The Ascorbates 8/28/73

1CC

Substance

Ascorbic Acid

Sodium Ascorbate

Isoascorbic Acid

Sodium Isoascorbate

Calcium Ascorbate

Ascorbyl Palmitate

#51

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THE ASCORBATES

Submitted to:

GRAS Review Branch (BF-335)
Bureau of Foods
Food and Drug Administration
200 C Street, S.W.
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Att. Mr. Alan Spiher
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Substance	CAS No.	Accession No.
Ascorbic Acid	000050817	93
Sodium Ascorbate	000134032	558
Isoascorbic Acid	000089656	258
Sodium Isoascorbate	977052064	575
Calcium Ascorbate	005743271	115
Ascorbyl Palmitate	000137666	94

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THE ASCORBATES

Summary - Toxicological Information

Although this monograph is entitled Ascorbates, it also includes a great deal of information on ascorbic acid because of the intertwining of the work on these compounds. Where any of the synonyms, D-isoascorbic acid, erythrobic acid, D-araboascorbic acid, or isoascorbic acid were encountered in the literature, they have been used in the monograph under the name of isoascorbic acid to avoid confusion in reading. The sodium salts of these synonyms have been referred to as sodium isoascorbate. Other compounds included in the monograph are sodium ascorbate, calcium ascorbate, and ascorbyl palmitate.

The probable reason that a large percentage of the work on the ascorbates was done on guinea pigs is that they are one of the few animals that do not manufacture their own vitamin C (111a). Work on animals that do produce ascorbic acid must be interpreted in that light.

The acute toxicity of isoascorbic acid administered by stomach tube in water to 20 g adult male mice of the 1S-32 strain shows the LD_{100} to be 12,000 mg/kg, the LD_{50} to be 8,300 mg/kg, and LD_0 to be 5,750 mg/kg. For young adult Holtzman rats of about 200 g weight, the values are 42,000, 18,000, and 8,000 mg/kg for LD_{100}, LD_{50}, and LD_0, respectively*.

Isoascorbyl palmitate orally administered showed LD_{50} values greater than 8,000 mg/kg for mice and 6,000 mg/kg for rats (30a).

Sodium ascorbate oral LD_{50} was greater than 5,000 mg/kg for mice, rats and guinea pigs. Intravenous administration showed LD_{50}

*Private communication from unidentified source, received from FDA.

values of greater than 1,000 mg/kg for the mice and rats and greater than 500 mg/kg for the guinea pigs (110a).

An unspecified number of rabbits were intravenously dosed with sodium bicarbonate neutralized ascorbic acid with resulting LD_{100} values of between 6,400 and 7,300 mg/kg. The time of death after intravenous injection of 8,500 mg/kg and 10,400 mg/kg doses of a 20% solution of sodium bicarbonate neutralized ascorbic acid at pH 7.4 were sixty and forty minutes, respectively. However, the life span was extended by one hour for 5 rabbits receiving 8,500 mg/kg of sodium ascorbate by the administration of 50 ml/kg of physiological saline solution with a probe into the stomach. At the dosage level of 7,300 mg/kg, the rabbits died with convulsive attacks after about 5 hours. No toxic effects were evidenced at the 5,300 mg/kg level but at 6,400 mg/kg, tremors were observed in the limbs approximately one hour after injection (88).

The suggestion that fatty acid esters of ascorbic acid and isoascorbic acid may safely be used in fat and oil as antioxidants have been confirmed. Only at the 5% level were any harmful effects noted for ascorbyl palmitate. Significant retardation of growth and bladder stones were found in two rabbits in a 9-month feeding study. Long term studies of 2 years duration at the 0.25% dietary level brought about no deleterious effects for ascorbyl palmitate and isoascorbyl palmitate. Increasing the dosage to the 1% level for isoascorbic acid had no deleterious effects in the same period of time. In another study of 9-month duration at the 2% level of L-ascorbyl and D-isoascorbyl palmitate, no toxic symptoms were observed (16).

The acceptability of isoascorbic acid was also shown at dosages up to 7,500 mg/day being given orally for periods up to 240 days. No hematological, biochemical, or urine deviations or pathological changes in the test beagle dogs were produced. * No morphologically significant changes were found on histological evaluation of the kidney and liver of dogs that had received injections of 1,000 mg/kg/day of sodium ascorbate into the maximal vein for 20 days. The sodium ascorbate which was formed by neutralizing ascorbic acid with sodium bicarbonate was administered as a 20% solution at pH 7.4 (88).

To the contrary, isoascorbic acid and ascorbic acid at the 0.1% dietary level administered to 20 cooper deficient chicks caused decreased growth and either decreased the elastin content of the aorta or increased the mortality of the chicks. In a 25 ppm copper supplement study, the isoascorbic acid and ascorbic acid did not cause the above effects. Therefore, the above side effects may be due to physiological imbalances brought on by the copper deficiency (183).

Though oral feeding of the acids in doses of 25 and 50 mg/day for 14 days were without effect on cobalt chloride induced polycythemia, sodium ascorbate and sodium isoascorbate intravenously administered in doses equivalent to 25 mg of ascorbate per day caused a marked decrease in the hemoglobin concentration in rabbits. A smaller decrease in the hemoglobin concentration was evidenced when sodium isoascorbate was substituted for the neutralized ascorbic acid. Only

*Private communication from unidentified source, received from FDA.

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sodium glucoascorbate produced no effect on the polycythemia in the rabbits. There appears to be a parallelism between the antiscorbutic affect of these compounds and their ability to reduce the polycythemia. Note should be made that only 2 or 3 rabbits were used for both the oral and intravenous administration tests of the three compounds (209).

Researchers disagree on the antiscorbutic activity and body storage of isoascorbic acid as compared to ascorbic acid (185, 234, 230, 257). A decided difference was found in the deposition in the tissues when ascorbic acid and isoascorbic acid were administered to guinea pigs on a low control intake ascorbic acid diet. 1.5 - 0.1 mg of body weight of each of the compounds resulted in ascorbic acid deposition but not in isoascorbic acid deposition. In contrast with the daily supplement, the intramuscular administration of the two compounds produced some isoascorbic acid in the tissue. This was not to the same extent as the equivalent amount of ascorbic acid. No evidence of the conversion of isoascorbic acid to ascorbic acid was found. Isoascorbic acid is absorbed in the gastrointestinal tract, extracted from the blood or retained by the tissues less readily than ascorbic acid (185).

A lack of antiscorbutic action for isoascorbic acid was found when 250 mg/day did not support guinea pigs fed a vitamin C deficient diet. Guinea pigs that were depleted of vitamin C showed no therapeutic benefit to receiving isoascorbic acid. The main action of isoascorbic acid may be protective of the ascorbic acid (234). Opposite

results were obtained when guinea pigs that survived 18 days on a scorbutic diet showed no evidence of disease following 22 days of therapy with 15 to 30 mg doses of isoascorbate. The minimum curative dose was tested on 7 to 9 guinea pigs in each experiment and was found to be 20 to 25 mg/day for the surviving guinea pigs. Note must be made in evaluating this curative dosage level that those animals being supersensitive to scurvy by reason of death under therapy were deleted from consideration. The autopsy showed all of them to be scorbutic. In contrast, all of the guinea pigs receiving 30 mg/day of sodium isoascorbate survived. It may, therefore, be concluded that a larger dosage than the minimum curative dose be administered (150).

Evidence that isoascorbic acid can replace the antiscorbutic activities of ascorbic acid was based on the normal survival and lack of scurvy signs in guinea pigs fed 10 to 200 mg of isoascorbic acid per day (167). To prove that isoascorbic acid was retained by the tissues guinea pigs were fed a synthetic diet containing ascorbic acid and isoascorbic acid. The isoascorbic acid was found to be a replacement for ascorbic acid and could reversibly be replaced by ascorbic acid (230). In a parallel vein, the results on five human subjects in comparing the blood levels of ascorbic acid and sodium ascorbate after the administration of 300 mg doses of isoascorbate seemed somewhat contradictory. It has been found that an overestimation of the state of vitamin C nutrition as judged by blood levels can result from the ingestion of sodium isoascorbate which has less than 1% of the vitamin C activity. However, a rapid paper chromatography separation of these isomers showed no significant displacement

of the ascorbic acid by 300 mg doses of isoascorbic acid. As there is no displacement of the ascorbic acid, no overestimation for the vitamin C nutrition level can be stated (192).

The blood level as an overestimation of vitamin activity for isoascorbic acid stated above (192) may be tied in with its high excretion rate. This rate was 5 times that of ascorbic acid in 6 male humans . Most of it was found in the six-hour urine. Therefore, no appreciable resorption by the kidney tubular cells is indicated for isoascorbic acid (257).

The product label Nu-211 that was found to be 6-palmitoyl-L-ascorbic acid produced results similar to those received when vitamin C was administered to artificially vitamin C deficient guinea pigs. The guinea pigs were allowed to stay on the deficient diet through the initial weight loss period, a weight recovery period of two weeks, and another period of marked loss. At this point vitamin C or Nu-211 was administered. Weight loss ceased and gain followed to an extent that brought the weight back or almost back to that of the parallel controls at the end of the experiment. A series of tests showed that for both compounds the dosage other than having a minimum acceptable value does not seem to be of importance. It is interesting to note that all of the guinea pigs that were on the vitamin C deficient diet that received neither vitamin C or Nu-211 died within 6 1/2 weeks and that a few of the other guinea pigs also died by the end of the experiment. However, at least 7 of the 9 or 10 original animals in each group, except the negative control group, were still alive when the test terminated. *

*Private communication from unidentified source, received from FDA.

When compared to ascorbic acid, the amount of isoascorbic acid preserved in the human in vitamin C deficiency is extremely small both in duration and amount and its antiscorbutic activity is negligible (213). Based on the previously cited minimum curative dosages of 20 to 25 mg of sodium isoascorbate necessary to counter scorbutism in guinea pigs, 1,000 mg contains 2 to 2 and 1/2 human doses or 40 - 50 biological units (150).

An extract of plants called factor 2 has been found that contains ascorbic acid and isoascorbic acid which is believed to be a flavone that stimulates the ascorbic acid to produce more glycogen in the liver than does the acid alone. Some plants contain a third factor that mitigates the action of factor 2. Proof that the vitamin C action does not depend only upon ascorbic acid was seen when 5 mg of natural vitamin C plus 1 cc lemon juice caused production of more liver glycogen than 50 mg of pure ascorbic acid. Factor 2 which has been given the name vitamin C_3 is believed to contain two components (252).

A cure for allergic sensitivity appears to have been found in sodium ascorbate (130, 131). However, bradycardia which resulted when sodium ascorbate was administered to 16 human subjects was traced to the sodium ions (126). Cases of chronic asthma and hay fever that did not do well under therapy with ascorbic acid, did extremely well with the sodium ascorbate. These patients were administered 2,000 mg of sodium ascorbate per day. A marked freedom from gastric irritation and increased diuresis resulted (131). In 13 other

cases refractive to ascorbic acid, sodium ascorbate therapy produced dramatic results with little or no side effects. The dosage using a sodium bicarbonate neutralized ascorbic acid preparation was varied to suit the individual need of each of these 13 patients (130). The patient whose allergic symptoms tend to stem from adrenalcortical insufficiency benefited the most from sodium ascorbate therapy. Calcium ascorbate benefited to a greater degree those patients whose allergic symptoms seem to stem from enzymatic and toxic factors (131).

ACTH depleting effects can partially be overcome by an increased quantity of ascorbic acid available for uptake (138).

In a comparative study on the metabolism of ascorbic acid and its fatty acid esters, guinea pigs received orally quantities of each compound equivalent to 20 mg of ascorbic acid. Urinary ascorbic acid levels were found to be greater in the 0 to 24 hour period than in the 24 to 48 hour period and a direct relationship can be seen between the content of ascorbic acid in the urine and the content in the organs. The inverse was true in the groups administered the esters of the acid. L-ascorbyl monopalmitate and L-ascorbyl dipalmitate yielded 80% and 20% free ascorbic acid, respectively, in mixtures of homogenates of small intestines and pancreas. Based on these results, it is felt that these esters are enzymatically hydrolyzed (22).

