PLAINTIFF'S EXHIBIT 936

Biochemical Journal Vol 48 (1951)

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Studies in Detoxication

38. THE METABOLISM OF BENZENE. (*a*) THE DETERMINATION OF PHENYLMERCAPTURIC ACID IN URINE. (b) MERCAPTURIC ACID EXCRETION BY RABBITS RECEIVING BENZENE

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(*Received* 26 *October* 1950)

We have shown that some 60 % of a dose of benzene administered to rabbits can be accounted for by oxidation to phenols (Porteous & Williams, 1949*a*, b) and by elimination in the unchanged state (Parke & Williams, 1950). Benzene, however, is also excreted as phenylmercapturic acid and Zbarsky & Young (1943) have proved this by the isolation of the mercapturic acid in a maximum yield of 0·37 % of the dose from the urine of rats dosed with benzene. Although Witter (1945) failed to isolate phenylmercapturic acid from the urine of rabbits receiving benzene, Drummond & Finar (1937) have suggested that these animals also excrete this conjugate. In the present paper we shall show that rabbits do, in fact, excrete phenylmercapturic acid and that. determined by the quantitative methods developed here. the amount excreted is about 1 % of the dose.

EXPERIMENTAL

Preparation of phenyl-L-cysteine derivatives. Phenyl-L-cysteine has been synthesized by Zbarsky & Young (1943), but we were unable to reproduce their conditions exactly. The present method has produced rather better yields. L-Cystine (5 g.) was reduced by boiling for 2 hr. in 100 ml. N-H_2SO_4 with 3 g. of Zn dust, a few pieces (total, 1 g.) of granulated Zn (A.R.) being added periodically. The solution was filtered hot and, in order to facilitate the formation of the ~ ~ p r (mercaptide, 1 ml. of conc. HCl was added (cf. Pirie, 1931). The hot solution was now treated with freshly precipitated Cu_2O (3·5 g. in 20 ml. water) which slowly dissolved on shaking to give a green solution. When the addition of the ~ ride is near completion the mercaptide may tend to come out of solution as a white precipitate, but this can readily be redissolved by adding a little 2N-H_2SO_4. Excess Cu_2O should be avoided, since it tends to co-precipitate with the mercaptide as e dark-brown spongy solid. To the solution of cuprous mercaptide cooled to 0°, there was added a solution of diazotized aniline (8 g.) in 2N-H_2SO_4 (120 ml.). A brisk evoiution of N_2 occurred with the formation of a buff precipitate. After 30 min. at 0°, the solution was heated at 60–70° for 30 min., filtered and extracted with ether (50 ml.) to remove phenol. The resulting green solution was saturated with H_2S to remove Cu and, after filtration of the copper sulphide, the solution was boiled *in vacuo* to remove excess H_2S. The solution was then neutralized with conc. SH, solution followed by glacial acetic acid (5 ml.) and after keeping at 0° overnight, the precipitated phenyl-L-cysteine was collected by filtration. At this stage, the compound was heavily contaminated with cystine which was largely removed by repeated dissolution in N-NaOH (charcoal) and precipitation with 2N-acetic acid. The material was finally recrystallized from 50 % aqueous ethanol in which cystine is very sparingly soluble (Clarke & Inouye, 1931). The product (2·2 g.) was obtained as white glistening plates, m.p. 180–181°; $[\alpha]_D^{23°}$ +10° (*c*, 1 in 0·1N-NaOH). (Zbarsky & Young (1943) give lap. 170–172° and $[\alpha]_D^{25°}$ +11° in 0·1N-NaOH.) A sample prepared by debromination and deacetylation of biosynthetic *p*-bromophenylmercapturic acid (Zbarsky & Young, 1943) had m.p. 180–181° and $[\alpha]_D^{23°}$ +10° (*c*, 1 in 0·1N-NaOH).

