

Observations on Bound Ascorbic Acid in Guinea Pig Liver.* (22252)

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Sealock and co-workers(1,2) recently reported, the presence of bound L-ascorbic acid in guinea pig liver. In their study, free L-ascorbic acid was extracted from liver with 95% ethanol and bound L-ascorbic acid was liberated from the alcohol-precipitated protein fraction by heating in acid solution. These investigators used the colorimetric method of Roe(3,4) to measure L-ascorbic acid present in the free and bound forms. The availability of L-ascorbic-1- C^{14} acid(5,6) has allowed further study of the presence of bound L-ascorbic acid in guinea pig liver. In addition, radioactive tracer technics have made possible observations on the binding of D-ascorbic acid in guinea pig liver.

Material and methods. L-ascorbic-1- C^{14} acid and D-ascorbic-1- C^{14} acid had specific activities of 1.40 and 1.18 μ c per mg respectively. D-ascorbic-1- C^{14} acid was prepared by the method used for L-ascorbic-1- C^{14} acid (7-8) except that D-xylosone was used as the starting material instead of L-xylosone. Preparation of samples and assay for C^{14} were carried out as described previously(5). Male guinea pigs purchased from Rockland Farms, 2 to 3 months old and weighing 250 to 350 g, were used. Carrier dilution experiments(9) have shown that at least 95% of the C^{14} in the liver of guinea pigs 24 hours after intraperitoneal administration of L-ascorbic-1- C^{14} acid is present as L-ascorbic acid. It was therefore possible in the present study to determine the amounts of free and bound L-ascorbic acid by radioactive assay. Three normal guinea pigs and 2 guinea pigs maintained on a vit. C free diet for 14 days each received 3 mg of L-ascorbic-1- C^{14} acid by intraperitoneal injection. Twenty-four hours

later the animals were sacrificed and their livers removed. A portion of the liver was homogenized with water and the total C^{14} in the liver was determined on an aliquot of the resulting homogenate by a wet combustion method(5). The remaining liver (5 g) was extracted 6 times with 30 ml portions of 95% ethanol by the procedure employed by Summerwell and Sealock(1). Measurements of C^{14} in the different alcoholic extracts showed that essentially all the free ascorbic acid was recovered in the first 3 extractions. The resulting alcohol-precipitated protein fraction was treated with hot 5% metaphosphoric acid(1) and after centrifugation a suitable aliquot of the supernate was assayed for C^{14} by the wet combustion technic(5). The % of ascorbic acid in the bound form was taken as the ratio of the total amount of C^{14} recovered after heat treatment of the alcohol-precipitated fraction to the total amount of C^{14} present in the liver. Further confirmation of C^{14} in the bound fraction being present as L-ascorbic acid was obtained by a carrier dilution technic(6). 200 mg of non-radioactive L-ascorbic acid was added prior to heat treatment and the C^{14} present in this fraction was recovered as the 2, 4 dinitrophenylosazone derivative of ascorbic acid. This technic has been shown to be highly specific for ascorbic acid. The determination of free and bound D-ascorbic acid in guinea pig liver was carried out in the same way as described for L-ascorbic acid. Two guinea pigs maintained on a vit. C-free diet for 14 days each received 3 mg of D-ascorbic-1- C^{14} acid by intraperitoneal injection and the animals were sacrificed 2 hr later and their livers removed. With D-ascorbic acid it was necessary to carry out the analyses at 2 instead of 24 hours used for L-ascorbic acid, because of the rapidity with which the former

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TABLE I. Bound L-Ascorbic Acid and D-Ascorbic Acid in Guinea Pig Liver, 7 Experiments.

Compound	Animal	% in bound fraction*
L-ascorbic-1-C ¹⁴ acid	Normal guinea pig	14
<i>Idem</i>	<i>Idem</i>	23
"	"	20
"	Vit. C deficient guinea pig	31
"	<i>Idem</i>	25
D-ascorbic-1-C ¹⁴ acid	"	35
<i>Idem</i>	"	34

* Expressed as % of C¹⁴ recovered after heating in acid of the alcohol-precipitated protein fraction to the total C¹⁴ present in the liver.

compound is eliminated by the guinea pig (10). A carrier dilution technic similar to that used for L-ascorbic-1-C¹⁴ acid showed that all the C¹⁴ in the liver 2 hours after administration of D-ascorbic-1-C¹⁴ acid is present as D-ascorbic acid(10).

Results. An average of 19% of the total L-ascorbic acid in the liver of the normal guinea pigs was found in the bound fraction compared to an average of 28% for the vit. C deficient animals (Table I). Sealock *et al.* (1,2) found that about 15% of the L-ascorbic acid in normal guinea pigs was in the bound fraction compared to 37% for vit. C deficient guinea pigs. Following the administration of D-ascorbic-1-C¹⁴ acid to vit. C deficient guinea pigs, about the same fraction of the total D-ascorbic acid in liver as in the case of L-ascorbic acid was found to be bound, averaging 35%.

Discussion. The results obtained in this study using C¹⁴ labeled L-ascorbic acid are in agreement with those determined colorimetrically by Sealock *et al.*(1,2) in that there is a bound form of L-ascorbic acid in guinea pig liver. At present no information is available on the nature of this binding. Sealock *et al.* (1,2) have attached importance to this bound

form of L-ascorbic acid in the metabolism of tyrosine.

The results also show that D-ascorbic acid can exist in a bound form. In this connection it is of interest that D-ascorbic acid, which is reported to have no vit. C activity in guinea pigs(11), has recently been shown to be equally active as L-ascorbic acid in the *in vitro* metabolism of tyrosine(12).

Summary. Studies with C¹⁴ labeled L-ascorbic acid have yielded further evidence for the presence of a bound form of the vitamin in guinea pig liver. About 19% of the liver L-ascorbic acid is bound in normal and 28% in vit. C deficient animals. When D-ascorbic acid, the enantiomorph of L-ascorbic acid, is administered to vit. C-deficient guinea pigs, it was found in a bound form to about the same extent as L-ascorbic acid.

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