Two healthy men were used as subjects in feeding studies of ascorbic acid and ascorbyl palmitate. Two weeks of feeding of ascorbic acid followed by no addition to the normal diet was then followed by

feedings of 1180 mg of ascorbyl palmitate, the equivalent of 500 mg of ascorbic acid. Between dosages the levels of ascorbic acid in the urine returned essentially to normal.*

A difference has been found in the response of younger rabbits to varying the dosage of intraperitoneally injected ascorbic acid. A rise in the aqueous humor acid concentration with increased concentration in the blood exists over most of the range of 0 to 100 mg% in the serum for young rabbits. The maximum level of 80 mg% was reached in the 5 to 40 day old animals. However, in the adults,the maximum was only 50 mg% and independent of the serum concentration of ascorbic acid with a limiting minimum value of 3 mg%. Isoascorbic acid and D-glucoascorbic acid were also found in the serum of the adults but only isoascorbic acid was found in the aqueous humor. The results indicated that the secretory process was apparently selective for ascorbic acid or its optical isomer and suggests that ascorbic acid formed an intricate part of the secretory mechanism. However, in the young animals, it appeared that diffusion was the dominating factor and not the secretory process. The secretory process appeared to be unrelated to the antiscorbutic action (201).

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*Private communication from unidentified source, received from FDA.

Aqueous humor dynamic studies have been undertaken on 2-3 kg (sic) male albino rats. This was accomplished by administering sodium ascorbate, subjecting the rats to 19°C hypothermia and comparing them with normothermic animals. A single injection of 1000 mg of sodium ascorbate resulted in a plasma level of 160 mg%. Whereas the anterior chamber responded to a greater extent in the 10-20 eyes tested of the chilled rabbits subjected to 1000 mg sodium ascorbate, the posterior chamber sodium ascorbate increase was greatest at the lower dosages. The hypothermic animals at the saturation level of sodium ascorbate had showed the anterior chamber concentration to remain at a 3-fold greater magnitude than the posterior chamber concentration during the 300-minute duration of the experiment. It may have been that the posterior chamber failed to become saturated due to its large volume. In the hypothermic rabbits, the flow coefficient was 0.2% per minute and in the anterior chamber with a diffusion coefficient of 0.25% per minute. Along with the decrease in the ascorbic acid concentration in the hypothermic rabbit, there was an accompanying rise in the bicarbonate concentration of the aqueous humor. At the cold temperatures, there was a smaller decrease in the rate of aqueous flow, which may have been a reflection of changes in the blood, blood vessels and/or any other anatomical entities resulting therefrom. The normothermic coefficient of the aqueous blood flow of 1.6% to 0.25% per minute at 19°C for sodium ascorbate compared well with values derived from the ascorbic acid turnover rate of 1.7% of the normothermic and 0.2% for the hypothermic rabbits. At this concentration, it was possible to determine both the

anterior and posterior aqueous humor ascorbate levels. One group of 82 rabbits was first saturated with 200 mg sodium ascorbate by intravenous and subcutaneous injection. Samples of the aqueous humor from one eye were obtained and then the animals were subjected to hypothermia. The plasma levels remained at 10 mg% throughout the experiment. Posterior and anterior chamber turnover rates were noted as a function of time (86).

In an effort to find a clinically effective combination of drugs to reduce ocular pressure without adverse side effects, co-administration of glycerol in a 20% solution of sodium ascorbate at pH 7.4 was tested. Various ascorbate dosage levels from 0.4 to 1.0 gram/kg of body weight in humans were administered. Intravenously administered, this preparation proved successful in eliminating the hematuria produced by glycerol alone and to a significant degree reduced intraocular pressure. Paralled studies on rabbits had delineated comparative results (141). Using the above combination of glycerol at dosages of 600 mg/kg and of sodium ascorbate at the level of 280 mg/kg, the necessary volume was found to be much less than that required for similar results using other material tests. A rapid decrease in the eyeball pressure from 70 mm of mercury to approximately 10 mm of mercury resulted in one hour. This was followed by a gradual rise of the pressure over the next 7 hours which was probably due to the depletion of the therapeutic agents from the system. Although it seems to be an ideal mixture for reducing ocular pressure, one must take into account the development of a reduction in cardiac frequency which at times

reached bradycardia. The bradycardia was traced to the addition of sodium ions from the sodium ascorbate. This caused a migration of sodium into the cell and potassium out of the cell. Another point to be considered is that these 16 patients from 22 to 77 years of age had been fasted before treatment was begun (126).

Work on acute chronic, simple or irritative glaucoma has also been performed using sodium ascorbate at pH 7.4 formed from crystallized vitamin C by neutralization with sodium carbonate. The subjects were 18 patients from 30 to 70 years of age who were administered a 20% solution at the levels of 100 to 1000 mg/kg of body weight. The reduction in pressure increased with the dosage. Over 500 mg/kg of body weight, there was no further reduction in the ocular pressure and diuresis was experienced. The greatest reduction in ocular pressure was realized at approximately 1 to 2 hours after the end of the intravenous infusion with a gradual return to the pretreatment values over the next 6 hours. In contrast, the body osmotic pressure of all dosage levels increased to a maximum at the end of the infusion and took from 4 to 12 hours to return to normal. The osmotic pressure curve tended to parallel the ascorbinemia curve which had a maximum value of approximately 490 mg% at the end of the infusion at the two higher dosage levels. The ascorbinemia and the osmotic pressure values were less at the lower dosage levels. As there are so many physiological effects to be con-

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sidered, the varying results experienced among the individual subjects and the type of glaucoma were as anticipated. The ratios of the values of the ocular tension and blood osmotic pressure did vary and sometimes were quite wide for the same dosage. Some of these effects may have been due to the sodium bicarbonate which was used to buffer the vitamin C. Theoretical values have in general been found to agree with the results of the study. Besides the cases of glaucoma previously mentioned, there was also one case of Marchesani's syndrome and one case of hypotensive uveitis included in the 18 patients studies (117).

Another pressure measurement study was performed using a 20% sodium ascorbate solution. 500 mg/kg of body weight was administered by means of phleboclysis in 13 patients as a measure of the intracranial pressure. The retinal artery pressure was measured before and 1 hour after the end of the injection. Both eyes of the same patient experienced essentially the same reduction in pressure. Individual patients showed a pressure reduction range of 3 to 13 mm of mercury. The humoral artery pressure tended to remain essentially the same in these experiments for all patients. Along with the data for a 50% solution of glycerol administered at the level of 1,000 mg/kg of body weight, these experimental results were analyzed according to the Weigelin method. This involved the measuring of the diasystolic and systolic pressure of the retina as well as the arm. To establish the deviation from the norm in the test cases special tables were consulted. The real value decrease of the pressure due to sodium ascorbate was found to be from 1.1 to 14.7 mm of mercury. There appears to be no change in the cerebral circulation as examined by ophthalmodynamometry that could

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be attributed to complications resulting from the use of either ascorbate acid or glycerol. The data further shows that retinal arterial pressure values were independent of the endocranial and probably intraocular pressure values. Only the retinal humoral ratios were indicative of the circulatory conditions (123).

Arterial pressure in the rabbit was not affected by sodium ascorbate up to levels of 1,000 mg/kg given intravenously. On the basis of this and other animal experimentation previously cited, the use of sodium ascorbate in humans was found acceptable for clinical experimentation. Hospitalized patients with brain tumors that presented no edemata or other major alteration of the electrolytic fluid equilibrium were the subjects tested. Solution of pH 7.4 were tolerated locally and generally at the level of 1,000 mg/kg . Most of the 100% increase in diureses encountered waseliminated in the first 24 hours. Ascorbinemia increased rapidly but returned to normal within 24 hours. There was also an alteration in the potassium picture. The results indicate that the mechanism may be one of osmotic action (88).

In a series of tests on the effect of reducing agents on copper deficiency in chicks, the overall copper uptake by the liver did not appear to be significantly influenced by dietary ascorbic acid using copper -64. A decrease in liver copper was most noticeable in 6 hours regardless of the method of administration of the copper along with the 0.1% dietary ascorbic acid. The 50 chicks thus tested indicate that there may be an interference with the copper uptake or an increased depletion of copper from the liver. Zinc or iron deficiency was not influenced by the ascorbic acid. Isoascorbic acid was not studied regarding its influence on the liver uptake of copper, zinc, and iron (183).

The calcium ion apparently is more effectively absorbed from subcutaneous tissues when it is injected as the ascorbate. This is shown in the rate of regeneration of rat bone which was accelerated when calcium ascorbate was injected at doses equivalent to 45 mg of vitamin C. There were no effects when a comparative injection of vitamin C was given (71).

When vitamin B_1 deficient rats were administered ascorbic acid and isoascorbic acid, the creatinurea of these rats was similarly reduced. However, when hypercreatinurea was present, ascorbic acid was effective only in the young (253).

Detoxication of KCN, phenol and tetanus toxin and the conversion of folic acid into citrovorum factor has been found to be equal for both ascorbic acid and isoascorbic acid. Here again,the detoxifying activity is not explained on the basis of the scorbutic activity (99).

At pH 7.4, 10 mM sodium ascorbate prolongs the prothrombin time of fibrinogen. Calcium at the 0.521 millimolar concentration reduces the effectiveness of this and other inhibitors. The effects of the magnesium ion were less marked. Dog blood plasma was used for these determinations (92).

0.1 to 1.0 mM ascorbyl palmitate inhibited the esterase system from 8.5 to 66.1% in these liver, perirenal tissues and serum of fasting male Wistar rats. Therefore, it can be concluded that the fat metabolism may be adversely affected by ascorbyl palmitate. Other fatty tissues were not significantly affected by the group of antioxidants tested (43).

The activation of thiamine disulfide to growth factor toward Lactobacillus fermenti was studied under the influence of increasing concentration of ascorbic acid in the medium. The activity of thiamine or thiamine disulfide dropped off markedly at the higher concentration of 1, 900 mg/l of either of the two compounds. Except at the higher levels of concentration the growth pattern was not affected (151).

Further studies showed that when the amount of ascorbic acid was increased from 2 to 20 mM, there was a direct concentration dependence in vitro on the oxidation of the thio groups in human plasma. Similar results were found on testing isoascorbic acid. The readily available thio groups probably accounted for the rapid initial oxidation and those that became more accessible as the protein gradually denatured probably accounted for the subsequent lower oxidation which took place within about 24 hours (215).

In collagen explants of chick bone rudiments, isoascorbic acid at 50 μg/ml dosage was one-third as active as ascorbic acid in promoting collagen synthesis. However, dehydroascorbic acid failed to prevent hydration and did not promote collagen synthesis. (238).

The relatively high concentration of 200 γ per ml, isoascorbic acid had been found as effective as ascorbic acid on collagen synthesis and acid mucopolysaccahride production in a monolayer tissue culture of human dermal fibroblasts. Cysteine hydrochloride tended to increase the negligible dehydroascorbic acid effect on these tissue cultures.

However, the level was not brought up to the effectiveness of ascorbic acid. It has been hypothesized that the cysteine-hydrochloride has a sparing effect on the ascorbic acid (244).

The p-hydroxyphenolpyruvic acid oxidase antioxidization inhibition by extra tyrosine in vitamin C deficient animals has been found preventable. Administration of ascorbic acid, isoascorbic acid, D-glucoascorbic acid, and other compounds were effective both in vivo and in vitro. This inhibition in the liver and kidneys of the vitamin C deficient guinea pigs helps to explain the tyrosyluria. A direct relationship between tissue concentration of vitamin C and the load of tyrosine administered has been demonstrated. Induced tyrosyluria in premature infants have also been found correctable by the administration of ascorbic acid (262).

Potentiation or inhibition of smooth muscle of the guinea pig with concentration of sodium ascorbate is not modified by atropine, mepyramine, 2-bromolysergic acid or reserpine (90).

In a study of the antineoplastic activities of iron salts of the first oxidation products of the ascorbic or isoascorbic acid, the chief role of the associated metal to the oxidation-reduction systems seem to be the process of infiltration. There appears to be no influence of the structure of the fifth atom carbon. The asymmetry of the fourth carbon atom appears not to be concerned in the infiltration of the neoplasm since the metallocomplexes of hydroxy tetronic acid have anti-tumoral activity but not antiscorbutic activity (149).

Besides the small amounts of hydrogen peroxide which have been found to form during the spontaneous oxidation of ascorbic and iso-ascorbic acids in vitro (205), dehydroascorbic acid and 2, 3-ketoglutamic acid have been found to be the first and second oxidation products of ascorbic acid. The later reaction is irreversible (101a).

The maximal rate of oxidation of ascorbic acid by cytochrome C was markedly enhanced in a mixture of 1.5×10^{-2}M ascorbic acid, 3.1×10^{-5}M cytochrome C and about 1.1×10^{-3} of adrenaline. The experiments were held at pH values between 7.0 and 7.5 and the cytochrome C was free of cytochrome oxidase. Copper, iron, or hemoglobin as a replacement of cytochrome C had no accelerating affect. Oxidized ascorbic acid acted in a manner similar to ascorbic acid in this situation (182). Cytochrome C_5 in the pig adrenal was found to catalyzed a direct oxidation of DPNH in the presence of ascorbic acid. Here again the ascorbate could be replaced by isoascorbate (198).

