CXVI. THE BIOCHEMISTRY AND PHYSIOLOGY OF GLUCURONIC ACID.

VI. THE EXCRETION OF ETHEREAL SULPHATE BY THE RABBIT FOLLOWING THE ADMINISTRATION OF PHENYLGLUCOSIDES.

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FROM time to time it has been suggested that in the dog (and presumably the arguments apply to other animals) glucuronic acid for detoxication processes may be derived from body protein rather than from glucose directly [Thierfelder, 1886; Mandel and Jackson, 1903; Quick, 1926, 1]. Quick [1926, 2] states that "the precursor of glucuronic acid may be derived more readily from glycogen and glycogenetic amino-acids rather than from glucose". Miller *et al.* [1933] suggest that the dog possesses a store of glucuronic acid or can synthesise it from carbohydrates or amino-acids, but that, when this store is depleted, mucin, which contains glucuronic acid, is used as a source for detoxication purposes. In the case of the rabbit Miller and Connor [1933] have shown that this animal is unable to synthesise glucuronic acid from carbohydrates or amino-acids (or at least performs these syntheses very slowly), but that it can utilise glucuronic acid, obtained by digestive processes, when fed with mucin. Miller and Connor therefore suggest that glucuronic acid for detoxication purposes is derived from food sources. That the food material has an influence on the efficiency of another detoxication process has been demonstrated by Abderhalden and Wertheimer [1925] in the cases of the formation of mercapturic and hippuric acids in the rabbit. In the case of glucuronic acid detoxication a somewhat similar influence of diet has been demonstrated recently by Palladin and Persova [1928], by Kusnetzova [1930] and by Makarevitch-Galperin [1930]. From the above data the balance of evidence seems to indicate that the glucuronic acid utilised in detoxication is preformed either in the diet or in the body of the organism. Glucuronic acid itself, when fed alone, is metabolised to a very slight extent and is a comparatively inert substance physiologically [Biberfeld, 1914; Hürthle, 1927; Quick, 1928].

These conclusions are obviously at variance with the Fischer and Piloty [1891] theory of intermediate glucoside formation as a step in glucuronic acid conjugation. Indeed we have already shown [Hemingway *et al.*, 1934] that phenyl- and bornyl- β -glucosides, when perfused through the isolated liver of the dog, are not converted into the corresponding glucuronic acids, although the liver-kidney preparations used were capable of conjugating free phenol and borneol and of excreting these as the glucuronic acids. A repetition of these experiments (hitherto unpublished) using phenyl- α -glucoside likewise gave no glucuronic acid in the perfused liver.¹ None the less experiments have been

¹ This experiment was carried out with the co-operation of Dr A. Hemingway.

It is well known that phenol is detoxicated by conjugation with both sulphuric and glucuronic acids. It might therefore be expected that if phenylglucoside were directly oxidised to phenylglucuronic acid no increase in ethereal sulphate excretion would occur. This expectation is strengthened by the observation of Koike and Tosawa [1933] that oral administration of phenylglucuronic acid itself does not give rise to increased excretion of ethereal sulphate in the rabbit. On the other hand if phenylglucoside were first hydrolysed and then detoxicated an increase in ethereal sulphate excretion may be expected, and an observed increase could only be explained by such a hydrolysis.

Phenylglucoside————→phenylglucuronic acid.

Phenylglucoside $\longrightarrow$ Phenol + glucose
 |
 conjugation
 ↙ ↘
 Phenyl sulphate + phenylglucuronic acid.

The present experiments indicate that the second of the above alternatives holds in the case of the rabbit. Two female rabbits were used, one serving as control. In repeat experiments the rôles of control and experimental animal were reversed with the view of eliminating possible individual variations in the two animals. In each experiment the control animal was given a known amount of phenol, whilst the other was given phenyl- β -glucoside sufficient to yield an amount of phenol approximately equal to that given to the control. The phenol and phenylglucoside were fed to the rabbits on the same day, and each animal received food from the same stock, thus eliminating possible dietetic variations which might influence the detoxication process. The results indicate that phenyl- β -glucoside reacts, in regard to detoxication as ethereal sulphate, exactly as if it were free phenol; the percentage of phenol detoxicated and excreted as ethereal sulphate in the glucoside-fed rabbit was the same as that detoxicated and excreted in the control. In both cases very slight traces of free phenol were found in the urine after phenol or glucoside feeding, but the amount of conjugated glucuronic acid excreted, as indicated by the naphthoresorcinol test,

was very little more than normal in each case. The urines after boiling with hydrochloric acid gave a very slight reduction which was probably due to traces of conjugated glucuronic acid rather than to unchanged glucoside.

