LXI. VITAMIN C STUDIES IN THE RAT. THE EFFECT OF SELENIUM DIOXIDE, SODIUM SELENATE AND TELLURATE

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SELENIUM poisoning in rats, either from sodium selenite [Franke & Potter, 1935] or from certain natural plant intoxicants [Franke, 1934], results in extensive pathological changes in the liver. The severity of the pathological changes in other organs depends on the extent to which the liver cells are regenerated as the result of necrosis.

Reduction of the toxic selenite and tellurite to the insoluble and relatively non-toxic elemental form by living cells has been reported from many laboratories. Respiration studies show that the O_2 uptake of yeast cells [Potter & Elvehjem, 1936] and the rate of CO_2 evolution during yeast fermentation [Moxon & Franke, 1935] are inhibited by sodium selenite. The reaction between the selenite and glutathione to give oxidized glutathione and selenium [Bersin, 1935] has been suggested by Potter & Elvehjem [1936] as an explanation for the toxic action of selenite in glycolysis.

The respiratory mechanism of animal tissues is generally considered to be much more complex than that in yeast. The role of vitamin C in tissue respiration is not completely understood, but glutathione has been regarded as an essential factor in the stabilization of this vitamin in tissue extracts [De Caro & Giani, 1934].

The present investigation was undertaken to study the effect of certain salts causing selenium and tellurium poisoning in rats on the vitamin C content of organs and to determine whether glutathione exerts a stabilizing influence on the reduction of these salts by the vitamin *in vitro*.

EXPERIMENTAL

Young albino rats of 90 g. wt., previously fed exclusively on the complete stock ration sold as Ralston Purina Dog Chow, were placed on basal diets devoid of vitamin C consisting of:

(a) 6% casein, 45% cornstarch, 15% sucrose, 4% Osborne and Mendel salts, 5% cod liver oil, 20% lard and 5% dried yeast.

(b) 18% casein, 32% starch, 15% sucrose, 4% salts, 5% cod liver oil, 15% lard, 9% dried yeast and 2% agar-agar.

(c) 52% casein, 3% salts, 30% butterfat, 15% lard, with the addition of 0.4 g. dried yeast daily to each rat. This diet is referred to in Tables I and II as "carbohydrate-deficient".

Fresh food (10 g. per rat) and water *ad lib.* were given daily. Adequate growth response was shown to occur with these diets in a 10-day preliminary test period.

The addition of selenium dioxide at a level of 22–35 p.p.m. of Se resulted in the refusal of the rats to eat the diets. The basal diets were then given for 3 days and the increased body weight was taken as the initial body weight. The average age of the rats at this stage of the experiment was 7–8 weeks.

Subcutaneous injections of the salts at levels determined by their respective toxicity were then given. At the end of the brief experimental period, the rats were killed by stunning, the organs were immediately removed (unless otherwise noted), weighed, extracted with 4% trichloroacetic acid and analysed in the usual manner for ascorbic acid by rapid titration with 2:6-dibromophenolindophenol blue.

Experiments in vivo

In these tests the rats injected with selenium and tellurium salts showed at necropsy the general symptoms—garlic odour of the liver and autolysis of the gut—mentioned in detail by Sollmann [1936]. In such a brief experimental period the marked necrosis of the liver noted in the prolonged feeding of plant intoxicants by Franke [1934] was not observed, but definite and rapid increases in weight of this organ were recorded.

The data in Tables I and II indicate that the percentage decrease of ascorbic acid in the organs, in accordance with previously recorded work on growth response by Schneider [1936], was not influenced by the sulphhydryl composition of the diet but depended on the amount of toxic salt injected and on the duration of the test period. The concentration of the vitamin, in contrast to the results of giving appreciably greater quantities of metallic salts such as copper sulphate, lead acetate, arsenic pentoxide, mercuric chloride, cadmium sulphate and manganese chloride, which had no apparent effect [Svirbely, 1936], was generally decreased in all the organs. A possible explanation for this decrease will be discussed in the next section.

