intact cells and extracts. The number of carbon atoms causing maximum acceleration is sixteen in the case of cells and from sixteen to eighteen in the case of extracts.

2. A parallelism was observed between the ability of cationic detergents to form micelles in aqueous solution, and their ability to accelerate the decarboxylation of glutamate and glutamine. Maximum acceleration of the decarboxylase within the homologous series approximately coincided with the 'critical point of micelle formation' as determined by the colour change of indicators.

3. Anionic surface-active agents inhibited the decarboxylation of glutamate and glutamine. Non-ionic surface-active agents and fatty acids were without effect.

The author wishes to express his thanks to Prof. H. A. Krebs, F.R.S., for help and criticism and to Mr D. H. Williamson for technical assistance. Acknowledgements are due to Dr S. Ellingsworth, Messrs Imperial Chemical Industries (Pharmaceuticals) Ltd., for the compounds in Table 4 and for the ethanolamine esters and octadecyl amidine in Table 5. Thanks are also due to Messrs Glaxo Laboratories Ltd., for the three amines in Table 5.

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# Studies in Detoxication

## 30. THE METABOLISM OF BENZENE. (a) THE DETERMINATION OF BENZESE. (b) THE ELIMINATION OF UNCHANGED BENZENE BY RABBITS

BY D. V. PARKE AND R. T. WILLIAMS
*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*

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In general, the metabolism of benzene proceeds along four paths, namely, (1) oxidation to phenols, (2) elimination unchanged, (3) formation of phenylmercapturic acid, and (4) ring fission to muconic acid and other products. The first of these was quantitatively studied by Porteous & Williams (1949), who concluded that in the rabbit an average of 21% or, at most, about 30% of an oral dose of benzene at the level of 500 mg./kg. was metabolized to phenols. At least 70% of the administered benzene must take other paths. The present work deals with the second path, the elimination unchanged. It is found that at a level of 500 mg./kg. about 40% of orally administered benzene is eliminated unchanged through the lungs. Thus the first two paths can account for at least 60% or, taking the higher values of some experiments, up to 80–90% of the dose.

That orally administered benzene might be excreted unchanged through the lungs was first suggested by Schultzen & Naunyn (1867), but Munk (1876) doubted this possibility. In 1883, Nencki & Sieber, after experiments on men, dogs and rabbits, expressed the view that approximately one-third of benzene was excreted as phenol conjugates, one-third as catechol and quinol and the remaining third must come out unchanged. The first quantitative experiments on the elimination of unchanged benzene were carried out by Lehmann, Stohr, Kleiner & Gundermann (1910). Tracheotomized rabbits were allowed to inhale benzene-air mixtures, and from an analysis of the inspired and expired air, the amount of benzene absorbed was ascertained. During 1 hr. after benzene ceased to be inhaled, 25% of the absorbed benzene was exhaled unchanged. When the period was increased to 4 hr. then 34% was exhaled,

but elimination over longer periods was not studied. In man, 6% was exhaled during 15 min. after ceasing to inhale benzene. Peronnet (1935) found that guinea pigs inhaling benzene excreted 4 mg. through the lungs in 5 min., but the amount of benzene inspired was not known. There appears to be only one observation on the elimination of free benzene through the kidneys. Drummond & Finar (1938) stated that free benzene can be detected in the urine of rabbits only when the dose exceeds 3 ml.

Thus the only relevant determinations of the elimination of free benzene are those of Lehmann *et al.* (1910), but unfortunately their longest experiments only lasted 4 hr. and were concluded whilst excretion was at the appreciable rate of 3% of the dose/hr. Our experiments were carried out until only traces of benzene were found in the expired air and the benzene was administered in a more controlled manner by the oral or parenteral routes.

## EXPERIMENTAL

### A. *The determination of benzene*

Of several methods available for the determination of benzene the method of Schrenk, Pearce & Yant (1935) is very sensitive, and we directed our attention to this.

Peltzer (1933) described colour *reactions* between *m*-dinitrobenzene and ketones in the presence of concentrated alkalis, and from these Pearce, Schrenk & Yant (1936) developed a micromethod for the determination of benzene in blood and urine. In this method benzene is blown in a stream of air into a mixture of concentrated nitric and sulphuric acids, and the *m*-dinitrobenzene formed is then extracted from the neutralized mixture with methyl ethyl ketone. A purple colour is then developed by adding aqueous 40% (w/v) sodium hydroxide. Baernstein (1943), Alekseeva (1940) and Bykhovskaya (1945) described modifications of the method.

The method of Schrenk *et al.* (1935) is sensitive to 1 mg. of benzene with an accuracy of 10%. However, a long time (1·5 hr.) is necessary for development of the colour, which is produced in a two-phase system. We have now investigated the method in detail and studied aspects not appreciated by earlier investigators. An analytical procedure has resulted in which maximum colour production is almost instantaneous in a one-phase system without loss of accuracy or sensitivity. Our investigations have covered the influence of the nature of the ketone and of the alkali, the concentration of ethanol and of water, and the presence of *o*- and *p*-dinitrobenzene upon the production of the colour.

The mechanism of colour development, discussed by Baernstein (1943), is that, in the presence of alkali, the acid forms of certain nitro compounds condense with the enol forms of ketones *to* form coloured quinonoid structures.

(1) ***The choice of ketone.*** In order *to* extract *m*-dinitrobenzene from the neutralized nitrating mixture, whilst avoiding such solvents as ether, a ketone incompletely miscible with water was necessary. With aliphatic ketones it was found that the intensity of the colour diminished with increasing number of C atoms, and we were immediately limited to methyl ethyl ketone and *cyclo*hexanone. Since the colour developed in *cyclo*hexanone was much more sensitive to alkali concentration than that in methyl ethyl ketone (*Fig.* 1), the latter ketone became the obvious choice.

