Technical Papers

Effect of Some Sulfhydryl-containing Substances on the Toxicity of an Organic Mercurial Compound[1]

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This study was undertaken to test certain substances containing sulfhydryl groups as possible antidotes to the acute toxic action of an organic mercurial diuretic on the dog, cat, and mouse. The trial of such substances for that purpose was suggested by the recent reports on the effectiveness of 2,3-dimercapto-propanol (British anti-lewisite) in poisoning by various heavy metals (*5, 8*), by the reversal of the antibacterial action of inorganic mercury with sulfhydryl compounds (*3, 6*), and by the reactivation of certain sulfhydryl-containing enzymes poisoned with p-chloromercuribenzoate that can result *in vitro* from the addition of compounds containing –SH groups (*1, 7*).

Methods

The organic mercurial diuretic used in these experiments was Salyrgan.[2] The sulfhydryl-containing compounds tested for their antidotal action were cysteine hydrochloride, glutathione, and 2,3-dimercapto-propanol.[3] Cystine and methionine were also tested for possible antidotal action.

The acute intravenous toxicity of Salyrgan alone and of Salyrgan given one minute after the injection of sulfhydryl compounds was determined in mice, and the 50-per cent lethal dose (LD_{50}) was calculated by the method of Behrens (*2*).

Intact cats and dogs were anesthetized intraperitoneally with Dial-Urethane (0.7 cc. per kilogram of body weight). The blood pressure was recorded with a mercury manometer from the carotid artery; the venous pressure, with a water manometer from the right external jugular vein. Electrocardiograms were taken in lead 1 with an ink-writing, "Grass" oscillograph.

Two types of injections of Salyrgan were used: repeated injections of 10 to 25 mg., given into the femoral vein until definite acute toxic manifestations appeared; or constant infusion of Salyrgan into the femoral vein until rhythm irregularities and other toxic manifestations developed, at which point the substance to be tested for antidotal action was injected while the infusion of Salyrgan was continuing. The compounds tested for their antidotal action were injected either into the femoral vein or into the left jugular vein by means of a thin rubber tube which made it possible to inject the material close to the right auricle.

Tests were also made on the dog heart-lung preparation. The technic for this preparation was in general the same as that of Krayer and Mendez (*4*). The anesthetic was 35 mg. of sodium pentobarbital per kilogram of body weight. Arterial, right and left atrial, and pulmonary arterial pressures, cardiac volume, coronary sinus outflow, and systemic output (output of the left ventricle minus coronary flow) were recorded.

Results

The acute intravenous LD_{50} of Salyrgan in the strain of mice used was found to be 103 mg. per kilogram of body weight. Both cysteine hydrochloride and glutathione, given one minute before the administration of Salyrgan, markedly protected the mice against the toxicity of Salyrgan. The LD_{50} was shifted from 103 to 176 mg. of Salyrgan per kilogram by 50 mg. per kilogram of cysteine hydrochloride and to 216 mg. per kilogram by an equimolar amount of glutathione. Cystine and methionine were much less effective than either cysteine hydrochloride or glutathione, but showed a slight degree of protection.

In the intact anesthetized cat and dog, Salyrgan produced manifestations which were mainly referable to the circulation and here, more specifically, to the heart. A lethal dose given either by continuous infusion or by repeated single injections produced a sudden drop of blood pressure, a rise in venous pressure, an increase in heart rate, and electrocardiographic changes characterized by increased PR and QRS intervals, decreased amplitude of T waves, ventricular extrasystoles, and ventricular tachycardia. These effects were all promptly counteracted by cysteine hydrochloride, 5–10 mg. per kilogram; glutathione, 5–10 mg. per kilogram; and 2,3-dimercapto-propanol, 0.5–2 mg. per kilogram. The effect of these substances on ventricular fibrillation induced by Salyrgan is not clearly established, but it is doubtful that such a process, once started, can be counteracted by any of the sulfhydryl compounds.

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[2] Kindly furnished by Winthrop Chemical Company, Inc.

[3] Kindly furnished by Dr. E. S. Guzman Barron.