p-Bromophenyl-L-cysteine was similarly prepared from cystine (5 g.) and diazotized *p*-bromoaniline (7·5 g.) (yield, 2·6 g.). It formed colourless, glistening plates, m.p. 193° (not depressed by a sample, m.p. 194°, prepared by deacetylation of biosynthetic *p*-bromophenylmercapturic acid according to Baumann & Preusse, 1881), and $[\alpha]_D^{23°}$ +20° (*c*, 1 in 0·1N-NaOH).

p-Chlorophenyl-L-cysteine. This compound has not been, previously described, although the DL-form (m.p. 202°) has been synthesized by Behringer & Fackler (1949). It was prepared from cystine (5 g.) and diazotized *p*-chloraniline (5·5 g.) as described above for phenylcysteine. The yield was 2g. after recrystallization from 50 % ethanol. It formed colourless glistening elongated plates, m.p. 190°, $[\alpha]_D^{25°}$ +18° (*c*, 1 in 0·1N-NaO
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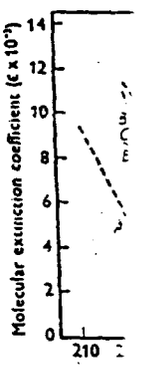

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(c, 1 in 0·1 N-NaOH). (Found: C, 46·9; H, 4·7; Cl, 15·1. $C_9H_{10}O_2NSCl$ requires C, 46·7; H, 4·4; Cl, 15·3 %.) A sample was also prepared by hearing biosynthetic *p*-chlorophenylmercapturic acid (0·1 g.) under reflux with 5 ml. 4 N-H_2SO_4 for 45 min. After cooling, the solution was neutralized with NH_3 solution, and after keeping overnight the precipitate of *p-chlorophenyl-L-cysteine* was washed with water and recrystallized from 50 % aqueous ethanol (yield, 50 mg.) (m.p. 189–190°; $[\alpha]_D^{22°}$ +19° (c, 1 in 0·14-% OH)). (Found: Cl, 14·9 %.)

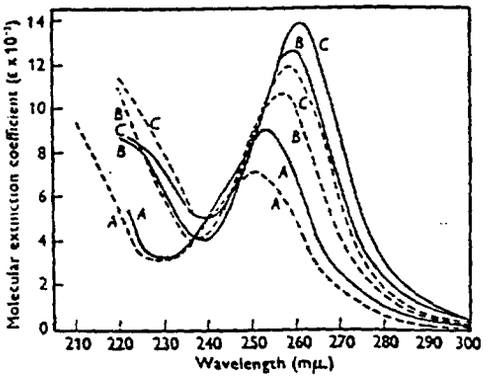

Fig. 1. Ultraviolet absorption spectra of phenyl-L-cysteine (*A*), *p*-chlorophenyl-L-cysteine (*B*) and *p*-bromophenyl-L-cysteine (*C*) in 0·1 N-NaOH (———) and 0·1 N-HCl (– – –). See also Table 1.

L-Phenylmercapturic acid (m.p. 142°; $[\alpha]_D^{22°}$ −21° in ethanol) was prepared by acetylation of phenyl-L-cysteine according to Zbarsky & Young (1943). The yield was 0·6 g from 1 g. of phenylcysteine. L-*p*-Chlorophenylmercapturic acid (m.p. 153°; $[\alpha]_D$ −20° in ethanol) and *p*-bromophenylmercapturic acid (m.p. 152° and $[\alpha]_D$ −13° in ethanol) were prepared biosynthetically from the urine of rabbits dosed with chlorobenzene and bromobenzene, respectively. The spectra of these compounds determined with a Unicam spectrophotometer (Model S.P. 500) are given in Figs. 1 and 2, and Table 1.

Alkaline hydrolysis of the phenylcysteines and mercapturic acids. Preliminary experiments showed that the phenylcysteines were more stable to alkaline hydrolysis than their *N*-acetyl derivatives, the mercapturic acids. These observations were investigated quantitatively as follows.

