid being destroyed within this period. Hydrogen peroxide accumulated in the solutions to reach concentrations of the order of 1:100,000 to 1:300,000.[1] Serum globulin or sodium diethyldithiocarbamate in appropriate concentrations inhibited the destruction of *l*-ascorbic acid and the solutions had little or no action upon the heart (Table I). The concentration of hydrogen peroxide was very low in the solutions in which the ascorbic acid destruction was markedly inhibited. The characteristic luminescence with 3-aminophthalhydrazide was either very weak or entirely absent, indicating concentrations of hydrogen peroxide of only one in 5 to 10 million.

These experiments show that *l*-ascorbic acid solutions (in the presence of oxygen and at pH 7.5 to 7.8) do not exhibit their characteristic positive inotropic and systolic effect upon the frog heart in the presence of copper inhibitors. Under this condition the hydrogen peroxide formation in the solution is insufficient to cause the heart effect.

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Preparation of and Cardiovascular Response to Neo-synephrine in Oil.

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Neo-synephrine HCl (laevo a-hydroxy-b-methyl-amino-3-hydroxy ethyl benzene hydrochloride) when used parenterally has been found to be a useful adjuvant in spinal anesthesia[1,2,3,4] and in cyclopropane anesthesia.[5] This substance raises and maintains blood pressure over a considerable period of time. Side reactions such as nervousness, anxiety, etc., are comparatively rare. In addition, neo-synephrine HCl has been used successfully in the treatment of some forms of shock.[6,7]

Blood pressure within a normal range can be maintained under various conditions by repeated intravenous injections of small amounts of neo-synephrine HCl, or by subcutaneous or intramuscular injections repeated as necessary. The latter methods of administration tend to establish a depot from which absorption takes place rather slowly into the blood stream. Inasmuch as substances in oil, when injected parenterally, are absorbed more slowly than those in aqueous solutions, the

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