As further evidence that dentin protein does not combine with phosphate at pH 7.5, denatured dentin protein[4] was agitated for 20 hours with a solution of labeled phosphate with the result that no active phosphate was present in the protein after thorough washing with water.

The greater rate of exchange of phosphate by dentin must, therefore, be attributed to the smaller size of the crystallites of the mineral phase of dentin, but more especially to the fact that dentin is permeated by the dentinal tubules which probably have the effect of permitting a more complete contact of the crystals of the mineral phase with the solution containing labeled phosphate than exists in the case of enamel. An alternative hypothesis might be that the higher activity of dentin is due merely to some active solution trapped in the dentinal tubules. Nevertheless, phosphate in solution which is not removed from the tubules by thorough washing would be expected, with time, to reach an exchange equilibrium with the phosphate of the mineral phase.

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Cinnamic Acid Metabolism in Man.

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The fate of cinnamic acid ($C_6H_5CH:CHCOOH$) after its administration to the animal organism has been examined repeatedly. The following facts are known. Cinnamic acid given by mouth to humans is oxidized to benzoic acid which is excreted in the urine linked with glycine in the form of hippuric acid.[1, 2] In cats and dogs after the administration of phenylpropionic acid not only hippuric acid but also small amounts of cinnamoylglycine are excreted.[3] After administration of cinnamic acid hippuric acid with small traces of cinnamoylglycine are found.[4] After administration of cinnamic

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[1] Erdmann and Marchand, *Liebig's Annalen der Chemie und Pharmacie*, 1842, **44**, 344.

[2] Knoop, F., *Beiträge zur Chemischen Physiologie*, 1905, **6**, 150, and 1908, **11**, 411.

[3] Dakin, H. D., *J. Biol. Chem.*, 1906, **5**, 173, 303.

[4] Dakin, H. D., *J. Biol. Chem.*, 1907, **6**, 203.

acid by mouth to dogs 65-70% of the excreted benzoic acid is present in the form of benzoylglycuronic acid and 30% in the form of hippuric acid.[5] The isolated kidney of calves and sheep is able to oxidize cinnamic acid to benzoic acid during perfusion.[6] However, the isolated kidney of the dog is not able to oxidize cinnamic acid but only conjugates the cinnamic acid with glycine to form cinnamoylglycine.[7] Excretion of cinnamoylglycuronic acid has not been observed previously. We have reëxamined the question whether after cinnamic acid administration to humans free or conjugated cinnamic acid could be found in the urine.

Experimental. Six g of cinnamic acid were given after dissolving in 200 cc of water and neutralizing. The urine excreted within the first 4 hours of the experiment was examined in order to compare the results obtained with Quick's hippuric acid test.

1. *Glycuronic acid* was excreted in considerable amounts. The urine always gave a positive naphthoresorcin reaction.[8] The amount of glycuronic acid varied between 300 and 800 mg determined with the Shaffer-Hartmann technic.[9]

2. *Hippuric acid.* To the 4 hours' urine concentrated HCl was added until the reaction became acid to Congo red. A considerable precipitate (4-5 g) was formed consisting of hippuric acid. After standing in the icebox for some hours the precipitate was filtered off. The filtrate still contained about 0.330 g of hippuric acid per 100 cc.

3. *Presence of free cinnamic acid.* Only small amounts of free cinnamic acid were excreted in this experiment. After chloroform extraction of the acidified urine, 10-15 mg of free cinnamic acid were demonstrated in the extraction fluid.

4. *Presence of a cinnamic acid compound.* Concentrated NaOH was added to the urine filtrate until the reaction was frankly alkaline. The alkaline fluid was boiled for one hour over a free flame and was then evaporated on a waterbath to a volume of 10-30 cc. After cooling, strong HCl was added. As soon as the reaction became acid, a thick white precipitate formed. After a few hours in the icebox, this precipitate was filtered off and dried in an incubator overnight. Next morning the precipitate was extracted on the waterbath for 2 hours with 150 cc of chloroform under a reflux con-

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[5] Quick, A. J., *J. Biol. Chem.*, 1928, **77**, 581.

[6] Snapper, I., and Grünbaum, A., *Bioch. Z.*, 1924, **150**, 12.

[7] Snapper, I., and Grünbaum, A., *Acta brevia Neerlandica*, 1934, **4**, 38, and *Pharmaceutisch Weekblad*, 1934, Jubileumboek Prof. P. van der Wielen.

[8] Tollens, B., Allen's comm. Organic Analysis, 5th edition, **1**, 496, and *Ber. d. Deutsch. chem. Ges.*, 1908, **41**, 1788.

[9] Quick, A. J., *J. Biol. Chem.*, 1926, **69**, 555.

denser. The chloroform was then filtered into a separatory funnel and extracted twice with 5 cc 8% NaOH.

To this alkaline solution, concentrated HCl was added until precipitation occurred. The precipitate was filtered off and dried. This precipitate contained a considerable amount of cinnamic acid. A. If, to a few mg of these crystals, one drop of Na_2CO_3 and one drop of $KMnO_4$ solution were added, the $KMnO_4$ quickly turned brown by reduction and a strong smell of benzaldehyde developed. B. By titration with bromine, considerable amounts of cinnamic acid were determined. For this titration a combination of the methods published by A. W. K. de Jong[10] and by Greenberg and Mackay[11] was used. C. Pure cinnamic acid crystals with a melting point of 133°C were recovered by recrystallization from boiling petroleum ether.