Mitochondria in the presence of ascorbic acid increased the change of DPNH to DPN from 33% to 95% and of TPNH to TPN from 35% to 90%. Isoascorbic acid proved only one-fifth as active as ascorbic acid in these conversions. Increased biological activity in this type of system was found when irradiated with UV light (199).

The release of epinephrine from the adrenal medulla chromatin granules by 10^{-5}M of ascorbic acid or isoascorbic acid was inhibited by 10^{-5}M reserpine and 10^{-4}M versene. This was accomplished by inhibiting the release or depletion action. Imidazole and adrenosine triphosphate acted similarly (215).

Similar action of ascorbic acid and isoascorbic acid have been found on the enzyme of Myrothecium verrucaria spores. Ascorbic acid has an absolute specificity and isoascorbic acid was irreversibly inactivated by the enzyme system. The results of this study indicated that a stable turnover complex was formed between the enzyme, isoascorbate and oxygen (211).

In an enriched synthetic basal media, ascorbic acid or isoascorbic acid at the level of 0.25 mg/ml caused a rapid initial increase in the growth of Lactobacillus casei. Their reducing effect is presumed to be the major factor (239).

Tyrosine oxidation was stimulated by a series of test materials including ascorbic acid and dehydroascorbic acid. Ascorbic acid was not consumed as it undergoes a cyclic oxidation and reduction (164).

Prewashed Allium cepa roots were tested for mutagenic effects by abnormal anaphase and telephase chromosone break assay system by soaking them in a 10^{-2}M aqueous ascorbic acid solution. The results showed no significant increase in chromosone damage or mitotic disturbances (93a).

Compounds of the ascorbic acid series in which the oxygen ring engages a hydroxyl group to the right of the carbon chain were directly oxidized at a much higher rate than were their optical partners. Results of the studies tend to indicate that the asymmetric carbon atoms outside of the ring condition the antiscorbutic activity but not the rate of the direct enzymic oxidation of ascorbic acid and its six carbon

analogs" (190). An L-configuration specificity has been found for the hydroxyl group on the second and fifth carbon atoms as well as a specificity for the D-configuration on the fourth carbon atom that must be present in the enzymes for catalyzing the transformation of aldonic ascorbic acid to ascorbic acid (187a).

A 5-fold increase in the production of ascorbic acid has been accomplished in cress seedling supported on a piece of fine nylon fabric. Extracellular melanin of the Canton in-bred line of D-melaninogaster sternites was inhibited in forming by the feeding of isoascorbic acid. Longer feeding periods caused collapsed abdomens and a reduction in the life span. These changes were attributed to the "processes modifying the formation or activity of tyrosinase and affecting the pattern of protein synthesis"(246).

The reduced form of ascorbic acid has been found to potentiate the spread of Evans blue dye and of sodium chloride whereas the oxidized form of the acid was not found to be a potentiating agent (169).

The total United States poundage in 1970 as reported by the NAS/FEMA were: (37a)

Isoascorbic Acid (Erythrobic Acid)	549,675
Sodium Isoascorbic Acid (Sodium Erythrobate)	656,575
Ascorbyl Palmitate	66
Sodium Ascorbate	896,526
Calcium ascorbate	No poundage was encountered in the available literature.

The levels of the ascorbates in food is from 1.5×10^{-3} to 0.1%, Table 3, page 71. In general, the ascorbates are used as an antioxidant for processed and unprocessed meats, pickling and brine solutions, beverages, baked foods and candies, Table 3, page 71 (37a).

A weighted mean maximum usage of sodium ascorbate in infant formulas and body foods is reported as 0.020% (Table 8, page 77) (37b).

The highest probable daily intake from all source by the 2-65+ age group are estimated as:

Isoascorbic Acid (Erythrobic Acid)	87.86 mg
Sodium Isoascorbate (Sodium Erythrobate)	55.2 mg
Ascorbyl Palmitate	negligible
Sodium Ascorbate	645.48 mg
Calcium Ascorbate	No data encountered in the available literature.

For further daily usage data consult Tables 4, 5, 6, 7, pages 72-77 (37b).

CHEMICAL INFORMATION

I. Nomenclature

A. Common names (116a, 95a)

Ascorbic Acid-

Vitamin C; L-ascorbic acid; L-xyloascorbic; antiscorbutic vitamin; cevitamic acid; Cebione; Cevalin; Cevatine; Cevimin; Cevitex; Cipca; Cebicure; C-Vimin; Cevitamin; Testascorbic; Allercorb; Cecon; Ce-Vi-Sol; Ascorin; Ascorteal; Cegioian; Adenex; Ascorvit; Cevex; Lemascorb; Ciamin; Hybrin; Vitacee; Cantan; Catavin C; Celin; Cenetone; Cescorbat; Cereon; Cergona; Cetemican; Cetamid; Planavit C; Concemin; Scorbacid; Davitamon C; Proscorbin; Redoxon; Scorbu-C; Ribena; Vicelat; Vitacin; Vitacimin; Vitascorbol; Xitiz; Cevitan; Laroscorbine.

Sodium Ascorbate-

Ascorbic acid sodium derivative; vitamin C sodium; Ascorbin; Sodascorbate; Natrascorb; Cenolate; Ascorbicin; Cebitate; Sodium L-ascorbate.

Isoascorbic Acid-

D-Araboascorbic acid; erythorbic acid; isovitamin C; saccharosonic acid; glucosaccharonic acid; ervcobin; Mercate "5"; Neo-Cebicure; D-isoascorbic acid

Sodium Isoascorbate-

Sodium erythorbate; Mercate "20"; Neo-Cebitate.

Calcium Ascorbate-

Ascorbic acid calcium salt.

Ascorbyl Palmitate-

Palmitoyl L-ascorbic acid.

B. Chemical names (116a)

Ascorbic Acid-

L-3-ketothreohexuronic acid lactone

3-oxo-L-gulofuranolactone (enol form)

Sodium Ascorbate-

None encountered in available literature.

Isoascorbic Acid-

D-erythro-3-ketohexonic acid lactone

D-erythro-3-oxohexonic acid lactone

Sodium Isoascorbate-

None encountered in available literature.

Calcium Ascorbate-

None encountered in available literature.

Ascorbyl Palmitate-

None encountered in available literature.

C. Trade names (125a)

Ascorbic Acid-

L-Ascorbic acid

Sodium Ascorbate-

No other trade names encountered in the available literature.

Isoascorbic Acid-

Mercate "5"

Sodium Isoascorbate-

Mercate "20"

Calcium Ascorbate-

No other trade names encountered in the available literature.

Ascorbyl Palmitate-

No other trade names encountered in the available literature.

D. Chemical Abstracts Service Unique Registry Number

Ascorbic Acid

000050817

Sodium Ascorbate

000134032

Isoascorbic Acid

000089656

Sodium Isoascorbate

977052064

Calcium Ascorbate

005743271

Ascorbyl Palmitate

000137666

II. Empirical Formula (95a)

Ascorbic Acid-

$C_6H_8O_6$

Sodium Ascorbate-

$C_6H_7NaO_6$

Isoascorbic Acid-

$C_6H_8O_6$

Sodium Isoascorbate-

$C_6H_7NaO_6 \cdot H_2O$

Calcium Ascorbate-

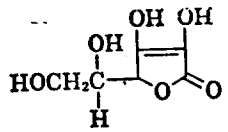
$C_{12}H_{14}CaO_{12} \cdot 2H_2O$

Ascorbyl Palmitate-

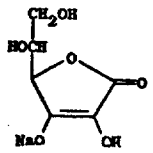
$C_{22}H_{38}O_7$

III. Structural Formula (95a, 116a)

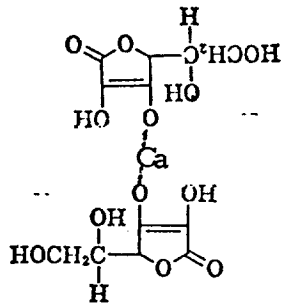
Ascorbic Acid-



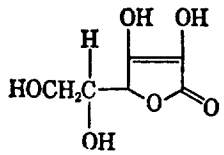
Sodium Ascorbate-



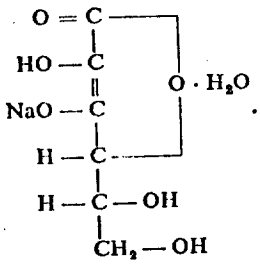
Calcium Ascorbate



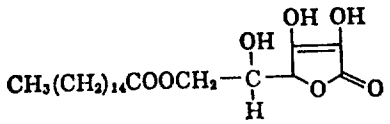
Isoascorbic Acid -



Sodium Isoascorbate-



Ascorbyl Palmitate-



IV. Molecular Weight (116a)

Ascorbic Acid-	176.13
Sodium Ascorbate-	198.11
Isoascorbic Acid-	176.13
Sodium Isoascorbate-	216.12
Calcium Ascorbate-	390.32
Ascorbyl Palmitate-	414.54

V. Specifications

A. Chemical (116a)

Ascorbic Acid-

C	40.91%
H	4.58%
O	54.51%

Sodium Ascorbate-

C	36.38%
H	3.56%
Na	11.60%
O	48.46%

Ascorbic acid 88.90%

Isoascorbic Acid-

C	40.91%
H	4.58%
O	54.51%

Sodium Isoascorbate-

None encountered in the available literature.

Calcium Ascorbate-

C	39.93%
H	3.61%
Ca	10.27%
O	49.19%

Ascorbyl Palmitate-

None encountered in the available literature.

B. Food Grade (95a)

Ascorbic Acid-

Assay. Not less than 99.0 percent of $C_6H_8O_6$.
Specific rotation, $[\alpha]_D^{25°}$. Between +20.5° and +21.5°.
Limits of Impurities
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).
Lead. Not more than 10 parts per million (0.001 percent).
Residue on ignition. Not more than 0.1 percent.

Sodium Ascorbate-

Assay. Not less than 99.0 percent and not more than the equivalent of 101.0 percent of $C_6H_7NaO_6$ after drying.
Specific rotation, $[\alpha]_D^{25°}$. Between +103° and +108°.
Limits of Impurities
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).
Lead. Not more than 10 parts per million (0.001 percent).
Loss on drying. Not more than 0.25 percent.

Isoascorbic Acid-

Assay. Not less than 99.0 percent of $C_6H_8O_6$.

Specific rotation, $[\alpha]_D^{25°}$. Between −16.5° and −18.0°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Residue on ignition. Not more than 0.3 percent.

Sodium Isoascorbate-

Assay. Not less than 98.0 percent of $C_6H_7NaO_6 . H_2O$.

Specific rotation, $[\alpha]_D^{25°}$. Between +95.5° and +98.0°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 30 parts per million (0.003 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Oxalate. Passes test.

Calcium Ascorbate-

Assay. Not less than 95.0 percent of $C_{22}H_{38}O_7$ calculated on the dried basis.

Melting range. Between 107° and 117°.

Specific rotation, $[\alpha]_D^{25°}$. Between +21° and +24° calculated on the dried basis.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).

Loss on drying. Not more than 2 percent.

Residue on ignition. Not more than 0.1 percent.

Ascorbyl Palmitate-

Assay. Not less than 98.0 percent of $C_{12}H_{14}CaO_{12}.2H_2O$.

Specific rotation, $[\alpha]_D^{25°}$. Between +95° and +97°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 10 parts per million (0.001 percent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).

Oxalate. Passes test.

C. Official Compendia

Food Chemical Codex, 2nd Ed. 1972

VI. Description (95a, 116a)

A. General Characteristics

Ascorbic Acid-

White or slightly yellow crystals or powder, melting at about 190°. Gradually darkens on exposure to light; reasonably stable in air when dry, but rapidly deteriorates in solution in the presence of air. One gram is soluble in about 3 ml of water and in about 30 ml of alcohol. It is insoluble in chloroform, in ether, and in benzene.

Sodium Ascorbate-

A white or almost white crystalline solid. One gram is soluble in 2 ml of water. The pH of a 1 in 10 solution is about 7.5.

Isoascorbic Acid-

White or slightly yellow crystals or powder. On exposure to light it gradually darkens. In the dry state it is reasonably stable in air, but in solution it rapidly deteriorates in the presence of air. It melts between 164° and 171° with decomposition. One gram is soluble in about 2.5 ml of water and in about 20 ml of alcohol. It is slightly soluble in glycerin.

