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ASCORBIC ACID IN THE PREVENTION AND TREATMENT OF TOXIC EFFECTS FROM CHROMATES

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Abstract. This paper reviews experimental data on the uses of ascorbic acid in laboratory animals for the prevention and treatment of systemic chromate intoxication and skin ulcers, and for the prevention of allergic eczematous dermatitis due to chromates. The mechanism for the inactivation of hexavalent chromium by ascorbic acid involved reduction to trivalent chromium and subsequent complex formation of the trivalent species.

The purpose of this paper is to report experiments on the treatment of systemic chromate intoxication and skin ulcers in laboratory animals by ascorbic acid. Ascorbic acid was also used to prevent allergic eczematous dermatitis due to chromates. The applicability of these measures in industrial exposure and the chemical reactions involved for the inactivation of hexavalent chromium will be discussed.

Ascorbic Acid in the Treatment of Acute Intoxication in Rats

In a previous study (5) we reported on the use of ascorbic acid in preventing and treating acute toxic effect of chromates. Measured amounts of hexavalent and trivalent chromium solutions were given to young albino rats by gastric intubation. This technique insured the introduction of known amounts and therefore was preferable to the routine administration of chromium solutions in drinking water. Administration of chromium was followed by the introduction of 10% aqueous ascorbic acid at varying intervals, to determine the efficacy of the antidote in the prevention and treatment of toxic symptoms. Internal organs of sacrificed animals were studied for gross pathologic changes and were analyzed for chromium.

In these experiments, it was found that the lethal oral dose of potassium dichromate was 130 mg of Cr_6/kg. In a similar series of experiments, chromic chloride did not cause any toxic effects with amounts as high as 650 mg of Cr_3/kg. Trivalent chromium produced no pathologic changes and was not retained in the liver, lungs, kidney or spleen.

It was possible to prevent chromate poisoning in rats with ascorbic acid, if the antidote was administered before the stomach emptied. The emptying time of a rat's stomach is reported to be two hours (2). Of 28 rats which received a lethal dose of hexavalent chromium followed within two hours by ascorbic acid at varying intervals (immediately, $^1/_2$, 1 and 2 hours) 26 (93%) survived. Two rats died; one from aspiration pneumonia, and one from chromate poisoning or accidential trauma. Six rats died when given a lethal dose of hexavalent chromium followed three hours later by ascorbic acid (Table I). Gross autopsy findings of these six rats showed diffuse hemorrhage, gastritis and enteritis. Spectrographic analyses (emission spectrographic analyses carried out by Dalare Associates, Philadelphia, Pa.) revealed very high amounts of chromium in the liver, lungs, kidney and spleen.

Thus ascorbic acid, which has the capacity to inactivate chromates can be an effective antidote when administered promptly to rats poisoned with chromate. It is likely that these experimental findings would apply also to man, although acute intoxication from oral ingestion of hexavalent chromates is rare.

The Effect of Ascorbic Acid in Experimental Chrome Ulcers in Guinea Pigs

With a previously described technique (6) we studied the effect of topical ascorbic acid on chrome ulcers in guinea pigs (4).

Table I. *Results of animal studies using ascorbic acid as an antidote for chromate poisoning*

Group no.	No. of rats	Solution intubated	Results
1	2	1 ml stock Cr sol.	Died within 24 h
2	6	1 ml stock Cr sol. + 4 ml water	Died within 4 to 10 h [b]
3	4	5 ml water	Alive and well [a]
4	4	4 ml ascorbic acid sol. + 1 ml water	Alive and well [a]
5	8	1 ml stock Cr sol. + 4 ml ascorbic acid sol. immediately	Alive and well [a]
6	6	1 ml stock Cr sol. + 4 ml ascorbic acid sol. 30 min later	Alive and well [a]
7	7	1 ml stock Cr sol. + 4 ml ascorbic acid sol. 1 h later	6 rats alive and well [a] 1 rat died within 1 h
8	7	1 ml stock Cr sol. + 4 ml ascorbic acid sol. 2 h later	6 rats alive and well [a] 1 rat died within 10 h
9	6	1 ml stock Cr sol. + 4 ml ascorbic acid sol. 3 h later	All died with 8 to 20 h

Stock $K_2Cr_2O_7$: 25 mg Cr(VI)/ml.
Stock ascorbic acid: 200 mg ascorbic acid/ml (20% solution).
[a] Rats observed for two week periods following experiment except for two rats each in groups 3, 4, 5, 6, 7 and 8 sacrificed after 48 hours for gross pathology and spectrographic examination.
[b] Rats in group 2 were controls.

