FURTHER STUDIES ON THE DETOXICATION OF PHENYLACETIC ACID

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(Received for publication, May 4, 1933)

In the previous work done on the detoxication of phenylacetic acid in this laboratory, we have never been able to account for more than half of it as the glutamine conjugate in the human urine. We ascribed this to either (*a*) conjugation with other substances besides glutamine, (*b*) an incomplete detoxication, or (*c*) the methods of analysis.

Conjugation with Glucuronic Acid

Quick (1) believed that glucuronic acid may arise not from glucose itself, but from glycogen and sugar-forming amino acids when glucuronic acid is needed for detoxication. In his experiments depancreatized dogs were able to produce glucuronic acid the same as normal dogs, but under these conditions glucuronic acid is produced at the expense of glucose which would otherwise appear in the urine.

The ratio between the conjugation of benzoic acid with glucuronic acid and glycine in the case of animals was also determined by Quick (2). The results show that the amount combined with glucuronic acid in these cases was by far larger than had previously been suspected.

Brakefield (3) finds only traces of or no glucuronic acid after feeding benzoic acid to humans while Quick (4) under the same conditions reports 11 per cent conjugation. However, when phenylacetic acid is fed to dogs (2), it is detoxicated in a manner similar to benzoic acid.

Incomplete Detoxication

Human subjects were fed phenylacetic acid; the urine was acidified and extracted with various organic solvents, but no free phenylacetic acid was found.

Experiment 1—The object of our experiment was to determine to what extent, if any, glucuronic acid is employed by the human

TABLE I

Rate of Glucuronic Acid Excretion after Feeding Varying Amounts of Phenylacetic Acid

Date	Amount fed	Phenylacetic acid eliminated		Date	Amount fed	Phenylacetic acid eliminated	
		Per cent of total	Per cent combined with glucuronic acid			Per cent of total	Per cent combined with glucuronic acid
	gm.				*gm.*		
Nov. 19, 23	1	93.0	0.0	Jan. 27	7	99.1	4.5
" 30, Dec. 1–4	2	97.5	0.0	" 30	8	99.1	6.8
Dec. 5–8, Jan. 4, 5	3	98.0	1.0	Feb. 1	8	98.4	5.7
Jan. 6, 7, 10	4	97.9	4.0	" 2	8	97.4	5.0
" 11	5	97.0	4.9	" 3	10	98.8	5.3
" 12	5	98.2	4.6	" 6	10	98.7	5.2
" 13	5	98.5	3.5	" 8	10	99.5	5.3
" 14	5	98.4	6.4	" 9	10	98.4	5.3
" 16	5	98.2	7.1	" 10	10	98.9	5.2
" 17	5	98.4	4.9	" 11	10	99.0	5.6
" 18	5	98.4	4.9	" 13	8	98.5	5.6
" 20	6	98.2	5.6	" 15	8	98.5	5.2
" 21	6	98.6	5.7	" 16	7	98.5	5.1
" 23	6	97.9	6.6	" 17	7	98.0	5.5
" 25	7	98.9	5.7	" 23	6	98.7	6.5
" 26	7	99.0	5.4	" 24	6	99.3	6.8

subject for detoxication of phenylacetic acid and the conditions under which this might take place.

In these experiments phenylacetic acid was fed to human subjects as the sodium salt in increasing daily doses in order to place a continual and ever increasing strain on the defense mechanism of the body, in the hope that in this way we might overtax the

power of the body to produce glutamine and force it to resort to a glucuronic acid detoxication. Table I shows the results obtained.

Experiment 2—Small doses of phenylacetic acid, 3 gm., were fed to thirty-four normal students and the urine was collected hourly over a period of 5 hours. It was thought that the small dose might be detoxicated by means of glucuronic acid and if so we wished to ascertain the rate at which this combination took place. It seemed quite possible to us that small amounts of phenylacetic acid excreted during the early stage of detoxication

TABLE II

Glucuronic Acid Excretion after Feeding Phenylacetic Acid

5 gm. were given every 8 hours, a total of 45 gm. in 72 hours.

Time of feeding	Phenylacetic acid eliminated	
	Total	Combined with glucuronic acid
	gm.	*gm.*
9 a.m.	1.84	0.0
5 p.m.	4.74	0.807
12 "	4.33	0.252
9 a.m.	2.68	0.602
5 p.m.	3.40	1.515
12 "	3.89	0.384
9 a.m.	3.34	0.301
5 p.m.	4.36	1.566
12 "	6.10	0.231
Total.....	34.68	5.658

79.29 per cent of the total phenylacetic acid fed was recovered. 16.00 per cent of the total eliminated was conjugated with glucuronic acid. 12.40 per cent of the total fed was conjugated with glucuronic acid.

might have been overlooked, when large volumes of urine were collected over a period of 24 hours. The results of the experiment, however, may be considered entirely negative as five subjects of the thirty-four showed less than 1 per cent of glucuronic acid conjugation at one time or another, and the others showed less or none at all.