5. *Presence of cinnamic acid as monocinnamoylglycuronic acid.* As the cinnamic acid compound present in the urine was very soluble in water, the possibility of the presence of cinnamic acid conjugated with glycuronic acid had to be considered. The following experiment makes the presence of a cinnamoylglycuronic acid compound seem probable.

The filtrate obtained after acidifying the urine with HCl was extracted with ether in a continuous Lind extractor for 15-20 hours. At that time the ether in the extraction flask showed a watery layer. This watery layer was separated from the ether and transferred to a 25 cc volumetric flask. After addition of 2 cc concentrated HCl, the volume was made up to 25 cc.

This solution was strongly reducing. Two samples of 4 cc each were used for the quantitative determination of glycuronic acid (Shaffer-Hartmann method). The remaining 17 cc were boiled for 30 minutes under a reflux condenser and then transferred while still hot to a separatory funnel in which 100 cc chloroform was already present. After cooling, the solution was shaken for 30 minutes. This was repeated twice with fresh chloroform. The 3 fractions of chloroform were mixed and shaken twice with 10 cc 8% NaOH. The NaOH was then made up to 25 cc in a volumetric flask. Two samples of 5 cc each were titrated with bromine. The remaining 15 cc were slightly concentrated by evaporation before a fan and then acidified with concentrated HCl. The precipitate was dried, weighed and titrated.

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[10] de Jong, A. W. K., *Am. J. Med. Sc.*, 1933, **185**, 630.

[11] Greenberg, D. M., and Mackey, M. A., *J. Biol. Chem.*, 1932, **96**, 419.

TABLE I.
Contents of Glycuronic Acid and Cinnamic Acid of the Water-soluble Fraction in the Extraction Flask of Lind Apparatus After Extraction for 20 hr.

	Glycuronic acid	Cinnamic acid	
	Found, mg	Found, mg	Calculated*
1	222	166.4	177.6
2	320	248.6	256
3	261	210	209
4	150.8	114.5	121.3
5	195.7	149	156
6	175.0	133	140

*From monocinnamoylglycuronic acid.

The glycuronic acid content of the 25 cc of watery solution was about equivalent to the amount of cinnamic acid found. When N mg glycuronic acid (M.W. 184) were found by reduction, the cinnamic acid content proved to be about 148/184 × N mg (Table I).

The stoichiometric relation existing between glycuronic acid and cinnamic acid in the fluid analyzed is a strong indication that monocinnamoylglycuronic acid was excreted after administration of cinnamic acid to humans, especially if one takes into consideration that the cinnamic acid compound is water-soluble, insoluble in chloroform, hardly soluble in ether and is easily hydrolyzed by boiling with HCl or NaOH.

6. *Absence of cinnamoylglycine.* Cinnamoylglycine, being ether soluble, if present should have been found in the ether after extraction in the Lind apparatus. In order to test for the presence of cinnamoylglycine, the crystals obtained after the distillation of the ether were dissolved in boiling water and crystallized. The fraction which crystallized when the solution was still warm was separated and recrystallized from hot water. After purification the crystals did not give the cinnamic acid reaction with Na_2CO_3 and $KMnO_4$.

Table II shows the excretion of hippuric acid in the four-hour period following the administration of cinnamic acid to humans. Six grams of cinnamic acid (equivalent to 5 g benzoic acid) brought about the excretion of 3.6-5.3 g of hippuric acid. These amounts are practically the same as those following the administration of equivalent amounts of benzoic acid.[12] These results indicate that the oxidation of cinnamic acid to benzoic acid occurs rapidly.

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[12] Snapper, I., *Klinische Wochenschr.*, 1924, **3**, 56; Quick, A. J., *Am. J. Med. Sc.*, 1933, **185**, 630.

TABLE II.
Excretion within 4 hr.

Diagnosis	After 5 g benzoic acid Hippuric acid g	After 6 g cinnamic acid Hippuric acid g	Cinnamic acid g	Glycuronic acid g
Sp. Reconvalescent Dysentery	5.00	4.31	0.216	0.596
L.S.S. Reconvalescent Diphtheria	4.32	3.60	0.294	0.458
F.L.Y. Healed Kala Azar	4.82	5.25	0.384	0.798
Y.H.S. Reconvalescent Lobar pneumonia	5.07	4.86	0.203	0.653
L.K.S. Reconvalescent Diphtheria	4.77	4.32	0.286	0.632
L.S.C. Avitaminosis A	5.12	4.72	0.202	0.635
C.H.C. Diencephalic Syndrome	5.89	5.28	0.367	0.654

During the same 4-hour period 400-800 mg of glycuronic acid were excreted. A small part of the cinnamic acid escapes β-oxidation. After giving 6 g of cinnamic acid to persons with normal liver function 200-400 mg of cinnamic acid, conjugated with glycuronic acid, were excreted within 4 hours (Table II).