Sodium Isoascorbate-

White, odorless, crystalline powder or granules. In the dry state it is reasonably stable in air, but in solution it deteriorates in the presence of air, trace metals, heat, and light. One gram dissolves in about 7 ml of water. The pH of a 1 in 20 solution is between 5.5 and 8.0.

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Calcium Ascorbate-

A white to slightly yellow, odorless, crystalline powder. It is soluble in water, slightly soluble in alcohol, and insoluble in ether. The pH of a 1 in 10 solution is between 6.8 and 7.4. A 1 in 10 solution gives positive tests for Calcium, and it decolorizes dichlorophenolindophenol T. S.

Ascorbyl Palmitate-

A white or yellowish white powder having a characteristic citrus-like odor. It is very slightly soluble in water and in vegetable oils. One gram dissolves in about 4.5 ml of alcohol. A 1 in 10 solution in alcohol decolorizes a 1 in 1000 solution of 2, 6-dichlorophenol-indophenol sodium.

B. Physical Properties (all ° are C)

Ascorbic Acid-

Density	1.65
pH, 5 mg/ml	3
50 mg/ml	2
pK_1	4.17
pK_2	11.57
UV max (acid sol)	245 mμ
(neutral sol)	265 mμ
Solubility (1 g in)	ml
H_2O	3
alcohol	30
absolute alcohol	50
glycerol U. S. P.	100
propylene glycol	20
hot H_2O (100°)	80.0%
(45°)	40.0%

Insolubility

ether, chloroform, benzene, petroleum, ether, oils, fats, fat solvents.

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Sodium Ascorbate-

Decomposes	218°
Solubility (g/100, ml)	
H_2O (25°)	62
warm H_2O (75°)	78
pH	5.6 - >7.0

Isoascorbic Acid- (208a)

mp 168-71°, very soluble H_2O, slightly soluble in alcohol, insoluble in acetone, very slightly soluble in ether.

Sodium Isoascorbate-

Soluble in water. pH of aqueous solutions of the sodium salt between 5 and 6. A 10% solution made from commercial grade, may have a pH of 7.2 to 7.9. The free acid is more soluble in water (40 g/100 ml H_2O) than the sodium salt (16 g/100 ml H_2O).

Calcium Ascorbate-

None encountered in the available literature.

Ascorbyl Palmitate-

None encountered in the available literature.

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VII. Analytical Methods

Electrometric	(192)
Glass fiber paper, silic acid-microfluorometric	(258)
Paper Chromatography	(192, 185)
Dichloro-inophenol	(201)

VIII. Occurrence and Levels Found in:

A. Plants (116a)

Ascorbic Acid-

Citrus fruits, bip berries, acerola, fresh tea leaves, paprika

Sodium Ascorbate-

None encountered in the available literature.

Isoascorbic Acid-

None encountered in the available literature.

Sodium Isoascorbate-

None encountered in the available literature.

Calcium Ascorbate-

None encountered in the available literature.

Ascorbyl Palmitate-

None encountered in the available literature.

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B. Animals

Ascorbic Acid-

Adrenal cortex of ox (116a)

"L-Ascorbic acid has a unique pattern of distribution in mammalian tissues. Several tissues have ascorbic acid concentrations several hundred times that of the plasma, i. e., hypophysis, adrenal, thymus, corpus luteum, and retina. A large number of tissues have concentrations 50 to 100 times the plasma level; these include the brain, testicle, thyroid, small intestinal mucosa, lymph glands, lungs, liver, spleen, white blood cells, pancreas, and salivary glands. A number of other tissues have only about 10 times the plasma concentration, i. e., kidney; skeletal, smooth, and cardiac muscle; and erythrocytes. Man, monkey, and the guinea pig are the only mammals known to be unable to synthesize L-ascorbic acid"(111a).

Sodium Ascorbate-

None have been encountered in the available literature.

Isoascorbic Acid-

None have been encountered in the available literature.

Sodium Isoascorbate-

None have been encountered in the available literature.

Calcium Ascorbate-

None have been encountered in the available literature.

Ascorbyl Palmitate-

None have been encountered in the available literature.

C. Synthetics

None have been encountered in the available literature.

D. Natural inorganic sources

None have been encountered in the available literature.

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BIOLOGICAL ASPECTS

I. Acute Toxicity

Table 1

Substance	Animal	Sex and No.	Route	Dosage mg/kg body wt.	Measurement	Ref.
Isoascorbic Acid	Mice	Male	Oral	5, 750	LD_0	*
	Mice	Male	Oral	8, 300	LD_{50}	*
	Mice	Male	Oral	12, 000	LD_{100}	*
	Rats	Male	Oral	8, 000	LD_0	*
	Rats	Male	Oral	18, 000	LD_{50}	*
	Rats	Male	Oral	42, 000	LD_{100}	*
	Rabbits		Oral	6,400-7,300	LD_{100}	(88)
Ascorbyl Palmitate	Mice		Oral	>8, 300	LD_{50}	(30a)
	Rats		Oral	6, 000	LD_{50}	(30a)
Sodium Ascorbate	Mice		Oral	>5, 000	LD_{50}	(110a)
	Mice		i. v.	>1, 000	LD_{50}	(110a)
	Rats		Oral	>5, 000	LD_{50}	(110a)
	Rats		i. v.	>1, 000	LD_{50}	(110a)
	Guinea pigs		Oral	>5, 000	LD_{50}	(110a)
	Guinea pigs		i. v.	> 500	LD_{50}	(110a)

II. Short Term Studies

The time of death after 8, 500 mg/kg and 10, 400 mg/kg doses were 60 and 40 minutes, respectively. However, the life span was extended by one hour for 5 rabbits receiving 8, 500 mg/kg of sodium ascorbate by the administration of 50 ml/kg of physiological saline solution with a probe into the stomach. In an attempt to establish the safety of sodium bicarbonate neutralized ascorbic acid in a 20% solution at pH 7. 4 for human testing, the acute toxicity of this mixture was studied on the rabbit and dog. At the intravenous infusion rate of 5 ml per minute up to a dose of 5, 300 mg/kg, no toxic effect occurred in the rabbit but at doses of 6, 400 mg/kg intra-

*Private communication from unidentified source, received from FDA.

venously, tremors were observed in the limbs approximately 1 hour after the injection. However, the animal intravenously injected with 7, 300 mg/kg died with convulsive attacks within 5 hours (88).

At the 5% level of ascorbyl palmitate and isoascorbyl palmitate there was significant retardation of growth. In two of the rats receiving ascorbyl palmitate, bladder stones were produced in a nine-month period. However, at the 1% level, isoascorbic acid had no deleterious effect in the same period. Both the L- and D- isoascorbyl palmitate at the 2% level were found to be non-toxic to animals in nine months. The palmitates were pre-treated by adding them to fresh lard and heat-treating the mixture before adding it to the diet (16).

Beagle dogs, found to have essentially normal hematology, biochemical parameters, and urine analysis were divided into three equiweight groups of 2 males and 2 females. Group 1 received 1, 000 mg/dog/day of isoascorbic acid orally for 240 days. Group 2 received 5, 000 mg/day for 50 days, and then 7, 500 mg/day for the remaining 190 days. The third group served as an untreated control group. The removal of one dog from the control group because of illness resulted in a higher initial body weight average for the controls. No hematological biochemical, or urine deviations or pathological changes were found in the test animals. *

*Private communication from unidentified source, received from FDA.

A food medium containing isoascorbic acid inhibited the formation of extra-cellular melanin of the canton inbred line of D. melanogaster sternites. In short period treatments with isoascorbic acid, a delay was caused in the time of the initial appearance of melanin pigment on the sternites and an increase in their life span. When the administration was for longer periods, in addition to the inhibition of melanin formation, collapsed abdomens and a reduction in the life span resulted in the treated animals attributed to the isoascorbic acid modifying the formation or activity of tyrosinase (246).

The plasma and adrenal ascorbic acid and adrenal cholesterol levels in hypopthysectomized male rats was measured three and six hours after the injection of ACTH. The expected adrenal ascorbic acid depletion was partially inhibited by oral administration of sodium ascorbate, while the decrease in cholesterol was unaffected. Therefore, the depleting effect of ACTH can be partially overcome by an increased quantity of ascorbic acid available for uptake (138).

Isoascorbic acid and ascorbic acid at the level of 0.1% in the diet fed to 2 groups of 20 copper deficient chicks decreased the growth, and either decreased the elastin content of the aorta or increased the mortality of the chicks. These effects did not occur when the diet was supplemented by 25 ppm of copper. The overall uptake of copper by the liver in 20 chicks in 3 weeks was not significantly reduced by dietary ascorbic acid. The results using copper-64 showed a decrease in liver copper which was most noticeable in 6 hours regardless of the method of administration of 5 microcuries orally or intraperitoneally along with

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0.1% dietary ascorbic acid to 50 chicks. The overall results in 48 hours confirmed no effect due to the route of administration of copper-64. This may mean an interference with copper uptake or an increased depletion of copper from the liver in the presence of ascorbic acid. Ascorbic acid had no effect on the zinc or iron deficiency. There was no mention in this report of any affect of isoascorbic acid on the liver uptake of copper, zinc, and iron (183).

Scorbutism in normal guinea pigs was induced by a diet of autoclaved hay, oats, and water. The animals that survived the 18th day were treated with 15-30 mg doses of sodium isoascorbate in groups of 7-9. No scurvy was found when the animals were sacrificed after 22 days of therapy. The minimum curative dose was reported as 20-25 mg/day for the surviving guinea pigs. Those animals deemed supersensitive to scurvy by reason of death under therapy were deleted from consideration. All of them were scorbutic. None of the guinea pigs receiving 30 mg/day of sodium isoascorbate succumbed to scurvy. Therefore, it was concluded that larger doses than the minimum curative dose, i.e., 25-30 mg, "may be considered to be fully curative" (150). Normal survival and the lack of the development of any sign of scurvy as discernible at autopsy was further evidence that isoascorbic acid can replace the antiscorbutic activities of ascorbic acid. This was based on the daily oral doses of 10 to 200 mg of isoascorbic acid administered to guinea pigs. Additional results verifying these findings were the similarity in the skin of the soluble and insoluble collagens of these animals (167).

In a study on the effects of the treatment of artificial vitamin C deficiency with vitamin C and material Nu-211 encoded from the article "to be ascorbyl palmitate," ascorbyl palmitate was found to have an activity the same as vitamin C. The data on the 75 William Wiersing guinea pigs put on an artificially deficient vitamin C diet lost weight and then gained weight for 2 weeks. At the third week, their weight gain stopped and dropped off as sharply as it had in the first period of the vitamin C deficient diet. Upon administration of vitamin C or Nu-211, 6-palmitoyl ascorbic acid, the weight of the guinea pigs returned almost to that of the control guinea pigs at the end of the experiment. At an extremely low dosage level, 0.5 mg/day, the effectiveness of the vitamin C was not as great as at the higher dosage of 1 mg/day. With the Nu-211, the dosages of 1.2, 2.4, and 4.8 mg/day produced about the same growth rate. "The daily dose of 0.5 mg of vitamin C is supposed to be about the minimal effective dose". The activity of Nu-211 through this experiment has been shown to be approximately or equal to that of vitamin C. In the negative control group, that is, in the group on the vitamin C deficient diet that received neither vitamin C or Nu-211, all of the guinea pigs died within 6 1/2 weeks. A few of the other guinea pigs also died but there were at least seven of the originally nine or ten guinea pigs in the group alive at the end of the experiment. *

Using previous experiments on animals (150) as justification and as there was an intended use of ascorbyl palmitate as emulsifiers and stabilizers for food in England, human trials on its metabolism were undertaken. Ascorbic acid was fed to two healthy men on a nor-

*Private communication from unidentified source, received from FDA.

mal diet for a period of approximately 2 weeks. Then, no addition was made to the diet. This was followed by the oral feeding of 1180 mg of ascorbyl palmitate, the equivalent of 500 mg of ascorbic acid, in single doses. Between dosages, the levels of ascorbic acid found in the urine returned essentially to normal. *

Tests were performed by the injection into the marginal vein of the ear of 1,000 mg/kg for 20 consecutive days. The kidney and liver tissues were determined to have no morphologically significant changes on histological examination when these rabbits were sacrificed at the end of the experiment with a lethal dose of curare, gallamine triiodide. A general anesthesia was not used because of the stress that would have been involved. There were no significant variations in the arterial pressure of the rabbits administered 500 to 1,000 mg/kg intravenously at the 20% sodium ascorbate solution as long as the pH value of the solution was maintained at 7.4. However, when the pH moved away from this value, there were substantial modifications of the arterial pressure in the animal. This would indicate that the pH of the sodium ascorbate used may be critical. Diuresis in proportion to the dosage of sodium ascorbate solution was found in the rabbits, i.e., the marked urinary value of 73.0 ml within one hour when 1,500 mg/kg were administered intravenously as a 20% solution. Dogs showed no adverse effects in 20 days to 1,000 mg/kg sodium ascorbate administered intravenously. Their livers and kidneys upon histological examination proved to be within normal limits. The dogs did have a marked increase in body weight from 6.2 kg to 8.4 kg during this period (88).