To 10 ml. portions of 0·1 % (w/v) solutions of each of phenyl-, *p*-chlorophenyl- and *p*-bromophenyl-mercapturic acids, there were added 10 ml. of 0·2, 0·3, 0·5, 0·7, 1·0 and 1·5 N-NaOH, respectively. A similar set of solutions of 0·1 % phenyl-, *p*-chlorophenyl- and *p*-bromophenyl-cysteines (made by dissolving the arylcysteine in hot water and adding

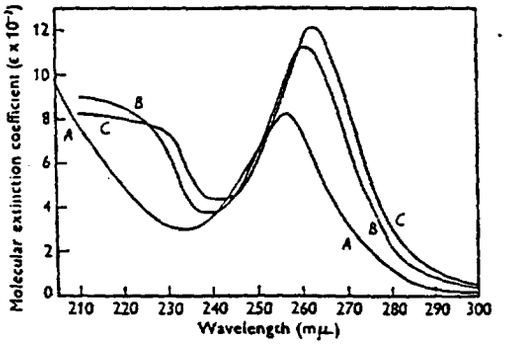

Fig. 2. Ultraviolet absorption spectra of L-phenylmercapturic acid (*A*), L-*p*-chlorophenylmercapturic acid (*B*) and L-*p*-bromophenylmercapturic acid (*C*) in ethanol. See also Table 1.

a few drops of ammonia) were also set up, but the NaOH concentrations used were 2, 4, 6, 8 and 10 N. The solutions were heated on a sand bath (105°) in conical flasks capped with small beakers for 30 or 60 min. The flasks were cooled in ice, treated with 10 ml. ethanol and enough 2 N-H_2SO_4 to neutralize the alkali and provide an excess of 2 ml. After the addition of 10 drops starch solution, the thiophenols set free were titrated with 0·01 N-I_2 (1 l. N-I_2 = 1 g.mol. of mercapturic acid). The results (see Fig. 3) show that the mercapturic acids are completely hydrolysed by alkali hydroxides of less than N concentration in 0·5 hr., whereas the aryl-

Table 1. *Optical rotations and absorption spectra of some mercapturic acids and derivatives*

Compound	Optical rotation: In 0·1 N-NaOH $[\alpha]_D^{22°}$	Optical rotation: In ethanol $[\alpha]_D^{22°}$	Absorption spectra in 0·1 N-NaOH: $\lambda_{max.}$ (mμ)	0·1 N-NaOH: $\epsilon_{max.} \times 10^{-3}$	0·1 N-HCl: $\lambda_{max.}$ (mμ)	0·1 N-HCl: $\epsilon_{max.} \times 10^{-3}$	Ethanol: $\lambda_{max.}$ (mμ)	Ethanol: $\epsilon_{max.} \times 10^{-3}$
Phenyl-L-cysteine	+10°	—	253	9·1	250	7·0	—	—
p-Chlorophenyl-L-cysteine	+18°	—	260	12·6	257	10·6	—	—
p-Bromophenyl-L-cysteine	+20°	—	262	13·9	259	11·9	—	—
L-Phenylmercapturic acid	—	−21°	—	—	—	—	256–257	8·1
L-*p*-Chlorophenylmercapturic acid	—	−20°	—	—	—	—	260–261	11·2
L-*p*-Bromophenylmercapturic acid	—	−13°	—	—	—	—	262–263	12·1

cysteines require up to 5N-alkali for complete hydrolysis. The substituted phenylmercapturic acids were mom readily hydrolysed than the unsubstituted derivatives.

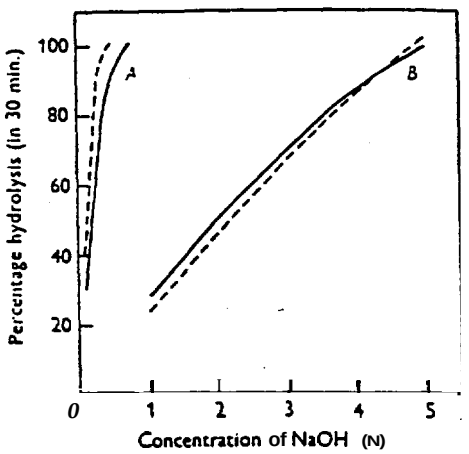

Fig. 3 Extent of hydrolysis to the thiophenols of phenyl. mercapturic acids (A; unsubstituted, ——; p-chloro- and p-bromo derivatives, – – – –) and phenylcysteines (B; unsubstituted, ——, p-chloro and p-bromo derivatives, – – – –) by various concentrations of NaOH in 30 min.