Albino male guinea pigs, weighing between 700 to 1200 gm, were shaved on the mid-backs with an electric clipper and the remaining hair removed with epilating wax. Superficial abrasions about 2 cm in diameter were produced by firm strokes with a serrated kitchen knife. Abrading was continued until the surface showed numerous discrete, bleeding points (about 40 strokes).

Table II. *Days required for healing of abrasions*

Site	Untreated	Immediate application of $K_2Cr_2O_7$
1	6	13
2	6	17
3	4	17
4	4	17
5	5	13
6	7	20
7	4	12
8	4	17
9	6	12
10	6	13
11	6	
12	6	
Mean	5.33	15.10

Table III. *The effect of washing the abrasions with 10% aqueous ascorbic acid at varying intervals after application of $K_2Cr_2O_7$*

	Healing time in days			
Animal	Immediate	5 min	15 min	30 min
1	4	6	6	6
2	6	6	6	6
3	6	6	6	6
4	6	6	7	11
5	6	7	8	11
6	6	7	12	11
7	7	13	7	12
Average	5.86	7.28	7.42	9.00

The ulcerogenic agent, 0.05 ml of 0.34 *M* $K_2Cr_2O_7$, was applied with a pipette 10–15 min after abrasion. The concentration of the ascorbic acid solution was 10%. When a wash solution (either H_2O or 10% aqueous ascorbic acid) was employed 1.0 ml was slowly run over the area. The animals were treated in a double blind fashion so that the technician did not know which animals received water or ascorbic acid washes. The degree of ulceration was evaluated by the number of days required for healing, with absence of crust or ulcer.

The experimental lesions were generally covered with large crusts. Reepithelialization occurred under the eschar, which was not cast off until the lesion was nearly healed. It was difficult to determine the severity of the ulcerative process from inspection and grading of the gross appearance of the lesion. Healing time proved to be a much more reliable measure of the extent of ulceration.

As was noted in previous experiments, trauma was a prerequisite in the production of chrome ulcers. Application of $K_2Cr_2O_7$ to abraded patches on guinea pig midbacks significantly prolongs the healing time. Untreated abrasions healed on the average in fifteen days (Table II).

One ml of aqueous ascorbic acid applied to abrasions treated with potassium dichromate significantly decreased their healing time. It was also noted that the longer time elapsed before application of ascorbic acid, the longer was the period required for healing (Table III). However, even with 30 min delay, the average healing time of treated ulcers was shorter than on the control sites. When water was substituted for the ascorbic acid solution, even when applied immediately following the dichromate, little effect was apparent. Ascorbic acid acts by reducing the hexavalent chromium salt to the less irritating trivalent state. This reduction could be observed in vitro as a distinct color change from orange to blue.

Use of Filters Impregnated with Ascorbic Acid for Protection Against Inhalation of Chromic Mist

This study (7) was designed to evaluate the degree of protection obtained by a worker wearing a dust respirator and to determine if this protec-

Table IV. *Effectiveness of filters impregnated with ascorbic acid*

Protective device	Cr found on tape mg CrO_3/m^3	Average
None	0.214, 0.250, 0.268, 0.270	0.250
600 A mask	0.090, 0.107, 0.090, 0.085	0.093
600 A mask with filter impregnated with 10% ascorbic acid solution	0.054, 0.054, 0.072	0.060
600 A mask with filter impregnated with 20% ascorbic acid solution	0.045, 0.050	0.048
45 D mask	0.107, 0.090, 0.090, 0.090	0.094
45 D mask with filter impregnated with 10% ascorbic acid solution	0.054, 0.072, 0.072	0.066
45 D mask with filter impregnated with 20% ascorbic acid solution	0.035, 0.045	0.040

tion was enhanced by incorporating ascorbic acid into the filters of the respirators.

The experiments were carried out in a special mist chamber into which a chromate mist was aspirated. The dust respirator was fitted to a manikin head mounted inside the chamber, and the chromate mist was drawn through the respirator by an air sampler (Model "D", Research Appliance Company, Allison Park, Pa.) at a rate of 0.260 cubic feet per minute. The flow rate of the aspirator was adjusted so that the air-borne chromium concentration inside the chamber corresponded to 0.025 mg CrO_3/m^3.

