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Antiscorbutic Activity of D-Araboascorbic Acid.* (32120)

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In 1933-36, Dalmer and Moll(1), Zilva(2) and Reichstein and Demole(3) recognized D-araboascorbic acid as a factor with antiscorbutic properties, although considerably less active than L-ascorbic acid.† Recent reports claimed that D-araboascorbic acid was not able to replace the antiscorbutic role of L-ascorbic acid(4,5). In view of the controversy concerning the vitamin activity of D-araboascorbic acid, we investigated this problem on guinea pigs fed a scorbutigenic diet supplemented with various doses of D-araboascorbic acid.

Methods and results. Experiments were performed on young adult male animals, 4-5 months-old, weighing 330 ± 40 g. The guinea pigs were initially fed Wayne Guinea Pig diet containing 40 mg L-ascorbic acid per 100 g for 2 weeks. After this adaptation period the animals were given our purified scorbutigenic diet(6,7) for one week (preparatory period) and thus depleted of ascorbic acid. The animals were then divided into 10 groups of seven each. For 38 days, 6 groups were fed the scorbutigenic diet supplemented with daily oral doses of 1, 2, 10, 50, 100 or 200 mg D-araboscorbic acid. However, 3 guinea pigs from the group that received 10 mg D-araboascorbic acid were kept in the experiment for 115 days. Three groups were fed the scorbutigenic diet and daily doses of 1, 2 or 15 mg L-ascorbic acid for 38 or 115 days. Animals of one group were fed the scorbutigenic diet exclusively until they died. In the above studies L-ascorbic acid and D-araboascorbic acid were dissolved in water and administered in volumes of 1 ml by pipet.

During the experimental period we observed the appearance of animals and recorded their weights regularly (Fig. 1 and 2); at autopsy

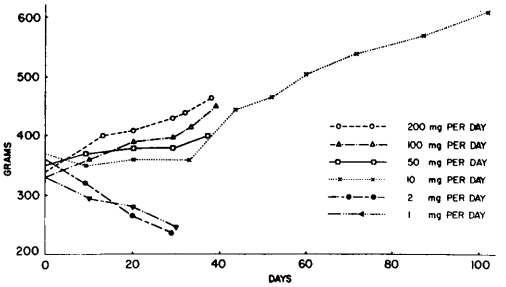

FIG. 1. Average body weight of guinea pigs receiving D-araboascorbic acid.

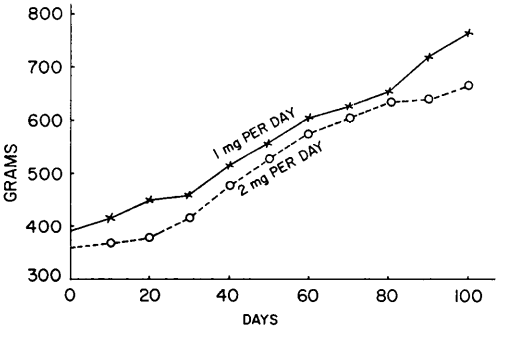

FIG. 2. Average body weight of guinea pigs receiving L-ascorbic acid.

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† L-ascorbic acid or ascorbic acid is also known as L-xyloascorbic acid or vitamin C. D-araboascorbic acid (known also as isoascorbic acid or erythorbic acid) differs from L-ascorbic acid only in the specific configuration of — H and — OH groups around the fifth carbon.

TABLE I. Hemorrhages Found at Autopsy of Guinea Pigs Receiving Oral Daily Doses of 1 Mg or 2 Mg D-Araboascorbic Acid.

Animal (No.)	Hemorrhages (localization and degree)			
	Articulations			
	Chondrocostal	Tibio-femoral	Intestines	Other organs
	Guinea pigs receiving 1 mg D-araboascorbic acid a day			
1	+	++	+	0
2	+	++	++	Hypertrophy of blood vessels (+++)
3	++	+++	+	Hypertrophy of blood vessels (++)
4	+	+	0	0
5	+	±	0	0
6	+	0	0	Hypertrophy of blood vessels (+++)
7†	0	0	+	0
	Guinea pigs receiving 2 mg D-araboascorbic acid a day			
1	0	+++	+++	0
2	0	0	0	Skin (petechiae) (++)
3	0	0	0	0
4*	0	0	0	Hypertrophy of blood vessels (++)
5	+	++	+	0
6	0	0	0	0
7	0	++	+	0

* Dead on 20th day.
† Dead on 28th day.

we examined them for signs of scurvy. In some animals the ascorbic acid content of various organs was determined. Animals receiving L-ascorbic acid or D-araboascorbic acid were sacrificed 24 hours after administration of the last dose.