Experiment 3—This experiment was undertaken in much the same fashion, except that the subject was fed 5 gm. of phenylacetic acid every 8 hours for a period of 72 hours, a total feeding of

45 gm., and the amount of glucuronic acid and phenylacetic acid determined at the end of each 8 hour period. (See Table II.)

Methods—Phenylacetylglutamine was prepared by the usual method (5). Total phenylacetic acid was determined by the method of Kingsbury and Swanson (6) as used by them for the determination of total benzoic acid, except that, instead of using quantities of urine as described in their method, the quantity varied inversely with the amount of phenylacetic acid ingested. Glucuronic acid was determined by its reducing power towards Somogyi's reagent (7).

From this work it is clearly seen that glucuronic acid plays a rather small rôle in the detoxication of phenylacetic acid in the human as compared with similar work on animals except when excessive amounts are ingested. Thus Quick (2) showed that phenylacetic acid is excreted by the dog, conjugated with glucuronic acid to the extent of about 34 per cent, the other 66 per cent appearing as the glycine conjugate. Table I shows that after repeated ingestions of phenylacetic acid at 24 hour intervals the body is still capable of furnishing glutamine for detoxication purposes and that the amount of glucuronic acid produced rarely exceeds a 5 per cent conjugation.

However, the same or smaller doses, but ingested at 8 hour intervals, required considerable amounts of this substance, as Experiment 3 (Table II) shows, but we were unable to complete the series as the subject became nauseated on account of the repeated ingestions. During the second period we obtained greater glucuronic acid conjugation, indicating the body's inability to synthesize sufficient glutamine, thus resorting to glucuronic acid conjugation. Phenylacetic acid retention was also observed.

Methods of Analysis of Phenylacetylglutamine

Folin and Flanders (8) have noticed that hippuric acid in the urine is appreciably hydrolyzed while evaporating on a water bath, so it is to be expected that phenylacetylglutamine, which is quite unstable, would also be destroyed to a considerable extent. In a series of four experiments, assuming that all the phenylacetic acid ingested should appear as a glutamine conjugate, an average of 51 per cent of phenylacetylglutamine was recovered from the urine. To check this by a recovery experiment, 16.4 gm. of

phenylacetylglutamine were dissolved in 2 liters of normal urine and the urine was treated in the usual way (5). Only 47.4 per cent of the original amount was recovered. Therefore, it seems that the large amount of phenylacetylglutamine unaccounted for is not to be ascribed to any extensive conjugation with any other substances, but rather to the losses inherent in the evaporation, as the extraction itself seems to give quantitative removal of the phenylacetylglutamine once it is in the apparatus.

In connection with this work we were able to get an approximate estimate of the time required for the body to detoxicate phenylacetic acid. A total of 20 gm. (in four portions) was ingested by two subjects, each taking 5 gm. in two doses 12 hours apart; 12 hour volumes of the urine, each including both night and day specimens, were evaporated and extracted; and the first 12 hour sample was compared with the following 12 hour sample. It was found that in the urine excreted during the first 12 hours following ingestion of the acid, 53.8 per cent of the theoretical phenylacetylglutamine was recovered, and in the second 12 hour period only 1.4 per cent. Thus over 95 per cent of the phenylacetic acid is taken care of by the body during the first 12 hours and less than 5 per cent remains to be detoxicated in the second 12 hours. Hence, whether we approach this detoxication problem from the glutamine conjugate or from that of glucuronic acid, the ratio of one to the other is very nearly the same, that is about 18:1.

In aqueous solution, pure phenylacetylglutamine shows a fairly strong acidity. Its hydrogen ion concentration, measured by indicators and checked approximately electrometrically, for 0.1 N solution is 6.9×10^{-3}, corresponding to an ionization of about 7 per cent, and for 0.01 N solution, 2.6×10^{-3}, corresponding to an ionization of about 25 per cent. Its warm 0.1 N solution decomposes the carbonates of alkalies and alkaline earths, also those of lead and copper. Its salts thus far studied are all soluble in water, making separation by precipitation thus far impossible. The insolubility of the barium salt in absolute alcohol (0.87 gm. per liter) is used to separate it from urea; the presence of 5 per cent of water in the alcohol raises its solubility over 400 per cent.

Decomposition by Evaporation—Two samples were dissolved in 25 cc. of water in small Kjeldahl flasks which were immersed in boiling water and a slow current of air passed through (over $2\frac{1}{2}$

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