Filters from commercial respirators (Style no. 600 A. Style no. 45 D-Willson Products Division, Ray-O-Vac Company, Reading, Pa.) were soaked in 10% and 20% ascorbic acid solutions for 30 min and dried at 100°C prior to use; control filters were unaltered. Each respirator was tested for 30 min at room temperature. The amount of chromium passing through the respirator was determined by chemical analysis of the air sampler tape with the technique of Urone & Anders. The results of these tests are presented in Table IV. They show that an approved type dust respirator reduces the chromate exposure to the threshold limit of 0.1 mg/m³. With the incorporation of ascorbic acid into the filter, the effectiveness of the respirator is enhanced 35% to 50%, depending on the concentration of ascorbic acid used.

As the ascorbic acid is used up in the course of its reaction with hexavalent chromium, the second phase of this study was directed to ascertain the "useful life" of the filters impregnated with ascorbic acid. For this purpose the amounts of chromium passing through the respirator were determined after $^1/_2$, 1, 2, 3 and 4 hours by chemical analysis of the air sampler tapes. The results are presented in Table V.

The enhancement by 10% ascorbic acid is of short duration, because the ascorbic acid is inactivated in its reaction with hexavalent chromium. Within three hours, filters impregnated with 10% ascorbic acid lost effectiveness and became equal to that of the untreated filters; filters impregnated with 20% ascorbic acid maintained effectiveness for three hours.

In the third phase of this study we determined the "shelf life" of filters impregnated with ascorbic acid. Several filters were soaked in 20% ascorbic acid and dried. One filter was selected at random each week and tested for a 30 min period.

Filters impregnated with 20% ascorbic acid retained their effectiveness during storage for at least four weeks.

Hexavalent chromium is inactivated by ascorbic acid by forming a red, water-soluble complex of trivalent chromium. In this form, the chromium probably has minimal toxic effects. As a trivalent compound, it does not possess the necrotizing action of the hexavalent species and thus does not produce nasal ulcerations. Its tendency to bind with tissue is decreased because its secondary valence is satisfied. The concentration of chromium in the tissues is probably decreased because of the high solubility of the compound in water.

The valence of the chromium ion is also important in controlling the occurrence and degree of its carcinogenic effect as emphasized by Hueper & Payne (3). According to these investigators, these highly water-soluble

Table V. "*Useful life*" *of filters impregnated with ascorbic acid*

Protective device	Cr found on tape mg CrO_3/m^3				
	0.5 h	1 h	2 h	3 h	4 h
None	0.252	—	0.250	—	0.265
600 A mask	0.107	0.090	—	0.107	0.107
600 A mask with filter impregnated with 10% ascorbic acid solution	0.054	0.071	—	0.107	0.107
600 A mask with filter impregnated with 20% ascorbic acid solution	0.045	0.052	0.060	0.065	0.090
45 D mask	0.107	0.107	—	0.107	0.107
45 D mask with filter impregnated with 10% ascorbic acid solution	0.054	0.071	—	0.107	0.107
45 D mask with filter impregnated with 20% ascorbic acid solution	0.035	0.060	0.060	0.065	0.090

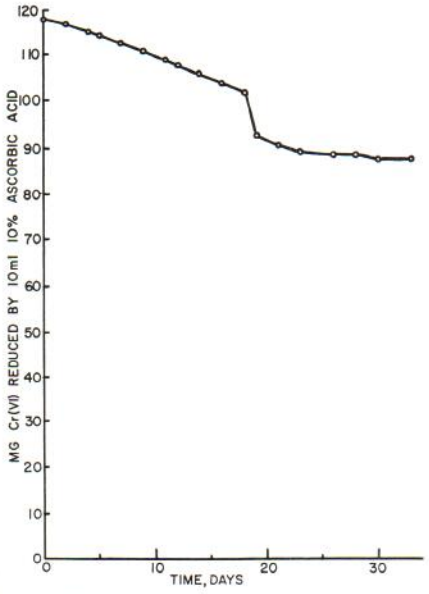

Fig. 1. Stability of ascorbic acid solutions with respect to chromium reduction.

and corrosive chromium compounds may exert or contribute to a carcinogenic effect upon the tissues of the respiratory tract, if such relatively mild exposures are repeated and prolonged, despite the essentially fleeting effect of contact. This type of exposure prevails in workers employed in plants which produce or use chromates.

Our experiments indicate that ascorbic acid enhances protection against inhalation of chromic acid mist. The impregnation of ascorbic acid into the filters of respirators would be of practical use in industries where chromic acid mist hazard is prevalent.

The Use of Ascorbic Acid as a Preventive Regimen in Chromate-sensitive Workers

In previous studies (8, 9) we reported that patch test reactions in chromate-sensitive patients could be inactivated by cysteine, glutathione, ascorbic acid, and pyrosulfite mixture. The stability and low cost of the latter two agents made them well-suited for practical use; of the two, the ascorbic acid solution had better patient acceptance.

