Studies on the Stability of Adrenalin Hydrochloride Solution.

Part I. The Influence of Various Compounds on the Stability of Adrenalin Hydrochloride Solution.

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It has been shown that chemical change of adrenalin hydrochloride solution is greatly retarded by the presence of an antioxidant, thus preventing loss of adrenalin. C. B. West and coworkers[1] have recently shown that the discolouration of adrenalin hydrorchloride solution can be prevented by the addition of an antioxidant such as sodium bisulfite or ascorbic acid.

In the present study we have worked out the influence of various compounds on the stability of adrenalin hydrochloride solution, making exact comparisons of their activities.

Experimental

(A) Stabilizing effects of various compunds upon aquas adrenalin hydrochloride solution.

(I) Chemical test:

A colorless standard solution (1 : 10 00) was prepared for the whole tests containing 50 milligrammes of adrenalin base in sufficient distilled water to furnish 50 cc of solution. Before bringing up to final volume, enough n/10 HCI (3.0 cc approximately) was added to complete solution and to produce a pH of 5. 2.

(a) On the discolouration of adrenalin hydrochloride solution at various kinds of concentration under the storage of 24 hours at 37° C :

The results are contained in Table I, and show the discolouration of adrenalin hydrochloride solution come out strongly in the concentration of 1:100,000.

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Table I.

Concentration of adrenalin hydrochloride solution. (pH 5.2)	Grade of discolouration	
1: 1,000	—	none
1: 10,000	++	strongly
1: 100,000	++	strongly
1: 1,000,000	+	slightly
1:10,000,000	—	none

(b) On the inhibitory effect of various compounds upon the discolouration of adrenalin hydrochloride solution.

The technique employed was as follows–Two cubic centimeters of varying dilutions of test compounds under study in adrenalin hydrochlotide solution of a 1:100,000 dilution, were placed in each of a series of test tubes, and the whole were gently mixed and placed for 24 hours at 37°C, after which the results were read and recorded (dilution causing inhibition of discolouration). In this test the control solution containing adrenalin hydrochloride alone changes its color into red after 24 hours, but in case of containing effective compounds, remains colorless. The results are indicated in Table II and III.

Table. II.

Concentration of compounds tested	Effect of compounds tested						
	$NaHSO_3$	$K_2S_2O_5$	Chloretone	Urethan	Urea	Quinine-HCl	Benzoic acid
1: 1,000	—	—	•	++	++	++	—
1: 2,000	—	—	++	++	++	++	—
1: 4,000	—	—	++	++	++	++	—
1: 8,000	—	—	++	++	++	++	—
1: 16,000	—	—	++	++	++	++	—
1: 32,000	—	—	++	++	++	++	—
1: 64,000	+	—	++	++	++	++	—
1: 128,000	++	++	++	++	++	++	+
1: 256,000	++	++	++	++	++	++	++
1: 512,000	++	++	++	++	++	++	++
Control	++	++	++	++	++	++	++

Teble. III.

Compounds tested	pH of 1% solution	Minimum concentration (inhibitory against the discolouration)
Na_2SO_3	8.6	1:32,000
$NaHSO_3$	5.4	1:16,000
$Na_2S_2O_3$	9.6	1:32,000
$K_2S_2O_5$	5.0	1:64.000
K_2SO_4	5.6	none
Chloretone	5.6	none
Urethan	5.6	none
Urotropin	6.6	1: 2,000
Quinine • HCl	6.2	none
Aminopyrine	6.2	1: 2,000
Benzoic acid	4.0	1:64,000
Cinnamic acid	5.2	1:16,000
Salcilic acid	5.2	1:32,000
p–Aminobenzoic acid	5.2	1:16,000
o–Aminobenzoic acid	5.2	1:16,000

None effective compounds were as follows: Camphor, cholesterin, menthol, coffein, tyrosin, taurin, phenylalanine and kreatin.

From the foregoing tabulated results, we are able to assert that:

(I) Benzoic acid and potassium metabisulfite have the most marked inhibitory power against the discolouration of aquas adrenalin hydrochloride.

Chloretone which is used for preserving hypodermic solutions, and solutions of adrenalin salts, does not act as the stabilizer for adrenalin hydrochloride solution.

(II) Biological test :

Protective effects of various compounds upon the loss of adrenalin were comfirmed by the blood pressure method using rabbits.

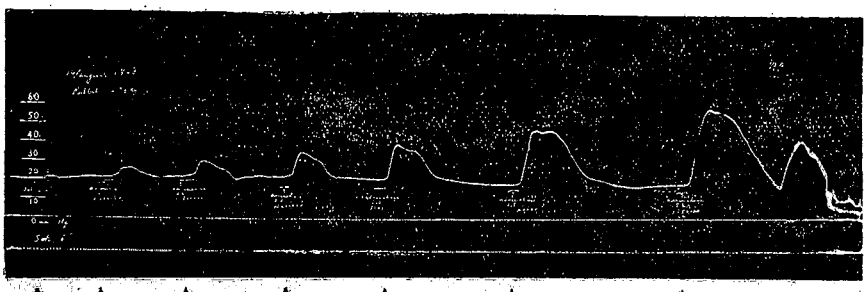
The standard solution used for these biological tests was the same as in the chemical test.