A positive silver nitrate test for ascorbic acid has been reported by Mouriquand & Couer [1935] to persist in the adrenals of normal guinea-pigs for as long as 4 days after the death of the animal. Tissue extracts of normal rats which had been killed by stunning and whose intact carcasses had been allowed to stand at room temperature or in the ice-box for a period of 14 hr. showed, in comparison with extracts from rats fasting a like interval but killed just prior to extraction of the organs, an appreciable decrease in the vitamin C content of the organs. This decrease is more marked per g. tissue if correction for the post-mortem autolysis of the liver and gut be taken into consideration. The time factor is not the sole explanation for the decrease in vitamin C concentration in the rats injected with lethal doses of the salts (Table I *), since the control rats in the same series were killed by stunning and allowed to stand at room temperature for 4 hr. before extraction of the organs was carried out. However, in order to avoid errors in the interpretation of data, the estimation of vitamin C should be made as soon as possible after death of the animals unless the carcasses are frozen thoroughly.

Estimation of vitamin C in vitro

Preliminary experiments indicated that reduction of selenium dioxide or sodium selenate to the colloidal state occurred more slowly with glutathione or cysteine (as the hydrochloride) than with ascorbic acid. Reduction of sodium tellurate in concentrations possessing the same degree of toxicity as the selenium salts was brought about more rapidly by cysteine than by glutathione, but did not occur with ascorbic acid. The rapid reduction of tellurate by cysteine, even in the presence of vitamin C, may account for the pigmented urine and dark

Table I. *Effect of subcutaneous injections of selenium dioxide, sodium selenate and tellurate in 7-8-week-old rats. Rats fasted during test period*

Vitamin C content of organs														Total amnt. in organs extrac- ted	% decrease
No. and sex of animals	Av. initial wt. g.	Av. final wt. g.	Amnt. salt injected mg.	Test period hr.	mg. per organ										
					Adre- nals	Liver	Gut	Spleen	Adre- nals	Liver	Gut	Spleen			
Rats had been fed dog chow															
Selenium dioxide	3♂*	128	115	0.5	15	1.0	0.08	0.19	0.17	0.02	0.53	0.75	0.05	1.35	22
Selenium dioxide	3♂*	121	105	0.5	15	0.5	0.08	0.22	0.17	0.01	0.54	0.81	0.06	1.42	18
Sod. selenate	3♂*	126	117	0.85	15	0.5	0.08	0.18	0.15	0.01	0.51	0.69	0.05	1.26	28
Sod. tellurate	3♂*	120	112	10.0	15	0.5	0.08	0.15	0.05	0.01	0.41	0.53	0.02	0.97	44
Sod. tellurate	3♀*	128	118	10.0	14	0.4	0.09	0.12	—	0.01	0.46	0.38	—	0.85	45
Controls	3♂*	120	103	—	19	1.25	0.17	0.21	0.27	0.03	0.78	0.84	0.07	1.74	—
Controls †	3♀	—	115	—	14	1.27	0.12	0.18	0.13	0.04	0.56	0.62	0.07	1.29	23
Controls ‡	3♀	—	118	—	14	0.78	0.11	0.24	0.17	0.02	0.59	0.77	0.06	1.44	11
Controls §	3♀	—	108	—	14	2.06	0.17	0.20	0.24	0.06	0.71	0.80	0.11	1.68	—
Selenium dioxide	5♀	120	107	0.3	16	1.11	0.10	0.20	0.19	0.03	0.51	0.75	0.06	1.35	17
Sod. selenate	3♀	119	102	0.5	16	1.00	0.07	0.16	0.18	0.03	0.35	0.70	0.05	1.13	30
Sod. tellurate	3♀	118	104	5.0	17	1.52	0.14	0.17	0.11	0.04	0.64	0.64	0.06	1.38	15
Controls	4♀	124	109	—	17	2.26	0.17	0.21	0.14	0.06	0.64	0.86	0.07	1.63	—

* Salts injected were in mg. per 100 g. initial body wt. All rats died on this dosage. The organs were removed and extracted about 4 hr. after death of the animals.