Fifteen lithographing and printing establishments participated in our study (10). The plants maintained excellent housekeeping, were well-ventilated, and lighted. The work areas were tested for the presence of hexavalent chromium; the degree of contamination was dependent upon the thoroughness of housekeeping and varied from plant to plant. The routine for prevention against solvent dermatitis and decontamination of chromates was readily accepted by both supervisors and workers.

In 11 plants, 22 workers with dermatitis were found. Patch tests carried out on this group of patients gave positive reactions to 0.25% potassium dichromate in 5 workers and negative results in 17 workers. The latter group had eruptions characteristic of irritancy due to solvents and soaps.

Preventive measures to control chromate dermatitis were instituted in five separate plants. Each plant had one chromate-sensitive worker. The chromate reactors were supplied with ascorbic acid, plastic buckets, measuring cups and instructions for preparation of the 10% ascorbic acid solution.

The antichrome solutions were kept in plastic containers near the work areas. The five chromate-sensitive workers were advised to soak their hands and forearms as promptly as possible after they had handled chromate mixtures. The workers were kept on their jobs and were reexamined at their plants at weekly intervals, for six months. During the first month of the study, the ascorbic acid solutions were assayed at weekly intervals to determine the stability and antichrome activity. Our studies showed that it was advisable to make fresh solutions weekly.

Three chromate-sensitive workers became symptom-free and stayed well as long as they continued the preventive routine. Two chromate-sensitive workers showed definite improvement in their hand eruptions without complete disappearance of the lesions; in one there was a marked recurrence of dermatitis when the preventive regimen was interrupted.

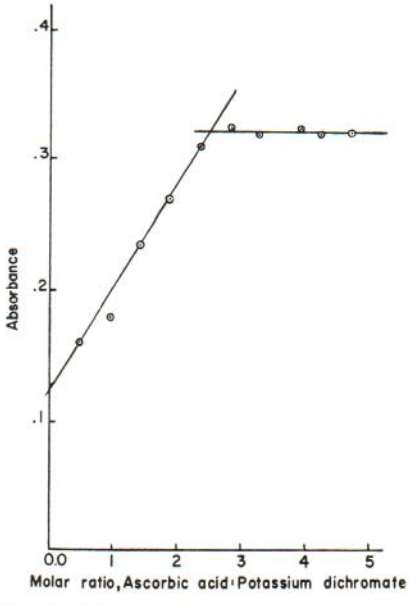

Fig. 2. Reduction of hexavalent chromium by ascorbic acid.

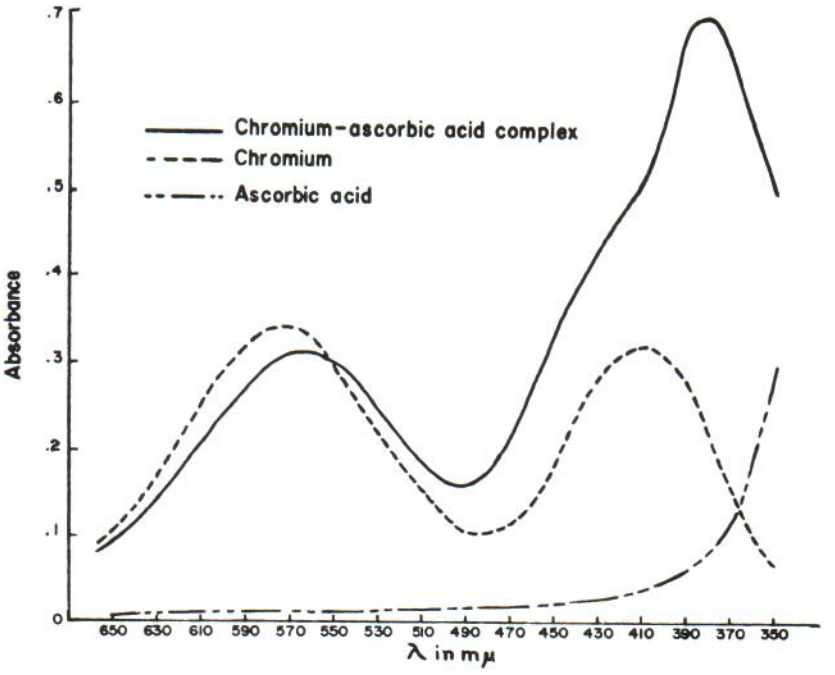

Fig. 3. Absorption spectra for trivalent chromium, ascorbic acid and the trivalent chromium ascorbic acid complex.