The determination of phenylmercapturic acid

Methods for the determination of p-bromophenylmercapturic acid have been devised by Stekol (1936) and Binkley (1949). In Stekol's method, the mercapturic acid was hydrolysed by 0·35N-NaOH to p-bromothiophenol which was then estimated iodometrically, or gravimetrically as p-bromophenylmercuric mercaptide. Binkley's method, which only indirectly involves the mercuric mercaptide. is colorimetric and uses Folin's reagent. Methods involving the formation of a phenylmercuric mercaptide are more specific than the iodometric method, but Stekol's gravimetric method cannot be conveniently used on a small scale. It is also clear from the preceding section that whereas 0·35N-NaOH completely hydrolyses p-chloro- and p-bromo. phenylmercapturic acids, mora concentrated alkali is necessary for phenylmercapturic acid, whilst for the phenylcysteines 5N-NaOH may be necessary.

The effect of diet on the figures obtained by the iodometric method. Whom mercapturic acids are estimated iodometrically in urine, it is necessary to determine a basal value in terms of the average iodometric titre of a day's output of normal urine before the compound to be studied can be administered. The I_2 titre for normal urine is variable unless great care is taken to maintain the animals on a constant diet. On a daily *diet* of 75 g. rat cubes (J. Thorley, Kings Cross, London). the average titre was 15 ml. 0·01N-I_2; on 75 g. rat cubes + 50 g. cabbage, 33 ml.; on 75 g. of an equal mixture of bran and oats, 7·5 ml.; and on 'Diet 41' (Associated London Flour Millers), 7 ml.

The iodometric method for all urines was carried out as follows. Each day's urine was made up to 200 ml. with water. To 100 ml. them was added 50 ml. 10% (w/v) $ZnSO_4$ and 50 ml. 0·5N-NaOH, the solutions mixed rad centrifuged The clear solution (40 ml.) mixed with 5N-NaOH (10 ml.) wm heated on a sand bath (105°) for 40 min. After cooling under the tap, the solution was acidified with 27 ml. of 2N-H_2SO_4. Ethanol (25 ml.) and starch solution (10 drops) were then added and the mixture titrated in an ice bath with 0·01N-I_2. A blank titration was performed on 40 mi. of the clear solution without hydrolysis with NaOH.

The turbidimetric method

The small amounts of phenylmercapturic acid expected in benzene urines rendered the iodometric method uncertain owing to the variability of the normal blanks and the presence of other iodine-consuming compounds in benzene urine (see later). Furthermore, the more specific gravimetric method was not adaptable to small amounts. However. an observation that when mercuric chloride was added to hydrolysed benzene urines stable suspensions were formed suggested a turbidimetric method.

Identity of the mercuric mercaptide. On alkaline hydrolysis phenylmercapturic acid gives thiophenol which reacts in acid ethanolic solution with $HgCl_2$ to give phenylmercuric mercaptide, $(C_6H_5S)_2Hg$. On standing, according to Lecher (1920), an equilibrium. $2C_6H_5SHgCl \rightleftharpoons (C_6H_5S)_2Hg + HgCl_2$, is attained. The mercaptide. which forms colourless plates (decomposes at 152°), prepared from phenylmercapturic acid was found to be identical with that prepared from authentic thiophenol and in a gravimetric experiment, 49·1 mg. pure phenylmercapturic acid yielded 43·5 mg. of the mercaptide (calc. for $C_{12}H_{10}S_2Hg$, 43·0 mg.).

Turbidimetric *estimation* of *phenylmercapturic* acid. *(a) In pure solution.* The principle of the method is that phenylmercapturic acid is hydrolysed to thiophenol by alkali. then neutralized by acid. The solution is then treated with ethanol to keep thiophenol in solution. and gelatin to stabilize the turbidity developed on adding $HgCl_2$. Turbidity is then measured in the Spekker absorptiometer.

