Effect of D-Glucuronic Acid and D-Glucuronolactone on Ascorbic Acid Levels in Blood and Urine of Man and Dog

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In 1953 Horowitz and King[1] demonstrated that the injection of C^{14}-labeled D-glucuronolactone in rats led to the formation of C^{14}-labeled L-ascorbic acid. Isherwood et al.[2] later showed that D-glucuronic acid was converted first to L-gulonic acid and then to L-ascorbic acid. Recently Touster et al.[3] further demonstrated that xylulose was excreted by human beings and guinea pigs fed large amounts of D-glucuronolactone.

That D-glucuronate was first converted to L-gluonate, then to 3-keto-L-gulonate and finally to L-ascorbate was demonstrated by Bublitz et al.,[4] using surviving tissue slices. In certain instances 3-keto-L-gulonate was decarboxylated and converted to L-xylulose.

During the course of studies in man on the renal excretion of ketopentoses, chiefly xylulose, it was noted that marked increases occurred in ascorbic acid levels in blood and urine following the ingestion of D-glucuronolactone. Therefore, it was decided to study the effects of the administration of D-glucuronolactone and D-glucuronic acid both in man and in a dog.

When given lactone all subjects excreted excess ascorbate, whereas when given the D-glucuronic acid only the dog excreted excess ascorbate. This suggests that man may synthesize ascorbic acid from certain precursors.

MATERIAL AND METHODS

D-Glucuronolactone and D-glucuronic acid used in the study were checked for purity by melting point measurement and by paper chromatography.

The method for measuring ascorbic acid levels in plasma and urine was that of Schaffert and Kingsley.[5] Urine ketopentose (xylulose) was determined by the procedure described by Futterman and Roe[6] as modified by Baker et al.[7]

The chromatographic method, except for a few details, was that of Mapson and Partridge.[8] Whatman No. 3 sheets were used in a descending solvent system of phenol, glacial acetic acid and water (100:1:100 by volume) and run in the presence of 10 to 20 mg. NaCN for a period of twenty to twenty-two hours. Ascorbic acid in an acid medium was shown to withstand shell freezing and lyophilization to dryness without loss or destruction. Therefore, since a spot of 40 μg. or more was required for positive identification, one-tenth of each urine sample was shell frozen, lyophilized to dryness and then the residue dissolved in 1 ml. of water; 0.1 ml. was immediately applied to the chromatographic sheets. A standard of ascorbic acid was dissolved in urine, as it was suspected that urine salts could possibly alter the R_f values.

Four men and two women served as experimental subjects. They fasted from midnight

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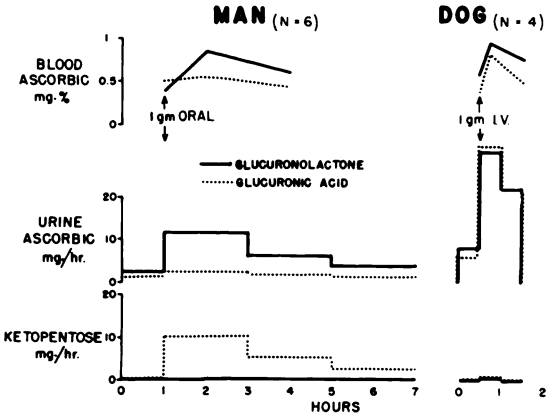

FIG. 1. Ascorbic acid levels in blood and urine, and ketopentose levels in urine, in man and dog following administration of D-glucuronolactone and D-glucuronic acid.

to morning. A one-hour control urine specimen was collected from 7 to 8 A.M. At 8 A.M. a fasting blood sample (control) was drawn, and then the subject swallowed 1 gm. of the glucuronolactone freshly dissolved in 25 ml. of water. Heparinized blood samples were obtained at one and three hours following ingestion of the lactone, and complete collections of urine were made at two-hour intervals for six hours. The subjects remained fasting but were allowed water during the experimental periods. The same procedure was used at later dates with 1 gm. D-glucuronic acid.

The dog used in this study was a male Boxer weighing 24.5 kg. The animal was given four separate intravenous infusions of 1 gm. of glucuronolactone and four intravenous infusions of 1 gm. of glucuronic acid. The animal was in a fasting state and was not anesthetized. On the morning of each test the bladder was catherized and a 200 ml. water load was given by stomach tube. After a half-hour control period of urine collection, a control blood sample was drawn and the animal was rapidly infused with 1 gm. of either the acid or lactone. Two half-hour periods followed, during which blood and urine urine samples were collected.