The Mechanism of Inactivation of Chromium by Ascorbic Acid

The chromium inactivating capacity of ascorbic acid was first studied in vitro by titrating 10 ml aliquots of a 10% ascorbic acid solution with standard dichromate solution with the Serfass titrimeter. The results of these titrations indicate that 1 gram of ascorbic acid reduced 0.118 grams of hexavalent chromium.

The stability of ascorbic acid solutions with respect to their chromium reducing ability was also determined by periodically titrating aliquots of the ascorbic acid solution. The results of this study are presented in Fig. 1.

While the mechanism by which ascorbic acid inactivates chromium is obscure, it has been proposed that the hexavalent form is reduced to the trivalent state by the ascorbic acid, and the resulting trivalent chromium is then complexed by the ascorbic acid (5). In a review of ascorbic acid as a reducing agent, no reference is made to the reduction of chromium (1).

We carried out recently experiments to define these chemical reactions (11). The reduction of hexavalent chromium by ascorbic acid was investigated by the molar-ratio method. Fig. 2 shows that ascorbic acid and hexavalent chromium react in a 5:2 ratio. This is slightly less than would be expected for oxidation of all the ascorbic acid to dehydro-ascorbic acid.

Spectra for the uncomplicated chromium and the chromium-ascorbic acid complex, recorded with a Beckman Model DB spectrophotometer, are presented in Fig. 3 and the formula of the complex was determined. The chromium-ascorbic acid complex exhibits a strong absorption near 390 mμ as evidence of complex formation.

The complex was evaluated by the method of continuous variation. Fig. 4 shows the relative concentrations for maximum complex formation. The seven to three ratio may indicate a polymeric species in which the chromium (III) is to more than one ascorbic acid molecule.

The mechanism for the inactivation of hexavalent chro-

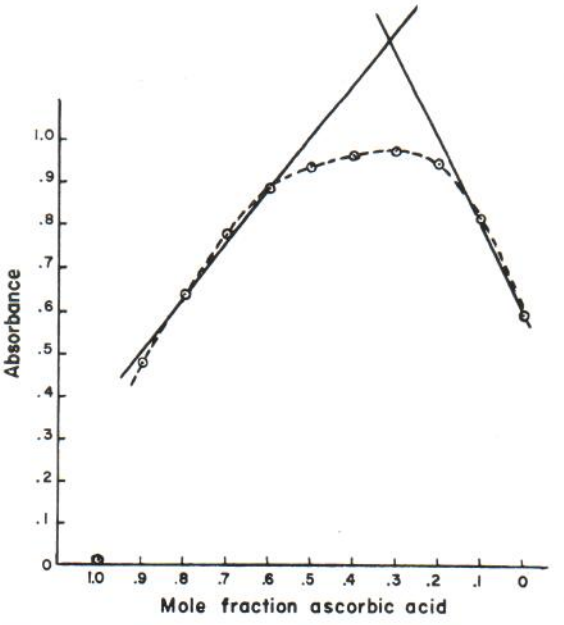

Fig. 4. Formation of the trivalent chromium ascorbic acid complex.

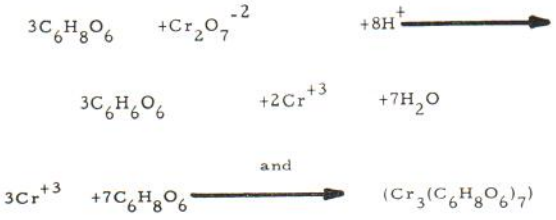

Fig. 5. Mechanism for the inactivation of hexavalent chromium by ascorbic acid.

mium by ascorbic acid involves reduction to trivalent chromium and subsequent complex formation of the trivalent species (Fig. 5).

CONCLUSIONS

The capacity of ascorbic acid to inactivate chromium compounds was studied with several experimental systems. Our findings show that ascorbic acid is effective in preventing and treating various adverse reactions to chromates. Acute chromate intoxication in rats was prevented when ascorbic acid was administered promptly; a preventive regimen with 10% aqueous ascorbic acid proved effective in protecting chromate-sensitive workers in the printing and lithographing industries; the impregnation of ascorbic acid into the filters of respirators enhanced protection against inhalation of chromic acid mist; the 10% ascorbic acid solution significantly shortened the time required for healing of skin ulcers produced by potassium chromate in guinea pigs.

The mechanism for the inactivation of hexavalent chromium by ascorbic acid involved reduction to trivalent chromium and subsequent complex formation of the trivalent species.

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