Careful studies, which need not be detailed here, were made of the effects of the concentration of gelatin, electrolyte.~,$HgCl_2$ and ethanol and of the effect of pH and temperature on the stability of the turbidity produced by the formation of the phenylmercaptide. Briefly, 0·005–0·02% gelatin gave complete stabilization, with no precipitation of the mercuric mercaptide in 1hr. Outside this range precipitation occurred. The electrolyte mixtures, H_2SO_4–Na_2SO_4, HCl–NaCl and HOAc–NaOAc were tested for their effect: the last produced unstable suspensions, the second caused slow development of opacity, whilst the first was the most suitable at a salt concentration of about 0·5N. A concentration of 0.1% $HgCl_2$ gave the best suspensions and below this concentration there was slow development and precipitation. A concentration of 16% ethanol or less did not greatly affect the stability of the suspension; below 10% the development of opacity became progressively slower and above 16% precipitation occurred. A concentration of 10% ethanol was adopted. The most stable suspensions were produced at a pH value of about 2. The best temperatures for development of opacity were between 10 and 20°; above 22° the mercaptide tended to dissolve and below 6° Na_2SO_4 tended to crystallize out of the solution.

Using 0·02% gelatin, 10% ethanol, 0·5N-Na_2SO_4, 0·1% $HgCl_2$, pH < 2 and temperature 10–20°, opacity was found to develop rapidly and to remain stable for several hours.

The description of the preparation of the standard curve which follows gives the method finally adopted.

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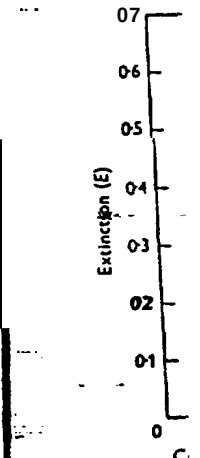

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Reagents. Phenylmercapturic acid; N-NaOH; 2N- and 5N-H_2SO_4; 1 % (w/v) aqueous $HgCl_2$; 0·2 % (w/v) gelatin; N-Na_2SO_4 and ethanol.

Method. Phenylmercapturic acid (100 mg.) was hydrolysed by heating (sand bath; 105°) in 50 ml. of N-NaOH for 40 min. The solution was cooled and made up to 200 ml. with N-NaOH. This solution contained the equivalent of 0·5 mg./ml. phenylmercapturic acid. A fourfold dilution with N-NaOH was also prepared.

Volumes of 1–12 ml. of the latter solution were added to a series of twelve 25 ml. graduated flasks each containing 2·5 ml. ethanol, 2·5 ml. 0·2 % gelatin and from 11 to 0 ml. of N-NaOH. The solutions were mixed and cooled in ice water during the addition of 3 ml. of 5N-H_2SO_4 and 2·5 ml. of 1 % $HgCl_2$. The solutions were removed to room temperature, made up to 25 ml. with water and mixed. After 10 min. the opacities were measured in the Spekker absorptiometer using 1 cm. cells and Ilford spectrum yellow filter no. 606 and heat filter H 503. The blank employed was prepared by diluting to 25 ml. a mixture of 2·5 ml. ethanol, 2·5 ml. 0·2 % gelatin, 2·5 ml. 1% $HgCl_2$, 12 ml. N-Na_2SO_4 and 2 ml. 2N-H_2SO_4. More concentrated solutions were set up by adding 5, 7·5, 10 and 12·5 ml. of the 0·5 mg./ml. solution of hydrolysed phenylmercapturic acid to 25 ml. flasks containing 7·5, 5, 2·5 and 0 ml. of N-NaOH and ethanol and gelatin solutions as before. The standard curve is given in Fig. 4.