† Room temperature 26° } Organs removed for extraction 14 hr. after death of animals.

‡ Ice-box

§ Fasting control rats

Table II. *Effect of selenium dioxide on rats fed on various diets. Rats fasted during test period.*
Av. age of rats in these tests was 59-62 days

Vitamin C content of organs																	
Diet and injections	No. and sex of animals	Av. initial wt. g.	Av. final wt. g.	Amnt. salt injected mg.	Test period hr.	mg. per g.						mg. per organ			Total amnt. in organs extracted	% decrease	
						Adrenals	Liver			Spleen	Adrenals	Liver					Spleen
							Gut	Gut	Gut			Gut	Gut	Gut			
Repeated injections of SeO ₂ *																	
6% casein	3♀	117	105	0.8	43	1.58	0.06	0.23	0.12	0.05	0.35	0.67	0.05	1.12	35		
6% casein	3♀	127	117	—	40	1.50	0.13	0.24	0.13	0.03	0.60	1.02	0.07	1.72	—		
18% casein	3♀	152	134	1.8	48	1.50	0.07	0.22	0.14	0.06	0.47	0.79	0.07	1.39	16		
18% casein	3♀	150	133	—	48	3.32	0.17	0.16	0.23	0.12	0.77	0.63	0.15	1.67	—		
Carbohydrate-deficient	4♂	167	143	2.8	48	2.03	0.11	0.27	0.11	0.06	0.89	1.09	0.09	2.13	35		
	3♂	188	164	—	48	3.57	0.28	0.28	0.09	0.10	1.69	1.42	0.08	3.29	—		
Dog chow	5♂	154	128	2.8	48	1.56	0.07	0.26	0.09	0.05	0.61	1.19	0.05	1.90	24		
Dog chow	4♂	155	122	—	48	3.00	0.20	0.28	0.16	0.07	1.03	1.30	0.10	2.50	—		
Single injection of SeO ₂																	
Dog chow	4♂	149	149	2.0	4	1.16	0.15	0.36	0.14	0.03	1.08	1.92	0.09	3.12	21		
Dog chow	2♂	151	151	—	4	2.05	0.26	0.32	0.13	0.04	1.79	1.95	0.17	3.95	—		
Carbohydrate-deficient	3♂	158	149	1.0	20	2.44	0.10	0.32	0.20	0.07	0.82	1.58	0.11	2.57	22		
	2♂	191	181	—	20	3.18	0.22	0.23	0.19	0.10	1.68	1.36	0.18	3.32	—		

* Subcutaneous injections: 0.3 mg. on first day at noon; 0.5 mg. 28 hr. after 1st injection; 1-2 mg. 45 hr. after 1st injection.

Table III. *Estimation of vitamin C in the presence of selenium dioxide, sodium selenate and tellurate*