Other experiments were performed in which phenyl- α - and β -glucosides were injected subcutaneously. There followed an increase in ethereal sulphate output similar to that observed after oral administration, whilst the urine gave very definite indications of the presence of free phenol.

It may therefore be concluded that, as in the case of the isolated liver, the intact animal fails to give any evidence of the direct formation of glucuronides from glucosides administered either orally or subcutaneously. Furthermore phenylglucosides are hydrolysed *in vivo* and, at the levels administered in our experiments, are detoxicated preferentially by conjugation with sulphuric acid, behaving in this respect like the equivalent amount of phenol.

EXPERIMENTAL.

(1) Feeding experiments.

Phenyl- α - and β -glucosides were prepared by the method of Helferich and Schmitz-Hillebrecht [1933] for the synthesis of phenolic glucosides. The final pure crystalline products had physical constants in accord with those already published. The female rabbits used were fed on a mixed diet of bran, oats and cabbage. Urines were collected in a beaker by means of large tinned copper funnels in which the rabbit cages were placed. Ethereal sulphate was estimated daily by the gravimetric method. Phenylglucoside and phenol were fed in small gelatin capsules. In the case of phenol, liquid paraffin was added to the capsule to reduce the corrosive action of solid phenol on the stomach. The animals suffered no apparent ill-effects during the entire course of the experiments.

The experimental period lasted 23 days on each of which ethereal sulphate was determined. The maximum value found, excluding that of the 1st day after each administration of phenol or glucoside, was for rabbit 11, 27.2 mg.,

Table I.

Rabbit 11, female, 2140 g.			Rabbit 12, female, 1860 g.		Diet, oats and cabbage.		
Controls. Phenol							
Rabbit	Phenol fed mg.	Normal ethereal SO ₃ mg.	Ethereal SO ₃ on 3rd day mg.	Increase in ethereal SO ₃	Phenol conjugated (calc.)	% phenol conjugated with SO ₃	
11	134	15	43	28	32.9	25	
11	195.5	12	82	70	82.2	42	
12	142*	11	30	19.1	22.3	16	
12	142	11	48	37	43.5	31	
Phenyl-β-glucoside							
Rabbit	Glucoside fed mg.	Phenol equivalent mg.	Normal ethereal SO ₃ mg.	Ethereal SO ₃ on 3rd day mg.	Increase in ethereal SO ₃	Phenol conjugated (calc.)	% phenol conjugated with SO ₃
12	397	145.5	14.6	41.4	27	31.7	22
12	474	174	11	69	58	68.1	39
11	379	139	12	38	26	30.6	22
11	387	142	14.6	58.5	44	51.7	36

* Some phenol lost during feeding; amount fed uncertain.

for rabbit 12, 23.4 mg. The minimum values were rabbit 11, 6.2 mg., rabbit 12, 6.6 mg. The normal value of the ethereal sulphate output was taken as the average for the 2 days immediately preceding, and for the 2nd and 3rd days following, the administration of phenol or glucoside. The experimentally induced increase was taken as the output observed on the 1st day immediately following the ingestion of phenol or glucoside. The results of the experiments are given in Tables I and II. Fig. 1 records graphically a result obtained for one 5-day period in which rabbit 11 received phenyl- β -glucoside and rabbit 12 phenol.

Table II.

Phenol-fed rabbit		Glucoside-fed rabbit	
Rabbit	% phenol conjugated with SO ₃	Rabbit	% phenol conjugated with SO ₃
11	25	12	22
11	42	12	39
12	16*	11	22
12	31	11	36

* See Table I.

Fig. 1. Increase in ethereal sulphate output following the administration of phenylglucoside contrasted with that following the administration of the equivalent amount of phenol.

(2) Injection experiments.

Phenyl- α - and β -glucosides were also injected subcutaneously and the ethereal sulphate increases were measured as in the experiments already described. Ethereal SO₃ was determined for 25 days. The maximum normal value found was 39.6 mg. and the minimum 7.4 mg. The results are given in Table III.

Table III.

Rabbit C, weight 2740 g.; at the end of experiment, 25 days later, 2690 g.

Glucoside g.	Normal E.S. mg.	E.S. 3rd day mg.	Increase mg.	% phenol detoxicated
0.5 β	22.9	78.1	55.2	35.4
0.4 α	22.8	60.2	37.4	30

SUMMARY.

Phenyl- α - and β -glucosides administered orally or subcutaneously to the rabbit are not converted into the corresponding glucuronides but undergo hydrolysis. The liberated phenol is detoxicated and excreted as ethereal sulphate. The phenylglucosides behave in this respect as the equivalent amount of phenol.

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