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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(Introduced by C. J. Farmer.)

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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.

the last 8 days the average daily excretion was 0.63 gm., which is lower than the average daily excretion for the entire period. The dogs were then fasted for 5 days, but the daily administration of borneol was continued. The average daily excretion for the 5-day period was 1.06 gm., an increase of 68% over the average daily excretion during the previous 8-day period. They were then given 10 gm. mucin and 5 gm. borneol daily, but no food, for 7 days. The average daily excretion of glucuronic acid during this period increased to 1.43 gm., an increase of 127% over the 8-day period. We then fed them the stock diet and stopped feeding borneol. The excretion of borneol glucuronic acid was negligible after 48 hours. The dogs apparently suffered no ill effects from the prolonged administration of borneol.

We interpret these findings to indicate that glucuronic acid obtained from the digestion of mucin is readily available for conjugation with borneol in the dog; that fasting dogs can conjugate glucuronic acid more readily than dogs given adequate food, due probably to the accelerated endogenous catabolism during starvation; that dogs either have stored in their bodies a considerable quantity of glucuronic acid, probably as a deposit protein in addition to their supply of mucin, or they can synthesize it from carbohydrates and amino acid metabolites; the loss of weight on an adequate diet makes us believe that the former view is more nearly correct; that dogs can excrete borneol glucuronic acid continuously for 43 days without causing any profound ill effects; that the efficiency of conjugation is dependent directly upon the total amount of glucuronic acid available, decreasing progressively with glucuronic acid availability, since the average daily excretion for the last 8-day period was less than the average daily excretion for the entire 31-day period.

Another group of 3 dogs having an average weight of 16 kg. was fed the stock diet and 10 gm. of mucin per day. The dogs were started on 5 gm. of borneol per day. The dosage was slowly increased until after 2 months, they were receiving 19-25 gm. of borneol per day, which was 1.3 gm. per kg. body weight. It is stated that dogs can tolerate 5 gm. of borneol per day without ill effects, the inference being that a quantity appreciably larger than this causes toxic symptoms to appear. Mucin was added to the diet with the expectation that it would protect the dogs against the toxic effects of large doses of borneol. During the third month, the dogs were fed 1.3 gm. borneol per kg. body weight for 24 days. The average daily excretion of glucuronic acid was 6.23 gm. Mucin

was then withdrawn from the diet. One dog had contracted distemper and was killed 2 days later. The daily excretion of glucuronic acid for the remaining 2 dogs continued at approximately the previous level. After 17 days, a second dog died, following a profound drop in glucuronic acid excretion. Death was probably due to the toxic effect of large doses of borneol without the protecting effect of mucin. After 21 days the third dog was fasted. The average daily excretion continued high for 7 days, when suddenly the glucuronic acid excretion dropped, the dog became toxic, and died within 3 days.

We interpret the results of these experiments to indicate that mucin protects against large doses of borneol because it supplies glucuronic acid to the organism; that the increase in average daily excretion over dogs not receiving mucin is due to the mucin itself; that the body is able to store a considerable quantity of glucuronic acid, probably as mucin or as a deposit protein, which can act as a source of glucuronic acid when needed; that the organism can be more or less depleted of this supply, and when this occurs, toxic symptoms appear.

A third group of 5 dogs was fasted. They were fed 5 gm. of borneol daily. Three dogs showed a progressive rise of about 400% in excretion of glucuronic acid in 2 weeks. One dog was pregnant and died after 2 weeks' fasting. Autopsy revealed a large prepyloric ulcer, 2 duodenal ulcers, and multiple ulcers in the pylorus. The areas involved were about ½ inch in diameter. A second dog became very weak and somewhat toxic after 14 days. It was fed 25 gm. mucin daily. During the remainder of the 37-day fasting period, glucuronic acid excretion dropped slightly and then remained constant. The third dog after the 20th day of fast showed a progressive drop in glucuronic acid excretion until the 34th day, after which there was a rise for 3 days.

The other 2 dogs of this group were fasted but given 10 gm. mucin daily for 37 days. There was a progressive rise in the glucuronic acid throughout the entire fasting period from an average daily excretion for the first 5 day period of 1.5 gm. to 2.96 gm. for the last 5-day period.

The glucuronic acid which is excreted during fasting may come either from the synthesis of glucuronic acid, from carbohydrates or amino acid metabolites, or from the metabolism of deposit protein in the accelerated endogenous catabolism. When fasting dogs are fed mucin there is a continual rise in excretion which lasts as long as 37 days. This indicates again that glucuronic acid arises from

mucin. If our previous hypothesis is correct, that the efficiency of glucuronic acid excretion is dependent upon the total amount of glucuronic acid available, then the progressive rise in glucuronic acid excretion during starvation when 10 gm. of mucin is fed indicates that glucuronic acid can be stored during fasting in some form and hence is not oxidized in the body for energy.

The autopsies on these dogs were made by Dr. S. J. Fogelson. With few exceptions all dogs that had excreted a large amount of glucuronic acid, showed a gastritis more marked in the pyloric area than in the fundus, and a marked duodenitis. The gastric mucosa was slimy, indicating that some mucin was still present. In one case, where a female was fasted for 2 weeks, one prepyloric, 2 duodenal ulcers, and multiple ulcers of the pylorus were found. We think that the gastritis and duodenitis result from the interference in the normal mucin metabolism of the mucosa incident to the loss of glucuronic acid. While it is possible that borneol may have an irritating action directly upon the mucosa and produce the gastritis and duodenitis, it seems that the feeding of borneol to dogs for over 100 days, during which time massive doses were fed for 40 days, would produce more ill effects than were noticed. Investigations on Pavlov pouch dogs bear out our conclusion.

Many more animal experiments must be performed before any final conclusions can be formed on the metabolism of glucuronic acid. We submit our findings and our tentative conclusions.

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Glucuronic Acid as a Growth Factor in Guinea Pigs.

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It is generally considered that the animal body can synthesize glucuronic acid either from carbohydrates or from amino acid metabolites. Miller and Conner have reported results previously which indicate that either the rabbit cannot synthesize glucuronic acid or that the rate of synthesis is low. A similar investigation on dogs did not give such definite indications. In comparing the chemical properties of glucuronic acid with the chemical properties of Vitamin