Ascorbic acid added mg.	Gluta- thione mg.	Selenium dioxide mg.	Ascorbic acid esti- mated mg.	% decrease	Remarks	Ascorbic acid added mg.	Gluta- thione mg.	Na selenate mg.	Ascorbic acid esti- mated mg.	% decrease	Remarks	Ascorbic acid added mg.	Gluta- thione mg.	Na tellurate mg.	Ascorbic acid esti- mated mg.	% decrease
Titrated within 2-5 min.																
0.60	—	—	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—
0.60	—	0.1	0.58	3	—	0.60	—	0.1	—	—	—	0.60	—	1.0	0.59	1
0.60	—	0.3	0.56	6	—	0.60	—	0.3	—	—	—	0.60	—	3.0	0.59	1
0.60	—	0.5	0.50	8	—	0.60	—	0.5	—	—	—	0.60	—	5.0	0.59	1
0.60	—	1.0	0.46	23	—	0.60	2.0	0.1	—	Less than 1.0	—	0.60	—	10.0	0.58	3
0.60	2.0	0.1	0.60	—	—	0.60	2.0	0.3	—	—	—	0.60	2.0	1.0	0.60	—
0.60	2.0	0.3	0.59	1	—	0.60	2.0	0.5	—	—	—	0.60	2.0	3.0	0.60	—
0.60	2.0	0.5	0.57	5	—	—	—	—	—	—	—	0.60	2.0	5.0	0.60	—
0.60	2.0	1.0	0.52	13	—	—	—	—	—	—	—	0.60	2.0	10.0	0.60	—
Titrated after standing 20 min.																
0.485	—	—	0.30	0.42	—	—	—	—	—	—	—	—	—	—	—	—
0.485	—	0.50	0.38	21	*	—	—	—	—	—	—	—	—	—	—	—
0.485	—	1.00	0.30	38	*	—	—	—	—	—	—	—	—	—	—	—
0.485	2.0	0.30	0.45	6	—	—	—	—	—	—	—	—	—	—	—	—
0.485	2.0	0.50	0.42	13	—	—	—	—	—	—	—	—	—	—	—	—
0.485	2.0	1.00	0.38	21	—	—	—	—	—	—	—	—	—	—	—	—
Titrated after standing 2 hr.																
0.60	—	—	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—
0.60	—	0.1	0.51	14	*	0.60	—	0.1	—	16	*	0.60	—	1.0	0.53	11
0.60	—	0.3	0.33	36	†	0.60	—	0.3	—	23	*	0.60	—	3.0	0.46	23
0.60	—	0.5	0.20	65	††	0.60	—	0.5	—	36	*	0.60	—	5.0	0.36	40
0.60	—	1.0	0.05	92	†††	0.60	—	1.0	—	52	*	0.60	—	10.0	0.34	43
0.60	2.0	—	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—
0.60	2.0	0.1	0.57	5	—	0.60	2.0	0.1	—	5	—	0.60	2.0	1.0	0.59	1
0.60	2.0	0.3	0.45	25	—	0.60	2.0	0.3	—	13	—	0.60	2.0	3.0	0.59	1
0.60	2.0	0.5	0.30	50	*	0.60	2.0	0.5	—	31	—	0.60	2.0	5.0	0.55	8
0.60	2.0	1.0	0.08	86	†	0.60	2.0	1.0	—	45	*	0.60	2.0	10.0	0.53	11
0.60	4.0	0.1	0.59	1	—	0.60	4.0	0.1	—	1	—	0.60	4.0	1.0	0.59	1
0.60	4.0	0.3	0.52	13	—	0.60	4.0	0.3	—	8	—	0.60	4.0	3.0	0.59	1
0.60	4.0	0.5	0.41	31	—	0.60	4.0	0.5	—	15	—	0.60	4.0	5.0	0.58	3
0.60	4.0	1.0	0.18	72	*	0.60	4.0	1.0	—	38	—	0.60	4.0	10.0	0.58	3
* Light yellow-coloured solution. † Yellow-coloured solution. ‡ Brown red to red solution—indicated colloidal state.																

† Brown red to red solution—indicated colloidal state.

† Yellow-coloured solution.

* Light yellow-coloured solution.

livers of the injected rats. Sollmann [1936] states that reduction of tellurium compounds occurs with all living cells and that the reduced tellurium is particularly abundant in the liver and kidneys, while the lungs, spleen and adrenals are relatively free. The liver and kidneys contain appreciable amounts of cysteine, which reduces the tellurate to the colloidal state even in the presence of vitamin C.

