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Origin of Glucuronic Acid in the Urine of Rabbits.

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Two theories have been proposed for the origin of glucuronic acid in the urine. Sundwick[1] and Fisher and Piloty[2] suggested that the toxic substance was conjugated with glucose by a glucoside linkage, followed by the oxidation of the terminal alcohol group to a carboxyl group. A second theory[3] states that the body is able to synthesize glucuronic acid from amino acid metabolites as it needs glucuronic acid for detoxification. Quick[4] thinks that the animal body is able to detoxicate itself in both ways. He[5] has reviewed the literature on the subject. One must conclude from reviewing the subject that the results of the investigations are inconclusive.

Glucuronic acid exists preformed in the body, in mucin. It is continuously being digested in the intestine, and its constituents are absorbed and restored to the body. It is reasonable to think that glucuronic acid would be one of the digestion products and that it is a normal constituent of the blood stream. It seemed to us that the glucuronic acid arising from mucin metabolism would be available for use by the body in detoxication when needed and that it is unnecessary to assume that the glucuronic acid appearing in the urine as conjugated glucuronic acids arises from the oxidation of carbohydrates or by synthesis from amino acid metabolites. Our experiments were carried out to see if mucin feeding had any significant effect upon the conjugation of glucuronic acid as indicated by the quantity excreted.

A group of 11 rabbits was fed a diet of oats, carrots, and water and each given 2 gm. of menthol in water by stomach tube for 3 days. Their average daily excretion of menthol glucuronic acid calculated as glucuronic acid was 0.41 gm. Glucuronic acid was determined by the method of Quick.[6] The rabbits were then given water and fasted for 5 days. They were each given 2 gm. of menthol in water by stomach tube. They showed signs of menthol intox-

[1] Sundwick, E., *Akadem Abhandlungen*, Helsingfors, 1886.

[2] Fischer, E., and Piloty, G., *Ber. Chem. Ges.*, 1891, **24**, 522.

[3] Lusk, Science of Nutrition, Saunders, 1928, 683.

[4] Quick, A. J., *J. Biol. Chem.*, 1926, **70**, 397.

[5] Quick, A. J., *J. Biol. Chem.*, 1924, **61**, 679; 1926, **70**, 59, 397.

[6] Quick, A. J., *J. Biol. Chem.*, 1924, **61**, 667.

ication during this period. During the third period of 3 days, they were each given water and fed 20 gm. of dextrin and 2 gm. of menthol by stomach tube. During this period, the average daily excretion of glucuronic acid was 0.25 gm. The quantity excreted decreased from day to day. The symptoms of toxicity increased during dextrin feeding. We interpret the difference in levels of average daily glucuronic acid excretion on a normal diet and a dextrin diet, and the increasing rather than decreasing symptoms of toxicity to indicate that carbohydrates are either not the precursors of glucuronic acid in the urine, or the rate of synthesis is too slow to protect rabbits adequately. This is in accord with the observations of other investigators.

A second group of 6 rabbits was used to determine whether mucin or proteins, like milk proteins, would have any effect upon glucuronic acid excretion. Five of the rabbits were 8 months old, and from one litter. The sixth was full grown. They were fed a commercial feed in pellet form, recommended as a balanced ration for rabbits, and 2 gm. menthol in water by stomach tube daily, throughout the experiment. During the first 4 days the average daily excretion dropped progressively. During the next 4 days, they were each given 2 gm. mucin in water by stomach tube. During this period, the average daily excretion increased progressively. There were no symptoms of toxicity. During the last period, they were given milk to drink instead of water. Each rabbit drank about 200 cc. daily. The average daily excretion of glucuronic acid dropped progressively, and they developed marked symptoms of intoxication.

We interpret this to mean that the glucuronic acid obtained in the digestion of mucin is readily available for detoxication purposes. Since there was a progressive decrease in glucuronic acid excretion during the first period when rabbits were given a balanced ration and since milk did not prevent them from developing symptoms of intoxication, we question whether the body can synthesize glucuronic acid from either amino acid metabolites or carbohydrates. At least, it indicates that the rate of synthesis is so slow that it does not prevent the development of symptoms of intoxication. Mandel and Jackson[7] found that feeding meat to fasting dogs caused an increase in the excretion of camphorol glucuronic acid. This has been interpreted as evidence of the ability of the body to synthesize glucuronic acid from amino acid metabolites. Since connective tissue of meat contains preformed glucuronic acid, it seems to us that the in-

[7] Mandel and Jackson, *Am. J. Physiol.*, 1902, **8**, XIII.

crease in camphorol glucuronic acid excretion was due to the availability of preformed glucuronic acid from the digestion of the meat and not to synthesis from amino acid metabolites.