(a) On the pressor action of adranalin hydrochloride under varying dilutions.

This experiment was first carried out as the control at all biological tests, for the purpose of measuring blood pressure activities of compounds tested. In this experiment onc cubic centimeter of each sclution as follows was received intra-

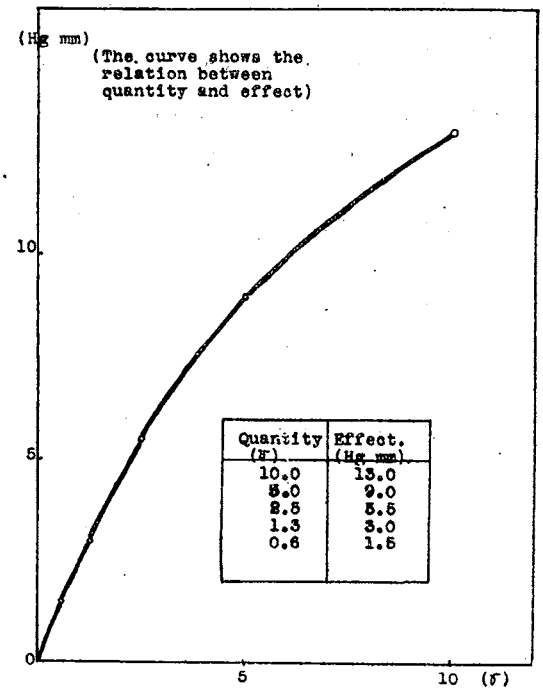
venously. From the results of the test a standard curve of pressor action of adrenalin is induced (in Fig. I and II), and the standard curve is useful for recording the relation between quantity and effect.

Fig I.



A . . . Saline solution
B . . . 1:1,600,000
C . . . 1: 800,000
D . . . 1: 400,000
E . . . 1: 200,000
F . . . 1: 100,000
G . . . 1: 50,000
} Dilute adrenalin hydrochloride solution of . . . .

Fig II. The standard curve of the action of adrenalin.

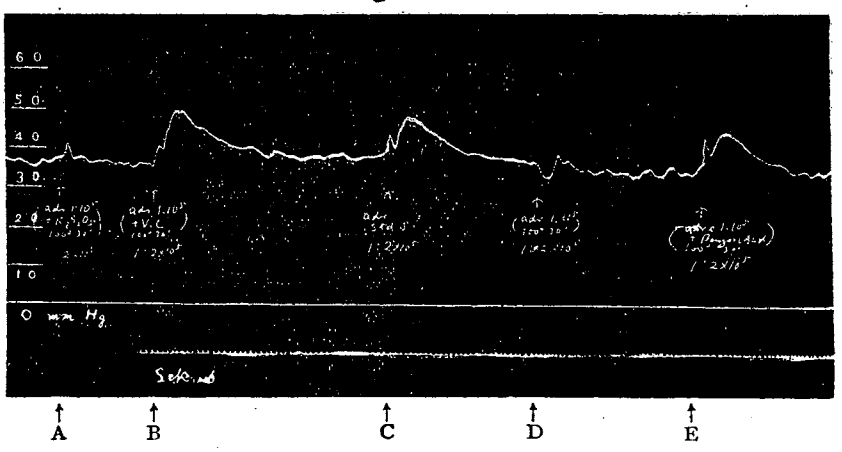


From the curve in Fig II illustrated the active quantity of adrenalin was calculated.

(b) On the influence of various compunds upon the destruction of adrenalin hydrocloride.

The solution tested of adrenlin hydrochloride under the addition of the tested compounds was heated at 100° C during 30 minutes. After the treatment it was added with equal volume of water. The solution prepared therefore must be looked upon as a 1 : 200,000 dilute solution of adrenalin hydrochloride. In this test one cc of the solution was received intravenenously.

Fig III.



A. . . .$K_2S_2O_5$ (1:1000), Adr. HCl (1:100,000); 100° C, 30.′
B. . . .Ascorbic acid (1:1000), Adr. HCl (1:100.000); 100° C, 30.′
C. . . .Control, none treated.
D. . . .Control, 100° C. 30.′
E. . . .Benzoic acid (1:1000), Adr. HCl (1:100,000), 100° C, 30.′

Results of another comparative test may be shown in the following table IV.

Table. IV.