Qualitative tests have indicated that the sulphydryl compounds, such as cysteine and glutathione, inhibit the reduction of silver nitrate [Svirbely, 1935] and of selenious acid [Emmerie, 1934] to the colloidal state by ascorbic acid. A quantitative study of these reductions by various salts was made by means of the indophenol titration method. Sulphydryl compounds were first added to the vitamin, then the salt, and, after a definite time interval (the solution being exposed to air in a 50 ml. beaker), titration was carried out rapidly in the presence of a few drops of 8 % acetic acid. The average results of three titrations, correction being made for any reduction of the dye by the sulphydryl compounds, are given in Table III. The salts themselves did not interfere with the titration.

The amounts of ascorbic acid removed by selenium dioxide, sodium selenate and tellurate paralleled the order of toxicity of the salts. Reduction of the salts (except the tellurate) to the colloidal state depended on the time interval elapsing before titration. Inhibition of this reduction in a 2 hr. interval was afforded by glutathione, cysteine (2 mg.) or a mixture of glutathione and cysteine (2 mg. each). Little protection to vitamin C was given by these sulphydryl compounds in the presence of the higher concentrations of selenium dioxide or sodium selenate, though protection was obvious with the lower concentrations and with sodium tellurate. No consistent titration values were obtained with larger amounts of cysteine since cysteine itself reduces the indophenol indicator.

The appreciable decrease in vitamin C with 1 mg. selenium dioxide, even when glutathione inhibited the assumption by the salt of the colloidal state, is of interest. Complete protection of ascorbic acid in a 2 hr. interval (estimated by the titration method) was afforded by these sulphydryl compounds (2 mg. cysteine or glutathione) in the presence of 1 mg. silver or uranyl nitrate. These salts, in the absence of cysteine or glutathione, produced a noticeable decrease in the amount of vitamin C, the percentage decreases (2 hr. interval) being 92 and 21 respectively. In confirmation of the work of Mawson [1935], it was found that the oxidation of the vitamin by 0.15 mg. Cu (as sulphate) was markedly decreased by glutathione. Such a variation in the ability to protect the vitamin, associated with inhibition of either the reduction of these metallic salts to the colloidal state or of the oxidation of the vitamin, may be explained by the formation of a stable complex of the sulphydryl compounds with the cations in the case of the heavy metals. With selenium salts, according to Bersin [1935], oxidized glutathione is formed, which may, in the glutathione-ascorbic acid system, lead to oxidation of the vitamin both *in vivo* and *in vitro*.

SUMMARY

1. Rats with symptoms of selenium and tellurium poisoning show marked decreases in the vitamin C content of the liver and adrenals.

2. The production of oxidized glutathione by selenium salts, in contrast to the stable complex which sulphydryl compounds form with the cations of heavy metals, may account for the decrease in ascorbic acid which is observed *in vitro* even when there is an inhibition of the reduction of the selenium to the colloidal state.

3. Selenium salts are reduced more readily by ascorbic acid than by glutathione or cysteine, but the reverse relationship holds true for sodium tellurate.

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REFERENCES

- Bersin (1935). *Ergebn. Enzymforsch.* **4**, 68.
De Caro & Giani (1934). *Hoppe-Seyl. Z.* **228**, 13.
Emmerie (1934). *Acta. brev. neerl. Physiol.* **4**, 141.
Franke (1934). *J. Nutrit.* **8**, 597.
—— & Potter (1935). *J. Nutrit.* **10**, 213.
Mawson (1935). *Biochem. J.* **29**, 569.
Mouriquand & Couer (1935). *C.R. Soc. Biol., Paris*, **119**, 615.
Moxon & Franke (1935). *Industr. Engng Chem.* **27**, 77.
Potter & Elvehjem (1936). *Biochem. J.* **30**, 189.
Schneider (1936). *Science*, **83**, 32.
Sollmann (1936). *Pharmacology*. Philadelphia and London, 5th ed., 960.
Svirbely (1935). *Biochem. J.* **29**, 1547.
—— (1936). *J. biol. chem.* **116**, 543.