*Private communication from unidentified source, received from FDA.

Because of the results in animals, administration of sodium ascorbate for clinical experimentation was felt to be permissible. Hospitalized patients with brain tumors that presented no edemata or other major alteration of the electrolytic fluid equilibrium were the subjects used for the sodium ascorbate test. As long as the pH of the solutions stayed at 7.4 and the rate of application did not exceed 60 drops per minute, levels up to 1,000 mg/kg intravenously were "tolerated locally as well as generally". Here again marked diuresis was encountered and was equal to about 100% on the average. The greatest increase occurred within the first 6 hours after the end of the infusion. This was accompanied by a clear and constant reduction of the potassium level in the serum. However, the sodium level of the serum remained practically unchanged while the sodium diuresis increased proportionly to the infusion of the sodium present in the liquid. However, the potassium diuresis did not parallel the serum potassium picture. These findings of potassiurea afford no final conclusion because the experiments were "carried out on patients with an already increased basal potassiurea." Based on the lack of a significant decrease in the hematocrit, extracellular dehydration appears to be absent. Although the urine pH tended to have a slight and inconsistent modification, on the whole, it remained at values of weak acidity. Most of the urinary elimination occurs within the first 24 hours for doses of 500 mg/kg of sodium ascorbate and slightly more than half was excreted at the dosage level of 1,000 mg/kg in this period of time. The blood ascorbinemia increased rapidly and returned to the starting values within 24 hours. Urea nitrogen

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remained unchanged from the normal values throughout. Glycemia had marked variations 2 hours after the end of the infusion, but the values returned to normal within 24 hours. The 2-hour values may have had a dependence on the method employed in the analysis due to interference from the ascorbic acid. The results showed that the mechanism may be one of osmotic action. However, at doses less than 500 mg/kg no significant increase in diuresis either in animals or in man was evidenced (88).

Table 2

Effects of intravenous administration of 1,000 mg/kg of sodium ascorbate (88)

Patient	Quantity		Specific gr.		pH		Na m.eq o/oo		K m.eq o/oo	
	Before	After	Before	After	Before	After	Before	After	Before	After
A) on human urine										
M.A.	1,350	2,250	1,020	1,020	7.8	8.1	215	281	623	643
A.Z. (*)	3,300	5,170	1,030	1,032	6.6	6.6	110	670	473	1,787
F.A.	1,250	2,450	1,022	1,032	7.2	6.6	197	392	54	63
R.I.	1,100	2,550	1,032	1,020	6.9	6.6	130	523	69	68
C.H.	1,100	1,830	1,028	1,012	6.4	7.2	88	146	23	43
I.E.	950	3,000	1,008	10.38	7	7	115	1,267	136	910
Mean	1,508	2,875					142	546	229	585
Mean (**)		1,150	2,416				149	521	181	343

Patient	Na (serum) m.eq o/oo			K (serum) m.eq o/oo			Hematocrit		
	Before	After 1 h	After 24 h	Bef.	After 1 h	After 24 h	Bef.	After 1 h	After 24 h
B) on human blood									
M.A.	141.6	141.6	136.9	5.1	5.1	3.8	19	21	19
Z.A. (*)	139.1	160.4	147.8	5.6	5.6	2.5	47	50	45
F.A.	141.6	149.1	143.4	--	--	5.3	42	40	41
R.I.	121.5	121.5	126	4.8	4.8	4.3	30	26	28
C.H.	96.9	105	93.4	6.1	6.4	3	37	39	38
I.E.	124.5	128.2	124.5	--	--	4.6	47	45	42
Mean	127.5	140.9	128.6	5.4	5.4	3.9	39	37	39
Mean (**)	125.2	129	124.8	5.3	5.4	3.5	37	34	33

(*) The patient was treated simultaneously with glycerol (Luxoral).

(**) Mean of the values excluding the patient marked (*).

Comparison of the metabolism of ascorbic acid and its fatty acid esters was carried out on guinea pigs by measuring the ascorbic acid levels in the urine, liver, adrenals, and blood plasma. The equivalence of 20 mg of ascorbic acid administered for mono-ascorbyl palmitate and the diascorbyl palmitate. In higher levels of urinary ascorbic acid excreted in the 0- to 24-hour period than in the 24- to 48-hour period, a direct relationship was seen between the content of ascorbic acid in the urine and the content of the organs. However, in the groups administered the esters of the acids, the inverse was true. Mixtures of homogenate of small intestines and pancreas yielded 80% and 20% free ascorbic acid when L-ascorbyl monopalmitate and L-ascorbyl dipalmitate, respectively, were administered. Therefore, these esters are believed to enzymatically hydrolyzed (22).

The relationship of the adrenal cortical hormone to sodium and vitamin C has led to the investigation of the use of sodium ascorbate as a substitute for ascorbic acid in the treatment of allergy. The response to sodium ascorbate was strikingly more beneficial than the response to ascorbic acid. In addition, there was the advantage of marked freedom from gastric irritation at the level of 2,000 mg/day and increased diuresis resulted. Even the patients with chronic asthma and hay fever that did not do well under therapy with ascorbic acid did extremely well with the sodium ascorbate. All eight cases cited were cases of severe and long suffering from asthmatic and other allergic symptoms. They reported

dramatic positive results under this form of therapy. Reference is also made to calcium ascorbate being an effective anti-allergent. It appeared to benefit both persons whose allergic symptoms seem to stem from enzymatic and toxic factors, whereas, the sodium ascorbate tended to benefit to a greater extent those whose problems stem from adrenal cortical insufficiency (131). Thirteen other allergy cases refractive to ascorbic acid reported dramatic results and little or no side effects with sodium ascorbate therapy. The dosage was varied with the individual needs of the patients. At that time, there was no stable sodium ascorbate preparation on the market. However, since then a stable product has been prepared that withstands heating in the oven, up to 500°C (130).

As a part of a series on the study of the dynamics of aqueous humor, 47 male albino rats each weighing 2 to 3 kg (sic) had their body temperature reduced to approximately 19°C as determined by rectal temperatures in a room maintained at 20°C. This degree of hypothermia could be sustained for 5 to 8 hours, using thiamylal sodium, Surital, to prevent shivering. The turnover rate of both ascorbic acid and sodium in the aqueous humor was measured under these conditions and compared with 30 normothermic determinations of the same values. The first attempts to measure the turnover rate by the ascorbates at 10 mg% blood levels of ascorbic acid obtained by the administration of sodium ascorbate intravenously were unsuccessful. Eighty-two rabbits were first saturated with ascorbate in the normothermic state by the administration of sodium ascorbate intravenously at the 100 mg level and also 100 mg subcutaneously. After 2 hours, both plasma obtained by heart puncture techniques, and aqueous humor

samples from one eye were obtained. These animals were then subjected to hypothermia and any noticeable change in the turnover rate of ascorbate in the posterior and anterior chamber was noted as a function of time. No further administration of sodium ascorbate was necessary as the plasma levels remained at approximatly 10 mg% during the experiment. Sodium ascorbate concentration of the posterior chamber in the normothermic state rose sharply and was maintained at a higher level than that in the anterior chamber. A dramatic difference was noted when the rabbits were subjected to hypothermia. Neither the anterior or the posterior chamber ascorbate level was increased and in fact, both declined slowly over the 300-minute duration of the experiment. After 1,000 mg of sodium ascorbate was administered to 60 hypothermic rabbits intravenously and the concentration in the blood rose to 160 mg%, an increase in both the posterior and anterior chamber ascorbic acid aqueous humor levels was noted. Where the posterior chamber concentration, therefore blood levels, of ascorbic acid increased more rapidly under normothermic conditions at lower dosages the anterior chamber aqueous humor ascorbate increased to a greater extent in the 10-20 eyes of the chilled rabbits subjected to the 1000 mg of sodium ascorbate (86).

In the animals administered 100 mg of sodium ascorbate intravenously, and 100 mg subcutaneously 2 hours before hypothermia was induced, the posterior chamber values averaged 66 mg% and the anterior chamber values 50 mg% while the plasma values were maintained at approximately 10 mg% for the average of 6 to 14 eyes used for each determination in the study. The time course of the decrease of the ascorbic acid levels in the chambers under hypothermia were then measured while

the plasma levels remained approximately 10 mg%. During the 300 minutes of the hypothermia, the posterior chamber levels of ascorbic acid at first decreased quickly and then tapered off to an approximately constant level. In the anterior chamber, it declined at almost a straight line relationship and remained at a greater magnitude, approximately 3 times that of the level in the posterior chamber throughout the experiment. The fact that the posterior chamber lost the ascorbates so rapidly "may be related to the adjacent large volume of vitreous which had failed to become 'saturated' in the brief two-hour normothermic period" in this experiment. The flow coefficient was 0.2% per minute in the anterior chamber with a diffusion coefficient of 0.25% per minute. It was noted in these experiments that the ascorbic acid concentration in the hypothermic rabbit falls and there is an accompanying rise in the bicarbonate concentration of the aqueous humor. "It may be reasonably accepted that hypothermia decreases the rate of aqueous formation. The decreased flow characteristics are probably a reflection of the changes in the blood, blood vessels, and/or any other anatomical entities resulting in a reduced blood flow induced by the cold. In general, the normothermic coefficient of aqueous flow of 1.6% to 0.25% per minute, for the anterior chamber at 19°C, can be compared to values derived from the ascorbic acid turnover of 1.7% for the normothermic and 0.2% for the hypothermic rabbits (86).

A variance between that of adult rabbits and young rabbits has been noted for the absorption or crossing of the blood aqueous barrier in the eye by ascorbic acid. When the adult rabbits were injected intraperitoneally with ascorbic acid, the maximum level of ascorbic acid in blood

withdrawn by heart puncture was reached in 20 minutes whereas the maximum concentrations in the aqueous humor withdrawn by micropipettes was reached in 60 minutes. As the level of ascorbic acid in the aqueous humor remained constant as long as the blood ascorbic acid remained constant, it was assumed or implied that a steady state exists. Further experiments have shown that when the serum ascorbic acid reached a level of 3 mg%, the aqueous humor concentration reached 50 mg%. Even though an increase in the concentration in the serum was elevated up to as much as 130 mg%, there was no increase in the amount of ascorbic acid in the aqueous humor. Using younger rabbits, the results were somewhat different. It was found that the concentration in the aqueous humor rises with increased concentration of the blood almost throughout the entire range of 0. to 120 mg% ascorbic acid in the serum. It is also interesting to note that in the young animals of 5 to 40 days of age, the ascorbic acid equivalency in the aqueous humor reached a maximum more gradually. The higher levels were more in the range of 80 mg% as compared to the adult of 50 mg% . When the experiment was repeated using isoascorbic acid and D-glucoascorbic acid with the adult rabbits, only the isoascorbic acid was found in the serum of both cases. However, in the young rabbits, D-glucoascorbic acid did result in titratable ascorbates in the aqueous humor in proportion to the concentration in the serum. In the adults, "the results indicate that the secretory process is apparently selective for ascorbic acid or its optical isomer" and they suggest that ascorbic acid "forms an integral part of secretory mechanism. This selectivity in the adult rabbits appears to be unrelated to the antiscorbutic action." A contrast was found in the young animals where the predominating factor tended to be diffusion (201).

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Sodium ascorbate at pH 7.4 formed from crystallized vitamin C by neutralization with sodium carbonate was administered in a 20% solution at levels of 100, 200, 500, and 1,000 mg/kg of body weight to 18 patient suffering from acute chronic simple or irritative glaucoma. These patients ranging in age from 30 to 70 years revealed that the reduction of pressure increased with dosage up to the 500 mg/kg level. Dosages over this level were contraindicated because there was no further reduction in the ocular pressure and the diuresis experience was greater with higher doses. In general, the greatest reduction in the ocular pressure due to the sodium ascorbate was realized at approximately 1 to 2 hours after the end of the intravenous infusion with a gradual return toward the pretreatment values over a period of six hours or greater. The osmotic blood pressure in all dosage levels increased to a maximum at the end of the infusion and gradually returned to normal in from 4 to 12 hours. The higher doses required a somewhat longer time to be excreted. A comparison of the maximum value of ascorbinemia between the 1,000 mg/kg and the 500 mg/kg dosages showed them to be almost identical with values of 495 mg% and 480 mg% at the end of the infusion. Values dropped off to 75 mg% at the 100 mg/kg dosage level. The osmotic pressure curve of the blood tended to parallel that of the ascorbinemia and lower values of osmotic pressure were noted below dosage levels of 0.5 g/kg of body weight. The ratio of the values of ocular tension and blood osmotic pressure tended to vary according to the subject and the type of glaucoma, and individual variations of osmotic pressure were sometimes quite wide for the same dosage. The increase of

the osmotic pressure of the blood most likely involved in some measure the sodium bicarbonate that was used to buffer the vitamin C and form the sodium ascorbate. Theoretically predictable values between a given concentation of vitamin C in the blood and the respective increase of osmotic pressure were in agreement with the results found in this study. Besides the types of glaucoma previously mentioned, there was a single case of Marchesani's syndrome and one case of hypertensive uveitis among the 18 patients studied (117).