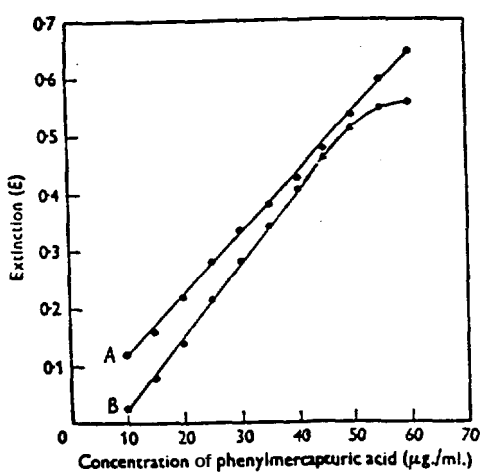

Fig. 4 Standard curves for the turbidimetric estimation of phenylmercapturic acid. *A* in pure aqueous solution, *B* in hydrolysed rabbit urine.

(b) *In urine.* If the above procedure is applied to hydrolysed rabbit urine diluted 1 *in* 6 the phenylmercuric mercaptide rapidly coagulates. Stable suspensions were readily obtained, however, if the gelatin were omitted. There is, therefore, a lyophilic substance in rabbit urine which is capable of protecting the phenyl mercuric mercaptide suspension and addition of 0·02 % gelatin acts in the same way as an excess of gelatin in pure solution and causes coagulation. The effects of pH, concentration of ethanol and $HgCl_2$ were similar to those found in pure solution.

The hydrolysed urine for the standard curve in urine was prepared by diluting a filtered 24 hr. sample of normal urine (about 100 ml.) to 200 ml. and adding 100 ml. each of 10 % $ZnSO_4$ and 0·5N-NaOH. After mixing, the solution was centrifuged and 200 ml. of the supernatant liquid hydrolysed by heating on a sand bath (105°) with 50 ml. 5N-NaOH for 40 min.

Solutions of hydrolysed phenylmercapturic acid in urine were prepared by heating phenylmercapturic acid (100 mg.) in a mixture of 40 ml. of the supernatant fraction of the urine and 10 ml. of 5N-NaOH. The solution was cooled and made up to 200 ml. with hydrolysed urine. This solution was equivalent to 0·5 mg./ml. phenylmercapturic acid. It was further diluted 1 :4 for estimation.

The standard curve in urine (see Fig. 4) was then prepared in an analogous manner to that for pure solution but omitting the gelatin, by taking 1–12 ml. of the solution of hydrolysed mercapturic acid (0·125 mg./ml.) in urine, and 11–0 ml. of the hydrolysed urine.

Recoverim of phenylmercapturic acid from urine were studied as follows. Rabbit urines (24 hr. samples) were diluted to 200 ml. and various volumes (up to 50 ml.) of a 5 mg./ml. aqueous solution of phenylmercapturic acid added, followed by 100 ml. of 10 % $ZnSO_4$ and 50 ml. N-NaOH. Each solution was diluted to 400 ml. with water, mixed and centrifuged. The supernatant solutions (160 ml.) were then heated on a sand bath with 40 ml. 5N-NaOH for 40 min. made up to 200 ml. with water and the phenylmercapturic acid estimated both turbidimetrically and iodometrically as above. If the opacities in the turbidimetric method were too great, suitable dilutions were made, prior to the formation of the phenylmercuric mercaptide, with hydrolysed urines. The I, titre of the normal diluted rabbit urine containing no added phenylmercapturic acid was also determined. In the turbidimetrio method the normal blanks were negligible.

The recoveries of phenylmercapturic acid from urine were 94 % (iodometric) and 93 % (turbidimetric) at levels of 100–250 mg./24 hr. urine and 91 % (iodometric) and 89 % (turhidirnetric) at 20 mg./24 hr. urine.

Experiments with benzene

Mercapturic acid excretion. Rabbits kept on a diet of 75 g. cubes (Associated London Flour Millers) were given benzene suspended in 50 ml. water, by means of a stomach tube made of polyvinyl plastic. Urine was collected every 24 hr. and analysed both iodometrically and turbidimetrically. For analysis each day's urine was filtered and made up to 200 ml. with water. To 100 ml. of the *diluted* urine there was added 50 ml. each of 10 % $ZnSO_4$ and 0·5N-NaOH. The solutions were mixed and centrifuged. The supernatant liquid (80 ml.) was then heated on a sand bath (at 105°) with 20 ml. of 5N-NaOH for 40 min. After cooling the solution was made up to 100 ml. with rater and 50 ml. was used for iodometric estimation and 12·5 ml. (in duplicate) for turbidimetric estimation. (During the titrations the odour of thiophenol was always evident.) For blank estimations, 40 ml. of the unhydrolysed urine, after treatment with $ZnSO_4$, was used for the iodometric blank and 10 ml. for the turbidimetric blank. Table 2 and Fig. 6 give examples of individual results, whilst Table 3 gives a summary of all results.