The concentration of "ascorbic acids" was determined in the adrenals, spleen and liver by our adaptation(8) of the 2,4-dinitrophenylhydrazine method of Roe and Kuether(9) using, respectively, L-ascorbic and D-araboascorbic acids as standards. In our preliminary studies the oxidized form of D-araboascorbic acid was found to react with 2,4-dinitrophenylhydrazine in the same way as the L-dehydroascorbic acid, but the "osazone" dissolved in sulfuric acid gave one third the color intensity obtained with L-dehydroascorbic acid.

All animals, except for the guinea pigs deprived of ascorbic acid and those on the scorbutigenic diet supplemented with 1 or 2 mg D-araboascorbic acid, presented a normal appearance and at autopsy showed no signs of scurvy or other diseases. The animals fed the scorbutigenic diet died between the 23rd and 31st days. Their average weight loss was 40% of the initial weight; they showed the typical signs of scurvy at autopsy(10,11). The guinea pigs receiving 1 or 2 mg of D-araboascorbic acid either died in 3 to 4 weeks (Table I) or were sacrificed *in extremis* on the 33rd day of the experiment. At that time, they had lost an average of 35% of their original weight. Their autopsy record is given in Table I.

In the case of guinea pigs receiving 10 mg of D-araboascorbic acid per day the "ascorbic acid" was stored in the tissues at approximately 20% lower level than that observed in guinea pigs given 15 mg of L-ascorbic acid (Table II). It was previously shown that the concentration of L-ascorbic acid in guinea pig tissues is proportional to the logarithm of the oral dose(8,12). This does not seem to apply to D-araboascorbic acid, since the concentration of "ascorbic acid" found in the tissues of animals fed 100 mg D-araboascorbic acid was only slightly higher than in those fed 10 mg (Table II). The method of analysis, however, does not reveal

TABLE II. Tissue Concentrations of "Ascorbic Acid" of Male Guinea Pigs Treated for 38 Days with Various Doses of L-Ascorbic Acid or D-Araboascorbic Acid.

Animals receiving:	"Ascorbic acid" (mg/100 g wet organ) found in: Liver	Spleen	Adrenals
L-ascorbic acid			
1 mg per day	.98 ± .1*	6.3 ± .7	8.6 ± 1.0
2 " " "	2.6 ± .5	10.4 ± 2.0	15.4 ± 2.1
15 " " "	5.4 ± .4	18.9 ± .2	61.3 ± 3.5
D-araboascorbic acid			
10 mg per day	2.7 ± .4	17.0 ± .7	49.1 ± 4.7
100 " " "	3.3 ± .6	19.6 ± .3	57.3 ± 2.9

Each group contained 4 male guinea pigs.

For determination of tissue "ascorbic acid" L-ascorbic acid and D-araboascorbic acid were used as standard, respectively.
* Standard deviation.

the nature of the stereoisomer of ascorbic acid stored in the organs of guinea pigs treated with D-araboascorbic acid. This problem is under study.‡

Discussion. It was previously shown that L-ascorbic acid disappears rapidly from the tissues of guinea pigs. After 8 days of deprivation the organs contain 1/8th the concentration of L-ascorbic acid found in control animals fed 20 mg of this vitamin per day(12, 15,16). Therefore, at the end of the one week in which the scorbutigenic diet was not supplemented with any form of ascorbic acid, the animals became practically depleted of this vitamin. We can hence attribute the normal survival of guinea pigs receiving 10 mg or more of D-araboascorbic acid per day to the antiscorbutic activity of this compound. Thus, after 115 days, we still found complete absence of hemorrhages at autopsy in guinea pigs given daily 10 mg D-araboascorbic acid. The rate of growth of guinea pigs receiving 10 mg D-araboascorbic acid was still below that observed for the groups fed 1 or 2 mg L-ascorbic acid (Fig. 1 and 2). Indeed, for the period of 115 days, the average daily gain by the animals given 10 mg D-araboascorbic acid was 2.2 g against 2.8 g for guinea pigs receiving 2 mg L-ascorbic acid.