The dosage levels using the above combination, 600 mg/kg of glycerol and 280 mg/kg of human body weight of sodium ascorbate was much less than those for urea, mannitol and sodium ascorbate alone which were also tested. Therefore, the volume necessary was only 140 cc. This factor meant that a lesser quantity of liquid needed to be eliminated when using the glycerol-sodium ascorbate solution compared with the other materials. The above method of treatment was accompanied by a reduction in the cardiac rhythm which at times could be classified as bradycardia. Heart changes were measured by electrocardiograms of the patients prior to, and 30 and 45 minutes after the intravenous introduction of the above combination of drugs. Upon other experimentation, the bradycardia was traced to the additional sodium ions from sodium ascorbate which caused further imbalance of the ions within the body. The excess sodium within the blood resulted in the migration of sodium into the cell and potassium from the cell. The tonometric studies of the eyeball pressure reveal a rapid decrease in the pressure from 70 mm of mercury to approximately 10 mm of mercury in 1 hour. The pressure tended to rise gradually over the next

7 hours. This was presumably due to the depletion of the therapeutic agents from the system. These 16 patients from 22 to 77 years of age had been fasted before the glycerol-sodium ascorbate was intravenously administered. From the above results, it is suggested that further studies on a combination of ascorbates, sodium and perhaps potassium salts in combination with glycerol be undertaken (126).

In the quest for a method of establishing changes in the intracranial pressure, experiments were performed by administering a 20% solution of sodium ascorbate in a dose of 500 mg/kg of body weight, by means of phleboclysis to 13 patients. The retinal arterial pressure was measured before and 1 hour after the end of the injection. The sodium ascorbate after 1 hour had caused a reduction in the retinal arterial pressure values as measured without any significant difference between the two sides. Some patients being more sensitive to the sodium ascorbate had a more marked reduction in the pressure. In general, these reductions ranged from 3 to 13 mm of mercury. The humoral arterial pressure tended to remain essentially the same in these experiments on patients from 13 to 62 years of age. This data along with the data for 50% solution of glycerol administered at a level of 1,000 mg/kg of body weight was also analyzed according to Weigelin method which involved the measuring of the diastolic and systolic pressure at the retina as well as the arm. Using these parameters, there is derived the mean arterial pressures which are compared to tables compiled by Weigelin using normal subjects, so as to establish the deviation from the norm in the cases under consideration. Real value decreases of the pressure due

to the sodium ascorbate of from 1.1 to 14.7 mm were shown. It is apparent from these values that one of 13 patients considered was not significantly sensitive to sodium ascorbate. "The cerebral circulation as examined by ophthalmodynamometry according to Weigelin suffers no changes that could be the cause of complications" resulting from the use of either sodium ascorbate or glycerol. However, "if such changes have occurred, they are well-compensated". The data further shows that "the retinal arterial pressure values are independent of the endocranial and probably intraocular pressure values". "Only the retinal humoral ratio is indicative of the cerebral circulatory conditions" (123).

Marked ocular hypotony in humans resulted within 60 to 90 minutes when 0.4 to 1.0 g/kg of sodium ascorbate as a 20% solution was administered intravenously. As glycerol had been found to be effective in reducing pressure exerted on the eyeball but was accompanied by changes in the kidneys that resulted in hematuria, the co-administration of the two materials was undertaken in an effort to find a safe-effective combination. Glycerol as a 30% solution combined with a 20% solution of sodium ascorbate at pH 7.4, intravenously administered proved successful in eliminating the hematuria produced by glycerol alone. The preparation was also effective to a significant degree in reducing the intraocular pressure. Parallel studies on rabbits delineated comparative results (141).

The rate of regeneration of rat bone was accelerated when calcium ascorbate was injected daily at doses equivalent to 45 mg of vitamin C. However, a comparative injection daily of vitamin C had no effect on bone generation (71).

Creatinurea was similarly reduced in vitamin B_1 deficient rats by both isoascorbic acid and ascorbic acid. In the young, there was a slight lowering of the hypercreatinurea but in the adult deficient in vitamin B_1, ascorbic acid was without effect (253).

The antineoplastic activities of the iron salts of the first oxidation product of ascorbic acid or of isoascorbic acid, indicated no influence of the sturcture of the fifth carbon atom. The asymmetry of the fourth carbon atom appeared not to be concerned in the infiltration of neoplasms since the metallocomplexes of hydroxytetronic acid have antitumoral activity although not antiscorbic activity. The chief role of the associated metals to the oxidation-reduced system seems to be the process of infiltration (149).

A false over-estimation of the state of vitamin C nutrition as judged by the blood levels could result from the ingestion of sodium isoascorbate which has "trifling" antiscorbutic activity. Separation of the isomers by quantitative paper chromatography showed no significant displacement of the ascorbic acid by 300 mg doses of the isoascorbic acid. Five normal human subjects were used in this comparative blood level testing (192).

III. Long Term Studies

Chronic toxicity studies for two years on L-ascorbyl palmitate and D-isoascorbyl palmitate administered as 0.25%, or less, of the diet caused no toxic effect in rats (16).

Life feedings to rats of isoascorbic acid at the levels of 1 and 2% of the diet produced no toxicity (30a).

IV. Special Studies

Guinea pigs on a low controlled intake of the ascorbic acid in their diet, 3 mg/kg of body weight, were given daily supplement of either ascorbic acid or isoascorbic acid at the 15 mg/kg of body weight level. The ascorbic acid was found deposited in the tissue but none of the isoascorbic acid was found there. However, on the intramuscular administration some isoascorbic acid was found in the tissues but not to the same extent as the equivalent amount of intramuscularly injected ascorbic acid. No evidence of the conversion of isoascorbic acid to ascorbic acid was found. Isoascorbic acid was absorbed from the gastrointestinal tract, extracted from the blood or retained by the tissues less readily than ascorbic acid (185). Further support that isoascorbic acid was poorly absorbed or did not have marked antiscorbutic action was evidenced when 250 mg/day of isoascorbic acid did not support guinea pigs fed a vitamin C deficient diet. However, such animals responded to some extent to isoascorbic acid when maintained on a suboptimal intake of ascorbic acid whereas in the guinea pigs that were depleted of vitamin C, the isoascorbic acid had no therapeutic effect. Although isoascorbic acid appeared to have no significant antiscorbutic effect of its own, these results suggest that isoascorbic acid may have had a protective effect on the ascorbic acid (234).

As a challenge to the theory that isoascorbic acid is not retained by the organs of animals, guinea pigs were fed a synthetic diet containing ascorbic and isoascorbic acids. Both compounds were retained in the organs as determined by the rate of ozasone formation of the oxidized

forms of these compounds, with 2, 4-dinitrophenyl-hydrazine. The amount of the isoascorbic acid that was retained was a replacement of ascorbic acid which could in turn be replaced by ascorbic acid when it was subsequently given in the diet (230).

When Evans blue was injected intradermally or isotonic sodium chloride was administered by subdermal infusion under constant pressure in guinea pigs and rats, ascorbic acid in its reduced form was active in potentiating the spread of the dye and sodium chloride. However, in the oxidized form, it was not active. Rutin and catechim similarly tested were also inactive. Parallel results were obtained on testing the degradation of hyaluronic acid in vitro (169).

Spasmatic responses of smooth muscle of guinea pigs are potentiated by sodium ascorbate in low concentrations and inhibited by high concentrations. These actions due to sodium ascorbate are not modified by atropine, mepyramine, 2-bromolysergic acid or reserpine (90).

When isolated frog hearts were placed in very dilute solution of ascorbic acid and isoascorbic acid in the presence of air, the amplitude and rate of contractions of the heart increased, followed by irreversible systolic standstill in 1-2 hours. This latter effect appeared to be due entirely to small amounts of hydrogen peroxide forming during the spontaneous oxidation of the acids and not to the acids (205).

When 7-day old chick limb bone rudiments were grown for 8 days in 50 micrograms per milliliter of isoascorbic acid, the activity was only about 1/3 the activity of an equal dose of ascorbic acid in promoting collagen synthesis. Dehydroascorbic acid under the same condition failed to prevent the hydration that takes place in vitro in bone rudiment and it did not promote collagenous synthesis. However, ascorbic acid at 50 micrograms per milliliter did prevent the hydration of the rudiments and stimulated collagen formation. Eighty micrograms per milliliter of ascorbic acid was the minimum dose that would support the maximum response (238).

Ascorbic or isoascorbic acids when administered to 5 rats per test by stomach tube in doses of 50 mg of ascorbic acid or its equivalent for 2 weeks accompanied by 1 mg/day of $CoCl_2$ did not influence the cobalt chloride produced polycythemia. D-glucoascorbic acid at a dose level equivalent to 25 mg ascorbic acid was also without effect. These three compounds at the 25 mg ascorbic acid equivalent neutralized by sodium bicarbonate level when injected intravenously had no effect on the polycythemia in 6 to 11 rats. However, the sodium ascorbate intravenously injected at the 50 mg ascorbate dosage level caused a marked decrease in the hemoglobin concentration in rabbits. A similar but smaller decrease in the hemoglobin concentration was brought on by the administration of sodium isoascorbate in place of the neutralized ascorbic acid. Of the three compounds tested using rabbits, only sodium glucoascorbate was without affect on the polycythemia in the rabbit. A preliminary conclusion was drawn from this study

"that there is a parallelism between the antiscorbutic effect of these compounds and their ability to reduce the hemoglobin concentration of the polycythemic rat". Only 2 or 3 rabbits were tested for both the oral and the intravenous administration of the three compounds. The same animals were administered the isoascorbic acid, ascorbic acid, and glucose ascorbic acid in series for varying periods of days alternating with varying rest periods (209).

In the extensively artificial medium of liver powder derived from liver acetone extracts, the oxidation of tyrosine was measured using ascorbic acid and dehydroascorbic acid. All of the test materials stimulated the oxidation but the ascorbic acid moiety was not consumed as it undergoes a cyclic oxidation and reduction (164).

Glycogen in the liver of rabbits was increased by the introduction of the food acids, ascorbic and isoascorbic. In addition, plants have been found to contain a flavone which further stimulated the ascorbic acid to produce glycogen. This material has been named factor 2. In some plants such as bitter oranges and green pea, a third factor exerted a negative action on Factor 2. Therefore, the vitamin C action did not depend solely upon the ascorbic acid in the plants. Verification of this theory was seen in experiments where more liver glycogen was produced by the approximately 5 mg of vitamin C in a 1 cc lemon juice and sugar infusion, than by 50 mg of pure ascorbic acid when given to rabbits. The flavone glycogenic substance which was believed to contain 2 components, one acting directly, the other indirectly on the glycogen deposition was given the name vitamin C_3 (252).

A comparison was made between isoascorbic acid and ascorbic acid as to their effect on the collagen synthesis and acid mucopolysaccahride production in a monolayer tissue culture of human dermal fibroblasts. It was found that isoascorbic acid was as effective as ascorbic acid if the comparison was made at the relatively high concentration of 200 γ per ml. Dehydroascorbic acid was ineffective when tested alone but when added with cysteine hydrochloride the effectiveness was increased but not to an amount equivalent to the effectiveness of the ascorbic acid. Therefore, cysteine hydrochloride appeared to have a sparing effect on the ascorbic acid (244).

A concentration dependence for ascorbic acid effect on human plasma thiol in vitro was seen when the amount of ascorbic acid was increased from 2 to 20 mM. The rate of the oxidation of thiol groups to SS bonds, therefore, the decrease in thiol concentration was accelerated with the increasing ascorbic acid. Similar results were obtained when isoascorbic acid was substituted for ascorbic acid. The initial rapid oxidation probably involved the reactive thiol groups and the subsequent slower oxidation which took place mainly within the 24-hour period involved the unreactive thiol groups that became more accessible to the ascorbic acid as the protein gradually became denatured (215).

