between the European strain and the Indian *Fasciola gigantica*[16]. This hypothesis is supported by the fact that the Indian Brahman cattle harboring *Fasciola gigantica* were imported in this area on a large scale between the years 1875 and 1906.

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FORMATION OF L-XYLULOSE FROM L-GULONIC ACID IN RAT KIDNEY

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SUMMARY

Evidence is presented for the presence of an active enzyme system in rat kidney for the conversion of L-gulonolactone or L-gulonic acid to L-xylulose. Identification of the end product was established by a carrier dilution technique, specific enzymatic and colorimetric assays, and column and paper chromatographic data. Evidence is also presented for the formation of a small amount of xylitol as a further product in this reaction. A scheme for the metabolism of L-gulonolactone involving the pentose phosphate pathway is presented.

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INTRODUCTION

L-Xylulose, a sugar excreted by patients with essential pentosuria, is present normally in urine of man[1], guinea pig[1] and rat[2]. D-glucuronolactone has been shown to be a precursor of L-xylulose in subjects with essential pentosuria[3,4]. L-Gulonic acid, which is excreted in urine after administration of D-glucuronolactone to rats and guinea pigs[5], has been postulated to be an intermediate in this reaction[1]. Other studies have shown that L-[1-^{14}C]gulonolactone is oxidized extensively to respiratory $^{14}CO_2$ in rats and guinea pigs and is converted to L-ascorbic acid in the former but not in the latter species[6].

The first clue to the mechanism for the formation of L-xylulose came from the tracer studies of RABINOWITZ AND SALL[7] who demonstrated that a particle-free enzyme system in rat kidney decarboxylated D-glucuronic acid and D-glucuronolactone. Although the intermediary step was not identified it now appears that D-glucuronic acid was most likely converted to L-gulonic acid before undergoing decarboxylation. Preliminary reports on the conversion of L-gulonic acid to L-xylulose in mammalian liver and kidney extracts have appeared recently[8-10]. It is the purpose of the present paper to present further details on the occurrence of this reaction in rat kidney extracts.

MATERIALS AND METHODS

Radioactive compounds

Uniformly and carboxyl-labeled L-[^{14}C]gulonolactone, having a specific activity of 0.20 μC per mg and 0.10 μC per mg respectively, were prepared by sodium borohydride reduction of the correspondingly labeled D-glucuronolactone*[6]. L-[1-^{14}C]-gulonic acid was prepared by treating L-[1-^{14}C]gulonolactone with a stoichiometric amount of NaOH in aqueous solution. L-[1-^{14}C]ascorbic acid with a specific activity of 0.10 μC per mg was synthesized by a previously published procedure[11].

Preparation of the enzyme system

All preparative manipulations were carried out at 4°. Kidneys from male rats of the Wistar strain were removed immediately after sacrificing. A 20-% homogenate (based on wet weight of tissue) was prepared using a Potter Elvejhem type homogenizer in pH 7.0 buffer which was 0.2 M with respect to phosphate and 0.06 M with respect to nicotinamide. The homogenate was centrifuged at 100,000 $\times$ g for 1 h and the supernatant fraction was used as the enzyme source.

Incubation procedure

The conditions employed were essentially those described by RABINOWITZ AND SALL[7] for the decarboxylation of D-glucuronolactone. Unless specifically noted, the following mixture was used for each incubation vessel. To 3 ml of the enzyme solution was added 1.2 μmoles uridine triphosphate, 1.0 μmoles diphosphopyridine nucleotide, 1.3 μmoles adenosine triphosphate, 1.8 μmoles thiamine pyrophosphate, 18 μmoles $MgCl_2$ and 2.0 mg of substrate and the mixture was adjusted to a total volume of 6.5 ml. The incubation was carried out aerobically for 90 min at 35°. The reaction

* Uniformly labeled D-glucuronolactone was obtained through the generosity of Dr. N. E. ARTZ of the Corn Products Refining Co., Argo, Illinois.