RESULTS

It was necessary to determine the effect of D-glucuronolactone, D-glucuronic acid and xylulose on the color development in the ascorbic acid method. It was found that 1 mg. of D-glucuronolactone gave a color equivalent of 0.005 mg. of L-ascorbic acid; 1 mg. of D-glucuronic acid gave a color equivalent of 0.05 mg. of L-ascorbic acid; 1 mg. of L-xylulose, although it formed the dinitrophenylhydrazone, when treated with the sulfuric acid gave no color at all; 1 mg. of D-glucose also gave no color.

Reductic acid, when assayed by this method, gave the same color equivalent as L-ascorbic acid. However, the chromatographic procedure readily differentiated the reductic acid and reductones from ascorbic acid.

Figure 1 demonstrates the mean results obtained when six human subjects ingested either D-glucuronolactone or D-glucuronic acid. When D-glucuronolactone was swallowed, the ascorbic acid level in plasma rose within one hour from a control of 0.39 (±0.30 S.D.) mg. per 100 ml. to 0.84 (±0.45 S.D.) mg. per 100 ml. and then fell to 0.62 (±0.46 S.D.) mg. per 100 ml. three hours after the administration of the D-glucuronolactone. These deviations were statistically significant when calculated as per cent change from control. Ascorbic acid levels in urine rose from a control of 2.25 (±0.96 S.D.) mg. per hour to 12.15 (±2.86 S.D.) mg. per hour during the first two-hour period following the administration of D-glucuronolactone. During the second two-hour period, the urine level dropped to 6.1 (±1.47 S.D.) mg. per hour and finally to 3.95 (±0.44 S.D.) mg. per hour during the third two-hour period. The mean milligram excess ascorbic acid excreted per hour during the six hours was 8 mg., whereas the milligram excess of ketopentose (xylulose) in urine was only 0.2 mg. per hour.

When the same test was performed using D-glucuronic acid instead of D-glucuronolactone, the ascorbic acid values in blood showed no significant increase from the control level. Also, the ascorbic acid values in urine did not show any marked change from the control level. The apparent milligram excess of ascorbic acid excreted during the test with D-glucuronic acid was only 0.6 mg. per hour. This level could be accounted for by

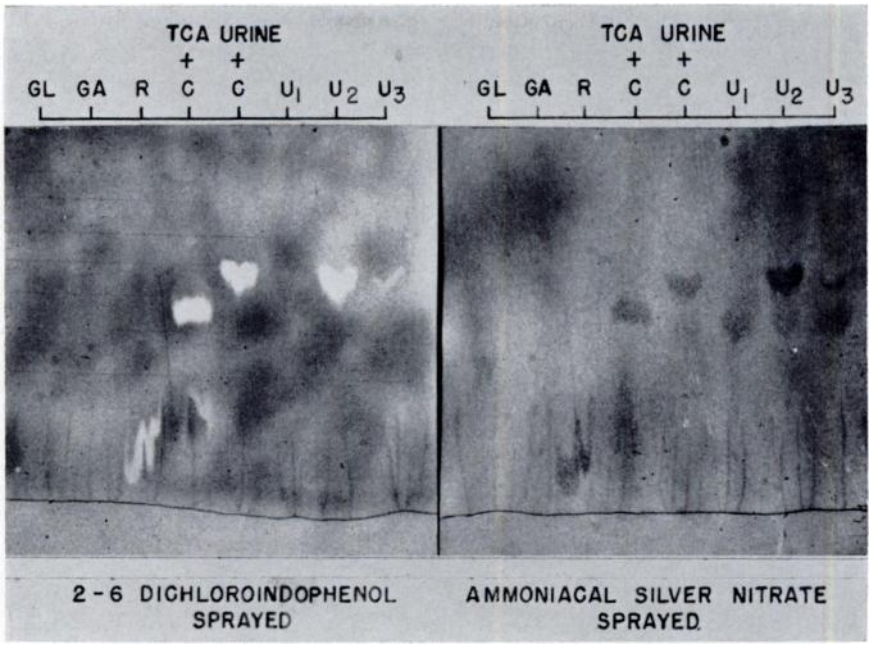

FIG. 2. A pair of partition diagrams demonstrating the presence and relative R_f values of reductic, ascorbic and dehydroascorbic acids. (For details see text.)

interference of the D-glucuronic acid in the chemical test. It is of interest that xylulose levels in urine rose from a control of 0.075 (±0.015 S.D.) mg. per hour to 10.3 (±1.10 S.D.) mg. per hour during the first two-hour period; this dropped to 5.25 (±0.63 S.D.) mg. per hour during the second two-hour period and then to 2.63 (±0.33 S.D.) mg. per hour during the third two-hour period. The milligram excess xylulose excreted by man during the tests with D-glucuronic acid averaged 8.1 mg. per hour.