Dalmer *et al*(1) found that D-araboascorbic acid administered orally was 40 times less active than L-ascorbic acid. These authors giving the two isomers for 4 weeks at insufficient dose to prevent scorbutic lesions, reached their conclusions by comparing the intensities of hemorrhages at autopsy in animals receiving daily 1.5 ml lemon juice (0.5 mg L-ascorbic acid) to the hemorrhages in animals fed the sodium salt of D-araboascorbic acid (20 mg per day, orally).

However, according to more recent work, D-araboascorbic acid has no antiscorbutic activity but exerts merely a protective effect on the residual stores of L-ascorbic acid in the tissues of humans(5) or guinea pigs(4). On the contrary, the present study indicates that D-araboascorbic acid when given in sufficiently high dose, is able to replace vitamin C.

Since one of the main functions of vitamin C is its participation in collagen synthesis (17), hydroxyproline contents were determined in the 0.154 M, 0.5 M NaCl, 0.5 M citrate (pH 3.6) extracts as well as on total skin of guinea pigs fed the scorbutigenic diet supplemented with oral doses of 10 mg D-araboascorbic acid for 115 days. These analyses showed that the levels of soluble and insoluble collagen fractions and of total hydroxyproline of dermal connective tissue in such guinea pigs were the same as those found in skin tissues of guinea pigs which

‡ So far we have been unable to determine the nature of ascorbic acid stored in the organs of guinea pigs treated with D-araboascorbic acid. Two methods, namely the paper chromatographic method of Miki *et al*(13) and the thin layer procedure of Brenner *et al*(14) gave satisfactory result with pure solutions of ascorbic acids, but no yield when carried out on tissue extracts. According to personal information from the authors, the methods were never tested on animal or vegetable tissue extracts.

received L-ascorbic acid (15 mg *per os*)(18).

Summary. D-Araboascorbic acid administered to guinea pigs in daily oral doses of 10 to 200 mg apparently replaced the antiscorbutic activity of L-ascorbic acid. The animals survived normally and the D-araboascorbic acid prevented them from developing any sign of scurvy discernible at autopsy.

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Immunity Against Experimental Cholera. (32121)

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Although cholera vaccines have been used since before the turn of the century, scientifically controlled field trials have only recently proven that they have any value in prophylaxis against cholera(1,2). The results of the field trials have been disappointing in that the vaccines which were sufficiently innocuous for wide scale usage in man conferred only a low degree of immunity of limited duration. However, one important fact emerged from these efforts: it is feasible, by means of parenteral immunization, to confer at least some degree of resistance against this bacterial infection which is restricted to the lumen of the intestine.

In previous studies on pathogenesis and immunity in experimental cholera(3,4,5), we have described a product, choleragen, which is elaborated *in vitro* under certain defined conditions by certain strains of cholera vibrios. This antigenic protein moiety, administered orally in microgram amounts with buffer, causes choleraic diarrhea in infant rabbits; it causes marked outpouring of fluid (enterosorption(6)) into the lumen of isolated loops of adult rabbit small bowel *in vivo;* in submicrogram amounts, it is an extremely potent permeability factor eliciting a delayed-sustained reaction in rabbit skin(7); and, it has been shown to cause a cholera-like syndrome in man(8). Our previous studies have shown that choleragen elicits the production of specific antibody which neutralizes and precipitates it(9). However, in our attempts to protect infant rabbits passively by parenteral administration of choleragen antiserum prior to challenge, little, if any, evidence of protection was obtained. The present communication presents evidence that parenteral administration of choleragen in adult rabbits gives rise to a highly effective degree of active immunity against intestinal challenge with either choleragen or a massive dose of living cholera vibrios. Some resistance can also be passively transferred to infant rabbits by means of serum from the immunized animals.