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ANTI-OXIDATIVE ACTION OF OXALIC ACID IN RELATION TO ADRENALIN AND ITS POSSIBLE IMPORTANCE IN ANIMAL ECONOMY *

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2 graphics on the text

It is a known fact that ascorbic acid is a protective agent against the oxidation of adrenalin; we tried to find out, in preliminary tests, if such protection would also remain when hydrogen peroxide is present. The results were satisfactory even when a great amount of peroxide was used. It is obvious that, if ascorbic acid acts when an excess of hydrogen peroxide is present, the protective action, for adrenalin could not be due exclusively to its molecular structure, because the peroxide changes it almost immediately into dehydro-ascorbic acid. On the other hand, from the oxidation of ascorbic acid the formation, *in vitro* as well as *in vivo*, of oxalic acid may result. For this reason it occurred to us to verify whether the oxalic acid itself was responsible for the protective action against the oxidation of adrenalin and of other substances to which the protective action of ascorbic acid has been, hitherto, attributed.

Our hypothesis was fully confirmed by our experiments.

EXPERIMENTAL

To be able to judge of the protective effect of adrenalin by oxalic acid, based on quantitative data, we obtained at first a curve from oxidated adrenalin, by reading such phenomenon in the Fisher electrophometer, using a filter B 525. The following schedule was used in our experiments:

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TABLE 1

OXIDATION OF ADRENALIN BY HYDROGEN PEROXIDE IN THE PRESENCE OF CUPRIC ION

Heated in water bath at 70°C for 10 minutes

Buffer (aceto-acetic), 0.1 N, pH 5, 5 ml;

$CuSO_4.5H_2O$ — 0.025% (w/v), 1 ml; H_2O_2 (10 vol.) 1 ml

Tube n.°	Adrenalin mg/3 ml "in" H_2O	k
1	—	blank
2	0.2	9.2
3	0.4	17.7
4	0.6	26.0
5	0.8	33.4
6	1.0	40.4
7	1.2	46.7
8	1.4	52.0
9	1.6	57.0
10	1.8	61.1
11	2.0	64.1

These results, which show a complete oxidation of different amounts of adrenalin, give us a curve, represented by Fig. 1.

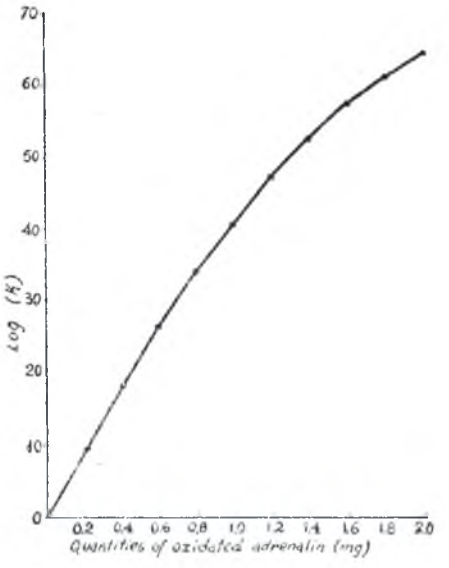

Fig. 1 - OXIDATION OF ADRENALIN-pH 5 FISHER ELECTROPHOTOMETER — FILTER B 525

To verify the protective action of oxalic acid in the oxidation of adrenalin, in the presence of copper and hydrogen peroxide, we used the following experimental schedule:

TABLE 2

PROTECTIVE ACTION OF THE OXALIC ACID AGAINST THE OXIDATION OF ADRENALIN BY HYDROGEN PEROXIDE IN PRESENCE OF CUPRIC ION

Heated in water bath at 70°C for 10 minutes

Buffer (aceto-acetic), 0.1 N, pH 5, 5 ml;

$CuSO_4.5H_2O$ — 0.025% (w/v), 1 ml; H_2O_2 (10 vol.) 1 ml

Tube n.°	Adrenalin mg/3 ml "in" H_2O	Oxalic acid mg	k
1	2	10	3.4
2	2	9	3.7
4	2	8	4.1
3	2	7	4.7
5	2	6	5.2
6	2	5	6.7
7	2	4	8.0
8	2	3	10.6
9	2	2	13.6
10	2	1	26.3
11	2	0.8	38.8
12	2	0.6	47.1
13	2	0.4	52.2
14	2	0.2	62.0
15	2	—	67.0
16	1	—	42.0
17	—	—	blank