The accuracy of the turbidimetric method is ±5 % at the levels of phenylmercapturic acid encountered in our experiments, but it is more specific than the iodometric method and gives no measurable blank with normal urines. The higher results given by the iodometric method appear to be due to

Table 2. *Mercapturic acid excretion by rabbits receiving benzene*

Rabbit no.	Dose of benzene (g.)	Day	Urine vol. (ml.)	Iodine titre (ml. 0·01 N-I_2)	Mercapturic acid iodometric (mg.)	Mercapturic acid iodometric % of dose	Mercapturic acid turbidimetric (mg.)	Mercapturic acid turbidimetric % of dose
19	3·18	1	75	25·2*	42	0·45	22	0·2
		2	55	30·7	54	0·6	13	0·1
		3	65	12·8	12	0·1	12	0·1
19	1·59	1	50	29·8†	56	1·2	43	0·9
		2	70	18·1	28	0·6	10	0·2

* Blank titre, 7·5 ml. 0·01 N-I_2/day.
† Blank titre, 6·0 ml. 0·01 N-I_2/day.

Table 3. *Phenylmercapturic acid excretion by rabbits receiving benzene*

Rabbit no.	Benzene fed (g.)		Phenylmercapturic acid (PA) iodometrically (mg.)	PA iodometrically % of dose	PA excretion estimated turbidimetrically (mg.)	PA turbidimetrically % of dose	
16	3·20	1 g./kg.	149*	1·5	115	1·2	
16	3·29		118†	1·2	72	0·7	
17	3·08		76†	0·8	46	0·5	
17	3·27		77†	0·8	63	0·6	Average 0·81
18	3·20		126†	1·3	M	0·9	
19	3·18		108†	1·1	47	0·5	
20	3·08		124†	1·3	67	0·7	
21	3·09		141†	1·5	54	0·6	
18	1·62	0·5 g./kg.	98*	2·0	60	1·2	
19	1·59		84*	1·8	53	1·1	Average 0.98
20	1·64		70*	1·4	37	0·8	
21	1·66		97*	1·9	38	0.8)	

* Two days. † Three days.

hydroxyquinol, a known metabolite of benzene (Porteous & Williams, 1949*b*). This conclusion was reached by testing the reaction of various benzene metabolites in the methods of estimation used. Aqueous ethanolic solutions of phenol, catechol, quinol, *cis-cis-* and *trans-trans*-muconic acid acidified with dilute H_2SO_4, do not react with I_2 or give a precipitate with $HgCl_2$. Hydroxyquinol, however, does react with I_2 for we found, for example, that 100 mg. hydroxyquinol in 50 ml. 10 % aqueous ethanol acidified with dilute H_2SO_4 immediately reduced 15 ml. of 0·01 N-I_2 and then absorbed further I_2 more slowly. Hydroxyquinol does not form a precipitate with $HgCl_2$.

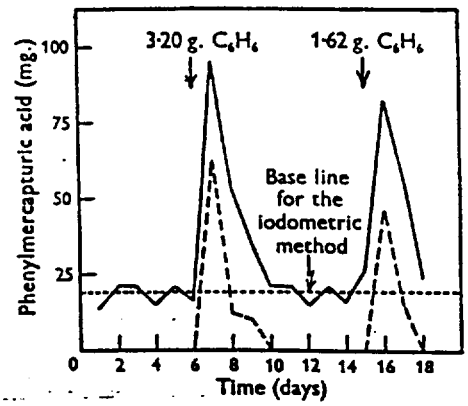

Fig. 5. The excretion of phenylmercapturic acid by rabbit no. 18 receiving benzene orally. ———, results obtained by the iodometric method; – – – –, by the turbidimetric method. The horizontal dotted line represents the average uptake of I_2 by normal rabbit urine; this uptake is not due to any phenylmercapturic acid in normal urine.