Rapid initial growth of Lactobacillus casei was obtained when ascorbic acid or isoascorbic acid, 0.25 mg/ml was added to the usual completely synthetic basal medium that had been enriched by the addition of DL-serine, 20 mg/10 ml; L-glutamic acid, 2 mg/ml; asparagen, 2.5 mg/ml; and 2x or greater increases in the content of the other vitamins. An increased rate of growth of Lactobacillus casei in the presence of ascorbic acid and isoascorbic acid was presumably due to their reducing effect. It appeared that the presence of trypsinized casein contributed no additional growth affect (239).

BIOCHEMICAL ASPECTS

I. Breakdown

Small amounts of H_2O_2 were formed during the spontaneous oxidation of ascorbic and isoascorbic acids (205). The first oxidation reaction of ascorbic acid to dehydroascorbic acid was reversible but the second oxidation reaction to 2, 3-diketogluconic acid was irreversible (101a).

Similarly, isoascorbic acid degrades in processing and use by way of a redox reaction resulting in the oxidized form of isoascorbic acid and dehydroisoascorbic acid (101a).

Ascorbyl palmitate fed to humans led to ascorbic acid in the urine * and (22).

No information on the breakdown of the sodium and calcium salts was encountered in the available literature.

II. Absorption - Distribution

The oral feeding studies on

ascorbyl palmitate	* and (22)
diascorbyl palmitate	(22)
isoascorbic acid	(185)

indicated that these compounds are absorbed intact from the stomach into the tissues.

In guinea pigs isoascorbic acid was absorbed from the gastro-intestinal tract, extracted from the blood or retained by the tissues less readily than ascorbic acid (185).

No information for sodium ascorbate, sodium isoascorbate, and calcium ascorbate was encountered in the available literature.

*Private communication from unidentified source, received from FDA.

III. Metabolism and Excretion

An average increase in diuresis was encountered when brain tumor patients were intravenously administered 1000 mg/kg of sodium ascorbate at pH 7.4. The urine in general remained weakly acid. Over 50% of the elimination took place in 24 hours. When the dosage reached 500 mg/kg, almost all of the elimination occurred in this time (88).

Feeding 1180 mg of ascorbyl palmitate in a single dose to 2 healthy persons caused the ascorbic acid level in the urine to rise. It then returned to normal. As the values were similar to those experienced administering ascorbic acid, it was assumed that the palmitate was split into ascorbic and palmitic acids. Thus the metabolism of the ascorbate radical of the palmitate was further assumed to be identical with ascorbic acid. *

A probable reason for the controversy cited under Short Term Studies on the relative activity of isoascorbic acid as an antiscorbutic agent was thought to be the high excretion rate. Therefore, two load tests of 165 mg and 250 mg were administered to 6 and 5 subjects, respectively. Blood plasma increments of total ascorbic acid were similar following either ascorbic acid or isoascorbic acid. A balance study using a crossover design of supplementation was performed on 6 young male college students. A 14-day period on 25 mg ascorbic acid was followed by 10 mg of ascorbic acid during 4-12 day experimental periods in which 100 mg of the two isomers were fed alternately. The mean fasting blood levels were: ascorbic acid 1.03 mg% and isoascorbic acid 0.62 mg%.

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*Private communication from unidentified source, received from FDA.

The approximately 5-fold increase of isoascorbic acid over ascorbic acid, 72.2 mg and 15.3 mg, respectively, in the 24-hour urine indicated the preferential resorption of ascorbic acid. These results indicated that a study be done of the peripheral uptake and excretion rate of the two isomers (257).

Six male adult subjects were fed a 25 mg supplement to a 10 mg ascorbic acid diet during three 7-day experimental periods. Prior to the experimental period, there was one week of adjustment and two weeks of exploratory work when all the subjects had a daily dietary ascorbic acid intake of 10 mg. On the 5th day of each experimental period, a test dose of 300 mg isoascorbic acid was given to the subjects; on the 7th day a test dose of the same amount of ascorbic acid was given. No increase of arterio-venous difference occurred during the elevation of plasma total ascorbic acid on either form of test dose. The arterio-venous difference method did not differentiate between the peripheral uptake of the two isomers, but it revealed a small sustained ascorbic acid uptake. Urinary collection was started after the ingestion of the test dose. The 24-hour collections divided into three periods were 32%, 12.6%, and 9% of the isoascorbic acid test dose; and 1.7%, 1.4%, and 2.2% for the ascorbic acid test dose. Most of the isoascorbic acid was excreted within the first six hours. The high and rapid isoascorbic acid excretion suggested no appreciable reabsorption by the tubular cells (257).

No information for sodium isoascorbate and calcium ascorbate was encountered in the available literature.

IV. Effects of Enzymes and Other Biological Parameters

Cytochrome C accelerated the oxidation of ascorbic acid to levels up to 9 times as high as did the adrenaline which only slightly accelerated the oxidation of ascorbic acid. At concentrations of 1.5×10^{-2}M of ascorbic acid, 3.1×10^{5}M cytochrome C and about 1.1×10^{3}M of adrenaline, the maximal rate of oxidation occurs. The concentration of adrenaline was approximate because of the formation of its oxidation products which were active in the system. The pH of the experiments was a dependent factor and was held at 7.0 and 7.5 throughout. Replacement of cytochrome C by copper or iron or by hemoglobin did not produce the accelerating effect . Oxidized ascorbic acid tended to act in a manner somewhat similar to ascorbic acid. The cytochrome C which was used for these experiments was free of cytochrome oxidase (182).

An enzyme was found in the pig adrenals which catalyzed the direct oxidation of DPNH in ascorbic acid. This material localized in the microsomal fraction was shown to be identical with cytochrome C_5. In this case, ascorbate could be replaced by isoascorbate but not by glutathione or adrenaline (198).

Homogenates of the fatty tissues, therefore; liver, perirenal tissue and epididymis, of fasting male Wistar rats as well as the serum was used to study the possible in vivo inhibition of antioxidant activity on the esterase system. Tween 80 in barbitural- HOAc buffer at pH 7.9 in the presence of calcium was used as a medium. In all cases, the serum esterase was markedly inhibited by the antioxidant studied. At concentrations of 0.1 - 1.0 mM, ascorbyl palmitate inhibited the

esterase systems from 8.5 to 66.1%. The other fatty tissues did not appear to be significantly affected by the group of antioxidant tested. It was, therefore, concluded that ascorbyl palmitate, one of the antioxidants tested, can effect the fat metabolism adversely by inhibiting the esterase system in the serum and the liver (43).

While the enzyme of Myrothecium verrucaria spores had an absolute specificity for ascorbic acid, isoascorbic acid was irreversibly inactivated by the same enzyme system. It was felt that this inactiviation was due to a chemical reaction with the enzymes. The factors which lead to this decision were the requirement for oxygen, the stoichiometrical relationship between the quantities of enzyme and isoascorbic acid that was necessary to effect a given degree of inhibition, the temperature coefficient requirement, and the irreversibility of the inactivation. There was a marked pH dependence which suggested that the reaction occurred only with the dissociated acids. It was felt that a stable ternary complex is formed between the enzyme, isoascorbate, and oxygen (211).

The p-hydroxyphenylpyruvic acid oxidase inhibition by tyrosine in vitamin C deficient animals was found preventable by the administration of ascorbic acid and the several other compounds, such as isoascorbic acid and D-glucoascorbic acid. These ascorbates also protected the enzyme from inhibition in vitro by excess substrate. The vitamin C deficiency on these guinea pigs was established by a vitamin C free diet for 2 weeks. Six to 10 animals were used in each experiment group. The tyrosine was administered as 400 mg suspended in 4 ml of sucrose sweetened water. This mixture was fed in 100 mg portions at hourly intervals for 4 hours. One hour after the last dose of tyrosine

the animals were killed. and the liver and kidneys rapidly removed and chilled. The ascorbates were administered intraperitoneally in a dose of 10 mg 30 minutes before the tryosine feeding and 10 mg more 2 hours later. The inhibition of the p-hydroxyphenolpyruvic acid oxidase in the liver and kidneys of the vitamin C deficient guinea pigs helped to explain the tyrosyluria. The degree of enzyme inhibition was found to depend upon the tissue concentration of vitamin C and the load of tyrosine given. The ability of vitamin C and several related compounds to protect p-hydroxyphenolpyruvic acid oxidase from substract inhibition was found both in vivo and in vitro. The 4- to 6-fold increased tyrosine transaminase activity found in the scorbutic guinea pigs fed tyrosine might help to maintain the metabolic defect. Tyrosyluria found in premature infants fed tyrosine or phenol alanine was correctable by the administration of ascorbic acid (262).

Compounds of the ascorbic acid series in which the oxygen ring engages a hydroxyl group to the right of the carbon chain, therefore, ascorbic acid, isoascorbic acid, and L-glucoascorbic acid, L-galacto-ascorbic acid, were directly oxidized at a much higher rate than were their optical partners. The oxidation in this group proceeded at a linear rate but that of their enantiomorphs fell off after a period of time. Of those compounds that were not oxidized at the linear rate, the six carbon members oxidized more rapidly than the seven carbon compounds.

Therefore, it appeared that the asymmetric carbon atoms outside of the ring condition the antiscorbutic activity but not the rate of the direct enzymatic oxidation of ascorbic acid and its fixed carbon analogs (190).

The action of ascorbic acid in the cell to reactivate hydrogen or to take part in electric transfer has been shown. The evidence was a marked increase in the dehydration of reduced diphosphopyridine nucleotide to triphosphopyridine nucleotide in the presence of mitochondria. With mitochondria alone 33% of DPNH changed to DPN after 30 minutes and 35% of TPNH to TPN. However, upon the addition of 25 micromoles of ascorbic acid, the conversion rate were 95 and 90%, respectively. Isoascorbic acid proved to be only 1/5 as active as ascorbic acid in these conversions. When irradiated with ultraviolet light, preparations containing DPNH mitochondria and ascorbic acid showed an increased biological activity (199).

Equally effective detoxifying activity for ascorbic acid and isoascorbic acid on KCN, phenol, and tetanus antitoxin has been found. They were also effective in the conversion of folic acid into citrovorum factor. This detoxifying activity of ascorbic acid cannot be used to explain its antiscorbutic activity (99).

At approximately pH 7.4, 10 mM of sodium ascorbate prolonged the prothrombin time of fibrinogen as did the other inhibitors tested. Calcium at the 0.521 mM reduced the effectiveness of the inhibitors but the effect of magnesium ion was less marked. Calcium ion was precipitated from the dog plasma by the use of sodium oxalate which also served as an anticoagulant for these tests (92).

Both isoascorbic and ascorbic acids had been found to liberate thiamine activity from the biologically inactive thiamine disulfide. Using 20 micrograms thiamine or 18.8 microns of thiamine disulfide, the activation of the thiamine disulfide to growth factor toward lactobacillus fermenti was studied under the influence of increasing concentrations of ascorbic acid in the medium. Steamed thiamine and thiamine disulfide were used as controls. At a higher concentration, approximately 1900 mg/liter of ascorbic acid or isoascorbic acid, the activity of the thiamine or thiamine disulfide dropped off markedly. Almost a normal growth pattern was observed using vitamin C or the ascorbates and the biologically inactive thiamine disulfide, except as already noted at the high concentration levels of the ascorbates (151).

10^{-5}M ascorbic acid or isoascorbic acid increased the release of epinephrine from rabbit adrenal medulla chromaffin granules. 10^{-5}M reserpine and 10^{-4}M versene in vitro served as inhibitors of the process. The release or depleting action of the ascorbates was antagonized by versene, imidazole, adenosine triphosphate and reserpine (214).

It has been found possible to cause the production of ascorbic acid in cress seedlings supported on a piece of fine nylon fabric kept moistened with a solution containing one of the four compounds, L-gulono-γ-lactone, L-galactone-lactone, D-glucurono-γ-lactone, or D-galacturonic acid methyl ester. These compounds stimulated or took part in, or were converted to ascorbic acid in excess of that produced by the seeds grown in water under these same conditions. D-mannono-γ-lactone in the rat and D-altrono-γ-lactone in cress seedlings were converted to isoascorbic acid (187a).

V. Drug Interaction

The actions of sodium ascorbate were not modified by atropine, mepyramine, 2-bromolysergic acid or reserpine (90).

Isoascorbic acid was as effective as ascorbic acid in detoxifying KCN, phenol and tetnus toxin (99). A synergism has been shown between thiamine and isoascorbic acid that was the same as found for ascorbic acid. With either combination of drugs in both *Lactobacillus fermenti* and rats, an increase in growth was noted (151).