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at 5° with 0.02 *M* borate. An aliquot of each tube was analyzed for both ^{14}C and for pentoses by the orcinol method. A peak was obtained by the ^{14}C and orcinol assay in the region expected for L-xylulose. The labeled L-xylulose in this fraction, was equivalent to 16% of the substrate incubated. The free pentose after removal of the borate esters[19] was chromatographed on paper in a solvent system consisting of ethyl acetate–acetic acid–H_2O, (3:1:3). Under such conditions, the unknown sugar co-chromatographed with authentic L-xylulose and exhibited the characteristic blue-gray fluorescence in the ultra violet after spraying with orcinol reagent[20]. In addition, the ketopentose in this fraction was characterized by its reaction with TPN-xylitol (L-xylulose) dehydrogenase. When the fraction was assayed with purified DPN-xylitol (D-xylulose) dehydrogenase[16] no D-xylulose was detected.

A second small peak of radioactivity was obtained which gave no reaction with the orcinol test. The fraction appeared to be a pentitol by its characteristic reaction in the periodate and chromatropic acid assays. The identity of the product as xylitol was established by addition of carrier xylitol to a pooled mixture of this fraction after removal of the borate esters[19]. The xylitol was isolated as its pentaacetate derivative[21] and it was found to contain 1.3% of the total ^{14}C incubated. The remaining radioactivity equivalent to about 25% of that incubated was recovered after total elution of the column with 0.1 *M* KCl. The identification of this material has not been established.

Possible implication of L-*ascorbic acid*

Experiments were carried out to see whether L-ascorbic acid was involved in this reaction. This was of importance since L-gulonolactone has been shown to be a precursor of L-ascorbic acid [6,22,23]. This was accomplished by incubating L-[1-^{14}C]-gulonolactone with the rat kidney system. Carrier L-ascorbic acid (200 mg) was then added to the trichloroacetic acid extract of the incubation mixture and the resulting solution was passed through an Amberlite IR-4B (acetate) column. The adsorbed material was eluted with 2 *N* formic acid and L-ascorbic acid was isolated from the eluate as the 2,4-dinitrophenylosazone derivative. From the amount of ^{14}C present in this derivative it was estimated that less than 0.17% of the incubated L-gulonolactone was converted to L-ascorbic acid. These results indicated that the vitamin did not accumulate in this system. However, the possibility that L-ascorbic acid might be actively metabolized, had to be considered since rat kidney homogenates have been reported to degrade L-ascorbic acid[12]. However, when carboxyl labeled L-ascorbic acid was incubated under the same conditions, used for the decarboxylation of L-gulonolactone, less than 0.10% of the vitamin was decarboxylated.

DISCUSSION

Evidence has been presented indicating that the soluble portion of rat kidney is capable of decarboxylating L-gulonolactone to form L-xylulose. This finding completes the following pathway for the metabolism of L-gulonolactone: L-gulonolactone → L-xylulose → xylitol → D-xylulose → D-xylulose-5-PO_4 → pentose phosphate pathway → glucose. TOUSTER *et al.*[24] have demonstrated an enzyme system in guinea pig liver capable of reversibly reducing both L-xylulose and D-xylulose to a common intermediate, xylitol, thereby providing a mechanism for the interconversion of the

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stereoisomers of this ketopentose. The subsequent finding by HICKMAN AND ASHWELL[25] of a specific liver kinase capable of forming D-xylulose-5-phosphate from the free sugar indicated that mammalian tissues possesses the complete enzymic structure necessary to carry out the conversion of L-xylulose to glucose via the pentose cycle as originally postulated by TOUSTER *et al.*[21]. Evidence for the occurrence of these reactions *in vivo* comes from recent findings that L-gulonolactone is converted to liver glycogen in accordance with this pathway[26].

NOTE ADDED IN PROOF

Recent studies[27] have provided experimental evidence for 3-keto-L-gulonic acid being an intermediate in the conversion of L-gulonic acid to L-xylulose by an enzyme in the soluble fraction of kidney. This enzyme, L-gulonic acid dehydrogenase, has been purified about 35-fold from hog kidney and a requirement for diphosphopyridine nucleotide has been demonstrated.

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