It should be pointed out, however, that the difference between the two methods is only appreciable where the mercapturic acid is a minor metabolite and is probably insignificant where mercapturic acids are formed in large amounts as in the case of the halogenobenzenes (see Stekol, 1936; Spencer & Williams, 1950*a*, *b*).

DISCUSSION

The present paper shows that phenylmercapturic acid is a metabolite of benzene in the rabbit. Estimation by the more specific turbidimetric method showed that about 0·8 % of the benzene fed at a dose level of 1 g./kg. and about 1% at the dose level of *0.6* g./kg. is excreted as phenylmercapturic acid. Using figures obtained in an earlier paper (Parke & Williams, 1960) it can be calculated that, allowing for the benzene eliminated unchanged, 2·2 % of the benzene catabolized is excreted as a mercapturic acid at a does of 1g./kg. and 1.7 % at 0·5 g./kg.

We can now add one further figure to our balance sheet for the metabolism of benzene at 0·5 g./kg.

	%
Benzene eliminated unchanged	39
Benzene oxidized (and excreted as glucuronide and ethereal sulphates)	21
Benzene eliminated as phenylmercapturic acid	1

preliminary results have shown that another metabolite of benzene, muconic acid, accounts for at least 8 further 1% of the dose.

SUMMARY

1. The excretion of phenylmercapturic acid by rabbits receiving benzene has been studied.

2. The presence of phenylmercapturic acid in urine after administration of benzene has been shown by developing a specific turbidimetric method of estimation, based on the formation of phenylmercuric mercaptide from phenylmercapturic acid by alkaline hydrolysis.

3. The iodometric method for the determination of mercapturic acid has been shown to be influenced by diet and by hydroxyquinol, a metabolite of benzene, and may give high results when small amounts of mercapturic acids have to be estimated in urine.

4. By the turbidimetric method about 1 % of the benzene fed at a dose level of 0·5 g./kg. and about 0·8 % at 1 g./kg. was excreted as phenylmercapturic acid, which was found in the urine for 2-3 days after dosing.

5. Some aryloysteines and mercapturic acids have been synthesized and their ultraviolet absorption spectra recorded.

The expenses of this work were in part defrayed by a grant from the Medical Research Council.

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Esterification of the Carboxyl Groups in Wool

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(*Received* 18 *July* 1950)

The carboxyl groups in wool were partially methylated with diazomethane by Rutherford, Patterson & Harris (1940) and with methyl sulphate and methyl iodide by Blackburn, Carter & Phillips (1941). In both cases titration showed that not more than 50 % of the carboxyl groups were esterified and that the reaction was non-specific and methylation of other groups occurred. Blackburn & Phillips (1944) reported that a mixture of methanol and acetic anhydride esterified the carboxyl groups, in part at least, but that some of the terminal amino groups in lysine and the peptide imino linkage were simultaneously acetylated. Fraenkel-Conrat & Olcott (1945) showed that anhydrous alcohols containing a catalytic quantity of mineral acid reacted specifically with the carboxyl groups in proteins without modifying any other group. Methanol containing 0·1 N-hydrochloric acid was used by Blackburn & Lindley (1948) to introduce 0·8 % of methyl groups into wool; this is equivalent to masking 65 % of the carboxyl groups.

Fraenkel-Conrat (1944) found that 1:2-epoxides readily esterified carboxyl groups in proteins and showed that, although epoxides also combine with amino groups, their basic nature is not suppressed and the reaction is therefore suitable for studying the effects of blocking the carboxyl group on the properties of proteins. Schlack (1947) discovered that the affinity of acidic dyes for wool was greatly increased by reacting the latter with an epoxide, and it will be shown that this results from the suppression of the ionization of the carboxyl groups.

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