The effects of 1 gm. infusions of D-glucuronolactone and D-glucuronic acid in the dog were similar (Fig. 1). After the dog was infused with the D-glucuronolactone, the mean ascorbic acid level in blood rose within 10 minutes from a control of 0.61 (±0.10 S.D.) mg. per 100 ml. to 0.96 (±0.10 S.D.) mg. per 100 ml. and in one hour fell to a level of 0.80 (±0.05 S.D.) mg. per 100 ml. The mean urine milligram excess ascorbic acid excreted by the dog was 10.3 mg. per hour, whereas the mean excess xylulose was only 0.25 mg. per hour.

The levels of ascorbic acid in blood and urine following the infusion of D-glucuronic acid matched those obtained with the D-glucuronolactone. In the blood the ascorbic acid level rose from 0.41 (±0.07) mg. per 100 ml. to 0.85 (±0.15) mg. per 100 ml. then fell to 0.53 (±0.06) mg. per 100 ml. one hour after the infusion. The excess ascorbic acid excreted during the experiments with D-glucuronic acid was 11 mg. per hour. The excess xylulose excreted during these same experiments was only 0.40 mg. per hour.

Chromatography revealed the presence of reduced ascorbic acid, as well as dehydroascorbic acid, in the urine of all subjects following the ingestion of D-glucuronolactone. However, reduced ascorbic acid could not be detected in their urine following the ingestion of D-glucuronic acid. Reduced ascorbic acid was not present in any of the control urine specimens, although traces of dehydroascorbic acid were present. The greatest concentration of reduced ascorbic acid appeared two hours after the ingestion of D-glucuronolactone and steadily decreased in proportion to dehydroascorbic acid until at the sixth hour the ascorbic acid present in the urine was almost wholly in the form of dehydroascorbic acid. This chromatographic test validated the results obtained using the ascorbic acid method of Shaffert

and Kingsley[5] when their chemical procedure was used to check the presence of both forms of ascorbate in several of the urine samples.

Chromatograms from a typical test with D-glucuronolactone in a human subject are illustrated in Figure 2. D-Glucuronolactone and D-glucuronic acid, when dissolved in 4 per cent trichloroacetic acid, did not react with the 2,6-dichloroindophenol, as can be seen by the total absence of white spots. Reductic acid (R) and ascorbic acid in the reduced form were indicated by white spots after spraying with indophenol. Pure ascorbic acid in urine (Urine + C) migrated more slowly than when dissolved in tricholoroacetic acid (TCA + C). The positions of the white spots of urine samples U_2 and U_3 have R_f values identical to the spot produced when pure ascorbic acid is added to control urine. The control urine sample, U_1, did not react with indophenol. However, when sprayed with ammoniacal silver nitrate, a light brown spot with an R_f of 0.41 was revealed, thus indicating the presence of dehydroascorbic acid. Following the ingestion of D-glucuronolactone, samples U_2 and U_3 indicated both dehydroascorbic acid, R_f 0.41, and reduced ascorbic acid, R_f 0.38, the latter having a rapidly developed black spot. The photograph also shows that most of the reduced ascorbate was excreted soon after the ingestion of the D-glucuronolactone. It was noted in other chromatograms that the R_f value of D-glucuronolactone was 0.64, of D-glucuronic acid 0.17, and of reductic acid, 0.82. However, these spots are difficult to see in Figure 2.

COMMENTS

Bublitz[4] demonstrated that D-glucuronate can be transformed to L-gulonate and 3-keto-L-gulonate and then to L-ascorbate in rats, and showed that in certain cases 3-keto-L-gulonate could be decarboxylated to L-xylulose (Fig. 3). Burns[9,10] found no detectable incorporation of C^{14} from D-glucuronolactone or L-gulonolactone into L-ascorbic acid in guinea pigs *in vivo*, the values obtained being less than one-twentieth of those shown in rats. Lehninger et al.[11,12] reported no net synthesis

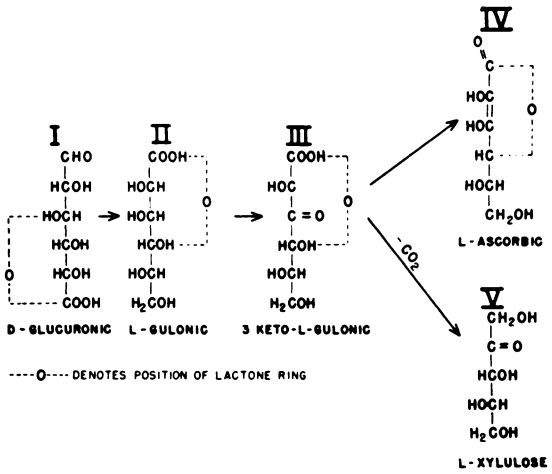

FIG. 3. Synthesis of L-ascorbic acid via D-glucuronic acid pathway.