These data show us that oxalic acid protects adrenalin against the phenomenon of oxidation in an efficient way, as can be seen by calculating the protection of adrenalin in milligram values, or in a percentage of the total quantity:

TABLE 3

PROTECTION OF ADRENALIN BY OXALIC ACID, CALCULATED IN PERCENTAGE AND IN WEIGHT

Oxalic acid mg	Protection	
	%	mg
0.2	7.0	0.140
0 4	26.1	0.522
0.6	39.2	0.784
0 8	54.0	1.080
1	69.5	1.390
2	84.6	1.692
3	88.4	1.768
4	91.2	1.824
5	92.7	1.854
6	94.3	1.886
7	95.9	1.918
8	96.6	1.932
9	97.0	1.940
10	97.4	1.948

From the data of this table Fig. 2 was drawn.

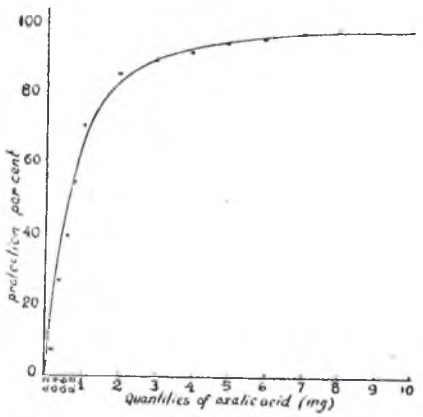

FIG. 2 - ANTI-OXIDATIVE ACTION OF OXALIC ACID FOR ADRENALIN

The observed protection is not specific to adrenalin, because we found out that it also acts with tyrosin, with phthalic acid and with p-aminobenzoic acid. On the other hand, the homologs of oxalic acid are inactive as we had presumed.

Results similar to those given on Table 2 were obtained also in other tests in which we changed the conditions of pH, from 1 to 7, but over pH 5 such a protection is largely diminished.

In the following table some data obtained by different pH with buffers of the same chemical nature are given briefly.

TABLE 4

INFLUENCE OF pH IN THE PROTECTION OF ADRENALIN BY OXALIC ACID, WITH ACETO-ACETIC BUFFERS

Adrenalin = 2 mg; oxalic acid = 5 mg; $CuSO_4.5H_2O$ 0.025% (w/v), 1 ml; H_2O_2 (10 vol.), 1 ml

pH	Protection %
3	100
4	100
5	91
6	58
7	13

DISCUSSION

Trying to find the possible protective mechanism of adrenalin and other substances, we observed at first, that it was not due to a simple reducing action because the oxalic acid proves to be more active than hydroquinones, formaldehyde and hydrosulfide in the protection of adrenalin against iron perchloride. On the other hand we also noted that the addition of oxalic acid to iron or copper solutions acts, by not allowing the ionization of the salts of these metals. In this way, the general oxidative processes, that are catalyzed by ions of copper or iron, are stopped or delayed in the presence of oxalic acid. We verified, for example, that ascorbic acid solutions get more stable by an addition of oxalic acid.

We can admit that the action of oxalic acid, by a weak dissociable combination, acting on the copper and iron present in the oxidative system may extend to the enzymatic processes in which the cause of action of the enzymes could be copper or iron. Later experiments confirmed such hypothesis as will be reported in another paper.

The fact that oxalic acid protects adrenalin and other substances of biological interest, against the phenomenon of oxidation, may open new possibilities for the

study and interpretation of animal metabolism. We believe that oxalic acid plays an important role in the animal economy, and should not be considered as an undesirable product of rejection of the organism.

SUMMARY

1. Oxalic acid protects adrenalin against oxidation, in the presence of copper, as well as other substances of biological interest, such as ascorbic acid, p-amino-benzoic acid and tyrosin.

2. The mechanism of action can be a combination of oxalic acid with copper and iron ions in the oxidative system, forming very little ionizable copper and iron oxalate.

3. Oxalic acid should not be considered as an undesirable catabolic product, but on the contrary, as an agent with active participation in the phenomenon of intra-organic oxido-reduction.