Compounds		pH	Pressor action (Hg mm)	Active quantity (exchange to adrenalin) (γ)	Per cent loss in activity (%)
Control	none treated	•	32.0	20.0	0
	treated	•	3.5	0.4	98
Benzoic acid		4.0	24.0	17.0	15
Ascorbic acid		4.0	34.0	20.0	0
$K_2S_2O_5$		5.4	23.0	16.5	18
Chloretone		6.4	4.0	0.6	97
α-Alanine		6.4	13.0	10.5	48
β-Alanine		6.4	7.5	4.5	78
Procain HCl		6.4	3.5	0.5	98
Quinine HCl		6.2	0	0	100
Pantocain HCl		6.2	2.0	0.2	99

In this experiment the adrenalin hydrochloride of 1:50, 000-diluted solution containing the compound tested at the rate of 1:500, was used.

The foregoing experiments conclude that :

1) The best results were obtained with ascorbic acid on the protection of loss of adrenalin.

2) Potassium metabisulfit and benzoic acid gave satisfactory results.

3) Procain hydrochloride and panthocain hydrochloride have no protective action against the destruction of adrenalin in water.

4) Quinine hydrochloride seems to possesses a destructive power against adrenalin in vitro.

(B) Accelerating effect of various compounds upon the discolouration of aquas adrenalin hydrochloride.

(I) Chemical test :

The technique for the test was almost in the same way at the section I. The control solution (1 : 1000 diluted solution of adrenalin hydrochloride) remaines colorless after the storage of 24 hours at 37° C, but in case of containing active compounds. the solution changes its color into red or yellow. Results are given in Tadle V.

Table. V.

Name of compound	pH	Minimum concentration on destructive effect
NaCl	5.8	1: 800
KCl	5.8	1: 400
K_2SO_4	6.0	1: 100
$CaCl_2$	6.4	1:1,600
$ZnSO_4$	5.6	1: 100
Procain HCl	5.2	1:3,200
Alypine • HCl	5.4	1:3,200
Tutocaine • HCl	5.2	1: 800
Ephedrine • HCl	5.4	1: 800
Antipyrine	5.4	1: 100
Quinine • HCl	5.5	1:6,400
Quinine Jobomethylate	5.4	1:1,600
α–Alanine	4.6	none
β–Alanine	5.6	1:6,400
Creatine	5.6	1:1,600

Results show that :

(1) Quinine hydrochloride and β-alanine are the most powerful in the compounds tested on the accelerating effect upon the discolouration of adrenalin hydrochloride solution.

(2) It is a suggestive fact that the β-type compound of alanine has no destructive effect, differing from the α-type.

(II) Biological test :

Accelerating powers of various compounds on the destruction of adrenalin hydrochloride were comfirmed by blood pressure method in the same way as at the section A.

Diluted adrenalin solution of 1 : 1000. containing 1 : 100 diluted solution of compound tested was heated for 30 minuts at 100° C; The mixture was added again with water to two hundred times of primary solution for use of intravenenously injection. (This solution prepared is equal to a 1 : 200, 000 dilute solution of adrenalin hydrochloride).

(a) On the accelerating effect of quinine hydrochloride and β-alanine on the destruction of adrenalin hydrochloride.

It was investigated here. as follows The solution tested containing adrenalin hydrochloride in the rate of 1 : 1000, and the compound tested in 1 : 100 in water heated during 30 minutes, at 100°C. After the treatment the solution was diluted with the physiological solution by 200 times, and this diluted solution was adminstered intravenenously.

The result of this test is given in Table VI.

Table. VI.

Compounds tested	Pressor action (mm Hg)
Quinine HCl	4.0
β-alannine	9.0
Control	9.5

The control solution is the saline solution of adrenalin hydrochloride in concentration of 1:200, 000.

(b) On the accelerating effect of sodium chloride upon the destruction of adrenalin hvdrochloride.

It is a noticeable fact that sodium chloride has a destructive power upon the adrenalin hydrochloride in water, A datum of the test is shown in the following table VII.

Solutions of 1:50,000 of adrenalin hydrochloride were treated, after which they were receieved as the saline solution.

Table. VII.

Solution tested	Condition of procedure	Pressor action (mm Hg)	Quantity (exchange to adrenalin) (γ)
Water solution	0° C, 24 hours	14,0	11.2
	100° C, 45 minutes	11.5	9.0
Physiological saline solution (0.85%)	0° C, 24 hours	6.0	2.5
	100° C, 45 minutes	3.5	0.5
Control	•	32.0	20.0

Conclusion

By summariziug all the experiments, the following results were found :

1) The water solution of adrenalin hydrochloride combined with ascorbic acid has a much greater stability than with any other compounds.

2) Procain hydrochloride accelerates the discolouration of adrenalin hydrochloride in water.

3) Quinine hydrochloride accelerates remarkably the destruction of adrenalin hydrochloride in water.

References

1) Pharm. J. 157, 54 (1949) ; C. A. 40, 4477.

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