of L-ascorbic acid from L-gulonolactone in human, monkey and guinea pig homogenates of liver; whereas significant synthesis of ascorbate acid occurred in rat, mouse, rabbit and dog homogenates of liver. Man does carry out the various steps required for the synthesis of ascorbate except for the conversion of the L-gulonate to ascorbate.[9] Therefore, it would be expected that glucuronic acid or glucuronolactone fed to man would be converted to the gulonate and then decarboxylated to L-xylulose. In the present experiments when glucuronic acid was fed, excess L-xylulose was excreted but little or no ascorbic acid, the reaction presumably proceeding *via* the decarboxylation of 3-keto-L-gulonate. When glucuronolactone, however, was fed, excess ascorbate was excreted with no change in xylulose excretion. The results suggest that man could convert lactonized L-gulonate to L-ascorbate. Bublitz and Lehninger[13] reported that rat liver enzymes converted L-gulonate to L-ascorbic acid from the lactone form but not from the free gulonate.

In the dog, which can synthesize vitamin C, both glucuronolactone and glucuronic acid could be converted to L-ascorbate. Little, if any, of either of the two compounds was diverted to xylulose.

Although these experiments suggest that man may synthesize ascorbic acid when given a proper precursor compound, they do not

exclude the possibility that the glucuronolactone displaced tissue ascorbic acid which passed into the blood stream and was excreted. It is also possible that the chemical and chromatographic procedures were detecting a "C"-like compound that was similar to the unidentified compound reported by Bublitz and Lehninger.[13]

SUMMARY

When human subjects were given 1 gm. of D-glucuronolactone orally the ascorbic acid levels in plasma increased. There was an excess renal excretion of 8 mg. per hour of ascorbic acid accompanied by an excess excretion of 0.2 mg. per hour of xylulose.

When the same subjects were given 1 gm. of D-glucuronic acid orally there was no increase in ascorbic acid levels in blood. Although no significant increase was noted in urinary ascorbic acid levels, the excess excretion of xylulose in the urine was 8.1 mg. per hour.

The infusion of a dog with 1 gm. of D-glucuronolactone or D-glucuronic acid caused an increase in the ascorbic acid levels in blood. The renal excretion of ascorbic acid was 10.3 mg. per hour during the experiments with D-glucuronolactone and 11 mg. per hour during the experiments with D-glucuronic acid. The xylulose excretion was not significantly changed.

REFERENCES

1. Horowitz, H. and King, C. Glucuronic acid as a precursor of ascorbic acid in the albino rat. *J. Biol. Chem.*, 205: 815, 1953.
2. Isherwood, F. A., Chen, Y. T. and Mapson, L. W. Synthesis of L-ascorbic acid in plants and animals. *Biochem. J.*, 56: 1, 1954.
3. Touster, O., Hutcheson, R. M. and Rice, L. The influence of D-glucuronolactone on the excretion of L-xylulose by humans and guinea pigs. *J. Biol. Chem.*, 215: 677, 1955.
4. Bublitz, C., Grollman, A. P. and Lehninger, A. L. Enzymatic conversion of D-glucuronate to L-ascorbate in animal tissues. *Fed. Proc.*, 16: 382, 1957.
5. Schaffert, R. R. and Kingsley, G. R. A rapid, simple method for the determination of reduced, dehydro-, and total ascorbic acid in biological material. *J. Biol. Chem.*, 212: 59, 1955.
6. Futterman, S. and Roe, J. H. The identification of ribulose and L-xylulose in human and rat urine. *J. Biol. Chem.*, 215: 257, 1955.
7. Baker, E. M., Bierman, E. L. and Plough, I. C. Factors influencing the urinary excretion of ketopentoses by normal men. *Metabolism*, in press.
8. Mapson, L. W. and Partridge, S. M. Separation of substances related to ascorbic acid. *Nature, London*, 164: 479, 1949.
9. Burns, J. J. Bio-synthesis of L-ascorbic acid. Basic defect in scurvy. *Am. J. Med.*, 26: 740, 1959.
10. Burns, J. J. and Evans, C. The synthesis of L-ascorbic acid in the rat from D-glucuronolactone and L-gulonolactone. *J. Biol. Chem.*, 223: 897, 1956.
11. Ul Hassan, M. and Lehninger, A. L. Enzymatic formation of ascorbic acid in rat liver extracts. *J. Biol. Chem.*, 223: 123, 1956.
12. Grollman, A. P. and Lehninger, A. L. Enzymatic synthesis of L-ascorbic acid in different animal species. *Arch. Biochem.*, 69: 458, 1957.
13. Bublitz, C. and Lehninger, A. L. The enzymatic conversion of L-gulonate to L-ascorbate by rat liver enzymes. *Biochim. et biophys. acta*, 32: 290, 1959.