The experiments on the heart-lung preparation of the dog clearly demonstrated the marked antidotal properties of sulfhydryl-containing compounds against the negative inotropic action of Salyrgan. In Table 1 it can be seen that severe heart failure produced by 150 mg. of Salyrgan could be promptly counteracted by 100 mg. of glutathione. Similar effects were

TABLE 1

THE EFFECT OF GLUTATHIONE ON HEART FAILURE PRODUCED BY TOXIC DOSES OF SALYRGAN*

Time in minutes	Systemic output cc./min.	Aortic pressure mm./Hg	Pulmonary pressure mm./H$_2$O	Right auricular pressure mm./H$_2$O	Left auricular pressure mm./H$_2$O	Heart rate per min.
0	400	105	136	19	32	140
5	Salyrgan, 50 mg.					
7	400	105	136	19	32	140
9	Salyrgan, 50 mg.					
11	400	105	136	19	32	140
14	Salyrgan, 50 mg.					
15	370	105	155	25	108	140
16	320	102	250	45	320	
18	100	95	545	94	478	
18.5	50	95	623	100	523	150
19	Glutathione, 100 mg.					
19.5	250	95	409	65	377	156
21	500	110	136	19	32	140
22	400	105	136	19	32	140
24	400	105	136	19	32	140
49	400	105	140	19	35	140

* Heart-lung preparation. Female dog, 10.6 kg. Arterial resistance, 72 mm. of mercury. Blood temperature, 39.0° C. Blood volume, 880 cc. Experiment 2, 14 January 1946.

obtained with 56 mg. of cysteine hydrochloride and 10 mg. of 2,3-dimercapto-propanol. Cystine in doses up to 200–300 mg. did not counteract Salyrgan-induced heart failure. Furthermore, in spontaneous heart failure and heart failure induced by sodium pentobarbital, neither cysteine, glutathione, nor 2,3-dimercapto-propanol had any positive inotropic effect.

SUMMARY

The acute toxicity of an organic mercurial as studied on the mouse, intact anesthetized cat and dog, and the heart-lung preparation of the dog can be readily counteracted by sulfhydryl-containing compounds such as glutathione, cysteine, and 2,3-dimercapto-propanol. Equimolar doses of 2,3-dimercapto-propanol are about five times as active as cysteine or glutathione.

References

1. BARRON, E. S. G., and SINGER, T. P. *J. biol. Chem.*, 1945, **157**, 221.
2. BEHRENS, B. *Arch. exp. Path. Pharmak.*, 1929, **140**, 237. 237.
3. FILDES, P. *Brit. J. exp. Path.*, 1940, **21**, 67.
4. KRAYER, O., and MENDEZ, R. *J. Pharm. exp. Therap.*, 1942, **74**, 350.
5. PETERS, R. A., STOCKEN, L. A., and THOMPSON, R. H. S. *Nature, Lond.*, 1945, **156**, 616.
6. SALLE, A. J., and GINOZA, Y. W. *Proc. Soc. exp. Biol. Med.*, 1943, **54**, 85.
7. SINGER, T. P., and BARRON, E. S. G. *J. biol. Chem.*, 1945, **157**, 241.
8. WATERS, L. L., and STOCK, C. *Science*, 1945, **102**, 601.

The Mechanism of Myotonic Contraction

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In 1942, while reviewing the mechanism of myotonias (*5*), the writer concluded that there were several reasons for admitting that the delayed relaxation of myotonic muscle was due to a pathological state of the muscle itself, independent of the central nervous system and of the neuromuscular synapsis.

The most important demonstration of the exclusion of the central nervous system has been furnished by Grund (*3*), Schäffer (*6*), Kennedy and Wolf (*4*), and Denny-Brown and Nevin (*2*), who showed that after nerve or spinal anesthesia, the myotonic reaction still exists in the anesthetized limb. Referring to the synapsis, Brown and Harvey (*1*) pointed out that in myotonic goats the completely curarized muscle shows myotonic phenomena.

The last experiment is crucial, and the only criticism of its generalization is that the experiments were performed on a condition supposed to be similar to that of myotonia in human beings. For these reasons, and counting on the willing cooperation of two patients affected by *Dystrophia myotonica*, experiments were planned to discover the effect of the exclusion of the synaptic mechanism in these patients.

The experiments were performed under penthotal anesthesia. Two shielded electrodes were placed in the median nerve, and two needle electrodes punctured the proximal and distal part of the thenar eminence, respectively. The nerve was stimulated supramaximally by a thyratron stimulator, and the source of the needle electrodes was an a-c potential reductor. The registration was recorded in a smoke drum through a myograph attached to the interphalangic joint of the first finger.

The experiment began with a two-second direct and indirect stimulation of 60 stimuli per second. A suitable dose of *Curare Brasiliensis* injected in the brachial artery, with a cuff to avoid the venous circulation during two minutes after the injection, completely suppressed the effect of the nerve stimulation. The direct stimulation, conversely, reproduced exactly the previous registration, showing that the myotonic delayed relaxation was not influenced by the synaptic exclusion. Also, the percussion of the muscles of the thenar eminence showed the same myotonic responses after full curarization.

In the second patient the effect of variations of frequency of stimulation was studied in addition, but a total curarization was not obtained because of tech-