Fromm and Clemens[8] state that a strong rabbit can tolerate 5 gm. of menthol daily. Our experience does not confirm this. We used young rabbits. The toxicity of menthol may decrease with age. Biberfeld[9] states that a rabbit can tolerate 2 gm. of menthol daily if it is fed greens. Our young rabbits could not tolerate 2 gm. of menthol on a diet that did not contain greens. This suggests to us that the greens may either contain preformed glucuronic acid or contain substances from which rabbits can readily obtain glucuronic acid. This also indicates that either the body is unable to synthesize glucuronic acid or that the synthesis proceeds slowly.

A third group of 2 rabbits was given water and fasted for 6 days. They were then given 2 gm. of menthol daily by stomach tube for 3 successive days. Their average daily excretion of glucuronic acid dropped from 0.96 gm. to 0 gm. in the 3 days. This indicates that the rabbit after a 6-day fast still contains glucuronic acid available for conjugation. The rate of decrease in menthol glucuronic acid excretion of fasting rabbits was faster than for rabbits that were well fed. The excretion of menthol glucuronic acid in rabbits receiving food never reached 0. The rate of decrease of menthol glucuronic acid may give some indication of the amount of mucin in the body. After the 3 days' fasting and menthol administration they were fed pellets which we had previously found did not protect rabbits from a decrease in glucuronic acid excretion, and 2 gm. of mucin per day, but no menthol, for 4 days. At the end of 4 days, they were given 2 gm. menthol each for 2 days. Their average daily excretion of glucuronic acid as menthol glucuronic acid was 0.53 gm. for the first day and 0.42 gm. for the second. This indicates that during the 4-day period of mucin feeding either glucuronic acid was stored in the body or more glucuronic acid already in the body was made available for conjugation. We think that the dietary mucin was the chief factor in raising the level of menthol glucuronic acid excretion.

We conclude from our experiments that glucuronic acid is liberated in the digestion of mucin and that it is readily available for conjugation with certain toxic substances when needed by the body. It seems unnecessary to us to assume that the detoxication mechanism necessitates the synthesis of glucuronic acid from carbohydrates

[8] Fromm, E., and Clemens, P., *Z. Physiol. Chem.*, 1901, **34**, 385.

[9] Biberfeld, J., *Biochem. Z.*, 1914, **65**, 479.

or amino acid metabolites. Our findings lead us to doubt if the rabbit can synthesize glucuronic acid from carbohydrates or amino acid metabolites.

Further investigations are in progress on the ways by which the body loses glucuronic acid and the importance of it in the animal economy.

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Studies on the Metabolism of Glucuronic Acid in the Dog.

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Since glucuronic acid and glucosamine are characteristic constituents of the prosthetic group of mucin, the biochemical synthesis of mucin can not take place without them. Very little is known about the metabolism of these substances, except that they are probably not glycogenic and are surprisingly inert in the body. We are interested in studying the metabolism of these substances because their metabolism is so closely related to the metabolism of mucin and gastro-intestinal function.

We wish to report our findings to date. When dogs are fed borneol, it is excreted as borneol glucuronic acid. In another paper of this series, Miller and Conner show that the glucuronic acid arising from the digestion of mucin in rabbits is readily available for conjugation with menthol, and that either the rabbit is unable to synthesize glucuronic acid from carbohydrates or amino acid metabolites, or that the synthesis takes place slowly. We were interested in determining if this applied to dogs.

Three dogs having an average weight of 9.5 kg. were fed a stock diet of bread and corn meal gruel containing a little meat broth. They were given 5 gm. of borneol in 90 cc. of 1% agar by stomach tube for 31 successive days. Their average total excretion of glucuronic acid as borneol glucuronic acid was 30.78 gm. Their average daily excretion of glucuronic acid was 1.11 gm.* At the end of the experiment their average weight was 8.12 kg. During

* Glucuronic acid was determined by the method of Quick, *J. Biol. Chem.*, 1931, **61**, 667.