No information was encountered on the drug interaction of sodium isoascorbate, calcium ascorbate or ascorbyl palmitate in the available literature.

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VI. Consumer Information

The levels of ascorbates used in processed and unprocessed meats, pickling and brine solutions, beverages, baked foods and candies as an antioxidant which ranges from 1.5×10^{-3} to 0.1% is shown in Table 3 (37a).

Tables 4, 5, 6 provide data on the probable average, high and maximum levels expected to be used by humans according to age. The usual use and maximum weighted mean % usages are contained in Table 7. Data for sodium ascorbate in usage by ingestion of infant products and body food products may be found in Table 8.

Table 3

Name	Function; Usage	Levels of Use
Ascorbate, Calcium Ascorbate, Sodium	Antioxidants	
	Concentrated milk products	
	Cooked, cured, or comminuted meat food products, per 100 lbs of the fresh, uncured, or comminuted meat and/or by-product.	7/8 oz
	Used in the pickle in which pork and beef products are cured or packed per 100 gal, up to	75 oz
Ascorbyl Palmitate	Candy	0.06%
Erythorbate, Sodium (Sodium Isoascorbate) Erythorbic Acid (Isoascorbic Acid)	Antioxidants	
	Pickling brine. Per 100 gal, up to	7.5 oz
	Meat products. Per 100 lbs, up to	3/4 oz
	Brine, solution. Up to	10.0%
	Beverages	0.006 - 0.01%
	Baked goods	0.02 - 0.05%
	Cured cuts; cured comminuted product to accelerate color fixing in curing, per 100 lbs, Sodium erythorbate, up to	7/8 oz
	Erythorbic acid, up to	3/4 oz
	Pickle in which pork and beef products are cured or packed, per 100 gal	75 oz

Table 4

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - -

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
SODIUM ASCORBATE NAS 0180	01	BAKED GOODS(R)	*	0-5 MO.	3.200080	4.235400	4.700160
				6-11 MO.	23.906480	48.754160	35.112960
				12-23 MO.	51.295400	84.519760	75.340800
				2-65+ YR.	129.132640	191.816560	189.665280
SODIUM ASCORBATE NAS 0180	02	BREAK CERLS(R)	8	0-5 MO.	.556320	1.576240	2.047500
				6-11 MO.	20.676560	55.446560	76.098750
				12-23 MO.	24.199920	47.194480	89.066250
				2-65+ YR.	18.544000	48.028960	68.250000
SODIUM ASCORBATE NAS 0180	05	MILK PRODS(R)	6	0-5 MO.	1.600560	1.185600	1.777140
				6-11 MO.	18.495360	88.940640	20.535840
				12-23 MO.	16.153800	51.692160	17.935950
				2-65+ YR.	11.707800	35.745840	12.999450
SODIUM ASCORBATE NAS 0180	08	PROCSD FRUT(R)	*	0-5 MO.	5.616500	15.057000	7.143530
				6-11 MO.	61.901000	154.155000	78.730020
				12-23 MO.	120.217000	238.641500	152.901940
				2-65+ YR.	141.368500	299.467000	179.804170
SODIUM ASCORBATE NAS 0180	10	MEAT PRODS(R)	14.	0-5 MO.	.363660	.958740	.543510
				6-11 MO.	6.643420	18.447480	10.227870
				12-23 MO.	9.984120	17.158140	14.921820
				2-65+ YR.	25.919040	43.011060	38.737440
SODIUM ASCORBATE NAS 0180	11	POULTRY(R)	*	0-5 MO.	.180000	.828000	.180000
				6-11 MO.	1.404000	4.752000	1.404000
				12-23 MO.	2.376000	6.624000	2.376000
				2-65+ YR.	4.644000	11.808000	4.644000
SODIUM ASCORBATE NAS 0180	13	FISH PRODS(R)	*	0-5 MO.	.036000	.108000	.036000
				6-11 MO.	.468000	1.764000	.468000
				12-23 MO.	1.944000	4.860000	1.944000
				2-65+ YR.	4.464000	11.124000	4.464000

Table 4 (con't)

SODIUM ASCORBATE NAS 0180	23	BEV TYPE I(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	.000000	.000000	.000000
SODIUM ASCORBATE NAS 0180	24	BEV TYPE II(R)	10	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	**********	.000800	**********
				12-23 MO.	**********	.001600	**********
				2-65+ YR.	.260000	.755200	.520000
SODIUM ASCORBATE NAS 0180	28	INIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	1.575000	2.587500	1.575000
				12-23 MO.	.900000	3.825000	.900000
				2-65+ YR.	1.012500	1.687500	1.012500
SODIUM ERYTHORBATE * NAS 0317	02	BREAK CERLS(R)	*	0-5 MO.	.012000	.034000	.045000
				6-11 MO.	.448000	1.196000	1.672500
				12-23 MO.	.523000	1.010000	1.957500
				2-65+ YR.	.400000	1.035000	1.500000
SODIUM ERYTHORBATE * NAS 0317	05	MILK PRODS(R)	*	0-5 MO.	.004960	.003600	.012960
				6-11 MO.	.056160	.270090	.149760
				12-23 MO.	.049050	.156960	.130800
				2-65+ YR.	.035550	.108540	.094800
SODIUM ERYTHORBATE * NAS 0317	10	MEAT PRODS(R)	17	0-5 MO.	.402930	1.062270	4.399340
				6-11 MO.	7.582410	20.439540	82.787500
				12-23 MO.	11.062260	19.010970	120.721880
				2-65+ YR.	28.717920	47.655630	313.552960
SODIUM ERYTHORBATE * NAS 0317	11	POULTRY(R)	*	0-5 MO.	.062500	.237500	.062500
				6-11 MO.	.407500	1.650000	.487500
				12-23 MO.	.825000	2.200000	.925000
				2-65+ YR.	1.612500	4.100000	1.612500
SODIUM ERYTHORBATE * NAS 0317	23	BEV TYPE I(R)	*	0-5 MO.	.008640	.012960	.008640
				6-11 MO.	.081720	.279720	.081720
				12-23 MO.	.195120	.585000	.195120
				2-65+ YR.	.374400	.999720	.374400
SODIUM ERYTHORBATE * NAS 0317	24	BEV TYPE II(R)	20	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	**********	.001390	**********
				12-23 MO.	**********	.002700	**********
				2-65+ YR.	.451750	1.312160	.592500
SODIUM ERYTHORBATE * NAS 0317	ALL CATEGORIES ********** ********** **********		37	0-5 MO.	.490930	1.400330	4.528440
				6-11 MO.	8.653790	23.836740	65.179060
				12-23 MO.	12.653430	23.073710	123.850300
				2-65+ YR.	31.592120	55.212050	317.487160

*sodium isoascorbate

Table 5

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

-- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - -

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
ERYTHORBIC ACID * NAS 0078	01 BAKED GOODS(R)	*	0-5 MO.	.008840	.011700	.026180
			6-11 MO.	.066040	.134600	.195580
			12-23 MO.	.141700	.233480	.419650
			2-65+ YR.	.356720	.529880	1.056440
ERYTHORBIC ACID * NAS 0078	04 FATS OILS(R)	*	0-5 MO.	.000200	.000200	.300000
			6-11 MO.	.001120	.003000	1.680000
			12-23 MO.	.002520	.004800	3.780000
			2-65+ YR.	.007000	.012640	10.500000
ERYTHORBIC ACID * NAS 0078	09 FRUIT ICES(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.012000	.032000	.012000
			12-23 MO.	.024000	.084000	.024000
			2-65+ YR.	.028000	.100000	.028000
ERYTHORBIC ACID * NAS 0078	10 MEAT PRODS(R)	*	0-5 MO.	.590260	1.556140	.591470
			6-11 MO.	11.107620	29.942280	11.130390
			12-23 MO.	16.205320	27.849540	16.238540
			2-65+ YR.	42.066440	69.811660	42.155680
ERYTHORBIC ACID * NAS 0078	14 PROCSD VEGS(R)	*	0-5 MO.	.000140	.000420	.000280
			6-11 MO.	.002400	.005600	.004800
			12-23 MO.	.003900	.006530	.007800
			2-65+ YR.	.008500	.014320	.017000
ERYTHORBIC ACID * NAS 0078	16 SOFT CANDY(R)	*	0-5 MO.	.000400	.004000	.001600
			6-11 MO.	.004400	.013600	.017600
			12-23 MO.	.007000	.018000	.028000
			2-65+ YR.	.011600	.039200	.046400
ERYTHORBIC ACID * NAS 0078	23 BEV TYPE I(R)	5	0-5 MO.	.122400	.183600	.234000
			6-11 MO.	1.157700	3.962700	2.213250
			12-23 MO.	2.764200	8.287500	5.284500
			2-65+ YR.	5.304000	14.162700	10.140000
ERYTHORBIC ACID * NAS 0078	27 GRAVIES(R)	*	0-5 MO.	.015000	.045000	.030000
			6-11 MO.	.210000	.585000	.420000
			12-23 MO.	.540000	1.530000	1.080000
			2-65+ YR.	1.245000	3.195000	2.490000
ERYTHORBIC ACID * NAS 0078	ALL CATEGORIES ********** ********** **********	11	0-5 MO.	.737240	1.801060	1.183530
			6-11 MO.	12.561280	34.678860	15.673620
			12-23 MO.	19.688640	36.014450	26.862490
			2-65+ YR.	49.030260	87.861400	66.433520

* Isoascorbic acid

Table 6

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

-- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II). PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - -

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	********** POSSIBLE DAILY INTAKE, MG. ********** (AGE)	AVERAGE	HIGH A	HIGH B
ASCORBYL PALMITATE NAS 0023	04 FATS OILS(R)	*	0-5 MO.	.050000	.050000	.150000
			6-11 MO.	.280000	.750000	.840000
			12-23 MO.	.630000	1.200000	1.890000
			2-65+ YR.	1.750000	3.160000	5.250000
ASCORBYL PALMITATE NAS 0023	34 INS COF TEA(R)	*	0-5 MO.	**********	**********	.000020
			6-11 MO.	**********	**********	.000530
			12-23 MO.	**********	**********	.000620
			2-65+ YR.	**********	**********	.012110
ASCORBYL PALMITATE NAS 0023	ALL CATEGORIES ********** ********** **********	*	0-5 MO.	.050000	.050000	.150020
			6-11 MO.	.280000	.750000	.840530
			12-23 MO.	.630000	1.200000	1.890620
			2-65+ YR.	1.750000	3.160000	5.262110

Table 7

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/01/72

USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
SODIUM ASCORBATE	01	BAKED GOODS(R)	*	.09412	.13824
NAS 0180 FEMA 3525	02	BREAK CERLS(R)	8	.09272	.34125
	05	MILK PRODS(R)	6	.02964	.03291
	08	PROCSD FRUT(R)	*	.11950	.15199
	10	MEAT PRODS(R)	14	.03306	.04941
	11	POULTRY(R)	*	.03600	.03600
	13	FISH PRODS(R)	*	.03600	.03600
	23	BEV TYPE I(R)	*	.00000	.00000
	24	BEV TYPE II(R)	10	.00080	.00160
	28	INIT DAIRY(R)	*	.11250	.11250
	30	HARD CANDY(R)	*	.12000	.30000
ASCORBYL PALMITATE	04	FATS OILS(R)	*	.01000	.03000
NAS 0023	34	INS COF TEA(R)	*	********	.00001
ERYTHORBIC ACID *	01	BAKED GOODS(R)	*	.00026	.00077
NAS 0078 FEMA 2410	04	FATS OILS(R)	*	.00004	.06000
	09	FRUIT ICES(R)	*	.00400	.00400
	10	MEAT PRODS(R)	*	.05366	.05377
ERYTHORBIC ACID * (CONT.)	14	PROCSD VEGS(R)	*	.00001	.00002
NAS 0078 FEMA 2410	16	SOFT CANDY(R)	*	.00020	.00080
	23	BEV TYPE I(R)	5	.00510	.00975
	27	GRAVIES(R)	*	.01500	.03000
SODIUM ERYTHORBATE **	02	BREAK CERLS(R)	*	.00200	.00750
NAS 0317	05	MILK PRODS(R)	*	.00009	.00024
	10	MEAT PRODS(R)	17	.03663	.39994
	11	POULTRY(R)	*	.01250	.01250
	23	BEV TYPE I(R)	*	.00036	.00036
	24	BEV TYPE II(R)	20	.00139	.00170

*isoascorbic acid

**sodium isoascorbate

Table 8

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0180	SODIUM ASCORBATE	83	FORMULAS(B)	4	.01620	.02004

THE ASCORBATES

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