COMMUNICATIONS TO THE EDITOR

BIOSYNTHESIS OF L-GULONIC ACID IN RATS AND GUINEA PIGS

Sir:

The most likely pathway for the biosynthesis of L-ascorbic acid in rats is D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid.[1-5] Unlike rats, guinea pigs are unable to convert L-gulonolactone to L-ascorbic acid, which may explain their inability to synthesize the vitamin.[6,7] In both species, administered L-gulonolactone is extensively oxidized to CO_2,[5] and a small fraction of the dose is excreted in urine as L-gulonic acid.[8] No evidence has been presented, however, for the biosynthesis and occurrence of L-gulonic acid or its lactone in animal species. The present studies show that D-glucose-1-C^{14} and D-glucuronolactone-6-C^{14} are converted *in vivo* to labeled L-gulonic acid.

The method for the isolation of labeled L-gulonic acid from urine is given[9]: 300 mg. of non-radioactive L-gulonic acid was added to a 24-hour urine sample which was passed through an Amberlite IR-4B column in the acetate form.[10] The adsorbed L-gulonic acid was eluted with 2 *N* formic acid and the eluate was evaporated rapidly to dryness *in vacuo* at 50°. L-Gulonic acid was converted to its lactone by crystallization from glacial acetic acid. The resulting L-gulonolactone was dissolved in water and the solution was passed through the Amberlite IR-4B column.[10] L-Gulonolactone was obtained after evaporation of the effluent to dryness, and its radioactive purity was established by finding constant specific activity on successive recrystallizations from glacial acetic acid and absolute ethanol. In addition, two derivatives, the potassium acid saccharate and the phenylhydrazide, prepared from the same sample of L-gulonolactone, had identical molar specific activities. Control experiments carried out by adding either D-gluconic acid-1-C^{14} or D-glucuronic acid-6-C^{14} to non-radioactive urine showed that these compounds did not contaminate the isolated L-gulonolactone.

D-Glucuronolactone-6-C^{14} was administered to rats[11] and guinea pigs in intraperitoneal doses of 20 mg. (0.5 μc./mg.) and labeled L-gulonic acid was isolated from urine collected over 24 hours. The results obtained showed that both species can convert D-glucuronolactone to L-gulonic acid the % conversion being 0.72 and 2.4 in two rats and 0.90, 1.3 and 2.9 in three guinea pigs.

The conversion of D-glucose-1-C^{14} to urinary L-gulonic acid was measured in rats[11] receiving either Chloretone or barbital to stimulate the synthesis of L-ascorbic acid[12] (Table I). Similar experiments also carried out in rats[11] not receiving drugs.

TABLE I

CONVERSION OF D-GLUCOSE-1-C^{14} TO URINARY L-GULONIC ACID IN RATS[a]

Drug	None		Chloretone[b]		Barbital[b]	
Conversion, %	0.04	0.03	0.34	0.54	0.22	0.16

[a] Urine was collected for 24 hours after 10 to 30 mg. intraperitoneal doses of D-glucose-1-C^{14} (1.0 μc./mg.). [b] Rats were fed daily either 150 mg. of barbital or 50 mg. of Chloretone for at least 5 days prior to the experiment.

It will be noted that the conversion of D-glucose-1-C^{14} to urinary L-gulonic acid averaged 0.3% in drug-treated rats, but no conversion was detected in animals not receiving drugs (<0.03%). Administration of Chloretone and barbital has been found also to produce in rats a similar increase in conversion of D-glucose-1-C^{14} to urinary D-glucuronic acid and L-ascorbic acid.[8] The possible mechanism by which drugs exert this effect on the formation of D-glucuronic acid, L-gulonic acid and L-ascorbic acid is now under investigation.

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A NEW METHOD FOR DEHYDROGENATION OF CORTICOSTEROIDS

Sir:

We wish to report a new, single-step method for the synthesis of unsaturated analogs of Δ^4-3-ketosteroids. It has been found that a variety of steroid Δ^4-3-ketones can be selectively oxidized with chloranil under mild conditions to Δ^6-dehydro derivatives. Detailed studies in the hydrocortisone series have revealed that more vigorous reaction conditions result in the formation of the corresponding $\Delta^{1,4,6}$-trienone derivative. The $\Delta^{1,4,6}$-3-one function is a previously unreported structural modification of glucocorticoids.

Hydrocortisone acetate (I), when treated with chloranil in refluxing xylene, yielded 66% of $\Delta^{4,6}$-pregnadiene-11β,17α,21-triol-3,20-dione acetate (II), m.p. 204.0–205.0°, $[\alpha]^{25}D$ +199° (dioxane), λ_{max}^{alc} 284 mμ (25,000); *Anal.* Calcd. for C_{23}-