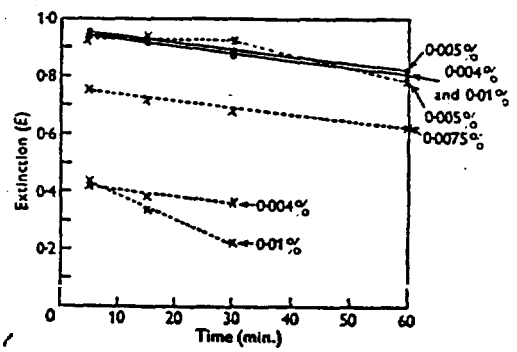


Fig. 1. The influence of alkali concentration on the stability of the colour formed between *m*-dinitrobenzene and methyl ethyl ketone or cyclohexanone. The data for this figure was obtained by taking 2 ml. of *m*-dinitrobenzene solution (40 μg./ml.) in methyl ethyl ketone or *cyclo*hexanone and adding 0·1 ml. of ethanolic NaOH (concentration 0·4, 0·5, 0·75 and 1% (w/v)) and making up to 10 ml. with the ketone. Readings were taken at intervals with the Spekker absorptiometer. Final alkali concentrations are given on the figure. Precipitation occurred in some of the *cyclo*hexanone solutions. Full lines, methyl ethyl ketone; broken lines, *cyclo*hexanone.

(2) ***The nature and concentration of the alkali.*** The strong organic bases piperidine and benzylamine gave no colour with *m*-dinitrobenzene in ray of the ketones tested. Na ethoxide in *dry* ethanol gave an unstable violet solution which rapidly turned brown owing to reaction between the ketone and ethoxide. In order to avoid obtaining a two-phase system which results from the use of aqueous alkali we tried ethanolic NaOH and KOH. In general, it was found that the colour was more stable with NaOH than with KOH. In methyl ethyl ketone the colour is completely destroyed in less than 5 min. if the alkali concentration is greater than 0·5%. Between 0·05 and 0·5%, there may be precipitation of alkali. Below 0·002% alkali the full colour is not developed. Some of our results are shown in Fig. 2 which again shows the difference between cyclohexanone and methyl ethyl ketone. The concentration of alkali finally adopted was 0·005% NaOH.

(3) ***The effect of ethanol.*** The stability of the colour is also affected by ethanol, some of which had to be used because the best alkali reagent found was ethanolic NaOH and some ethanol was necessary to maintain a homogeneous system. The effect of ethanol concentration is shown in Fig. 3. It was found that if the ethanol concentration is much below 1%

precipitation of the alkali occurred, whereas if it exceeds 2% the maximum development of colour is impaired. Methanol can be substituted for ethanol.

(4) *Effect of water.* Since, in the determination of benzene, the *m*-dinitrobenzene formed by nitration has to be extracted with methyl ethyl ketone from an aqueous solution, the influence of the concentration of water on the development of the colour *had* to be investigated. The results are

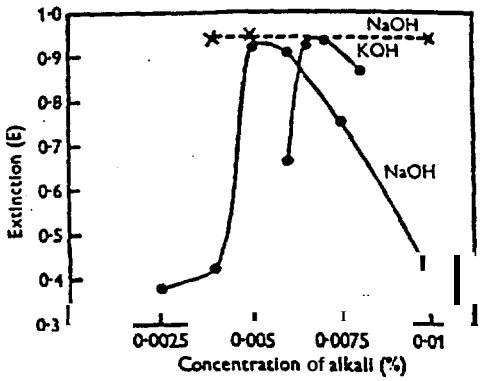


Fig. 2. Effect of NaOH or KOH concentration on the intensity of the colour from *m*-dinitrobenzene and methyl ethyl ketone (broken line) or *cyclo*hexanone (full lines). Solutions prepared as for Fig. 1 and read 5 min. after mixing.

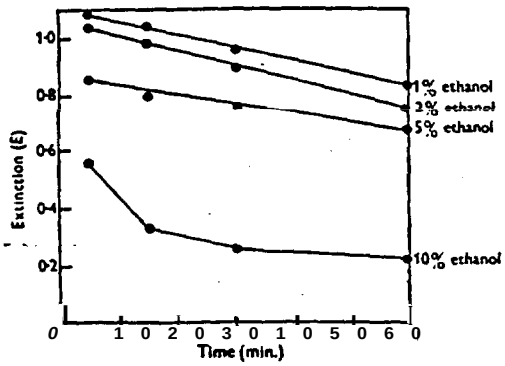


Fig. 3. Effect of ethanol concentration. The curves were obtained by adding to 1 ml. of *m*-dinitrobenzene in methyl ethyl ketone (100 μg./ml.), 0·1 ml. of 1%, *0.2* ml. of 0·5%, 0·5 ml. of 0·2% and 1 ml. of 0.1% ethanolic NaOH respectively and then making up to 10 ml. with the ketone. *The* colour was then read in the absorptiometer *at intervals.* Ethanol concentrations are given on the figure. The alkali concentration was constant throughout.

summarized in Fig. 4. Water in a concentration up to 0·5% has no influence on the colour. Above this concentration, the colour is slower to develop and there is a loss of intensity. Since the solubility of water in methyl ethyl ketone is 10%, the original ketone extract must be diluted at least 1 : 20 with ketone prior to colour development to ensure that the concentration of water is less than 0·5%.

(a) *The influence of o- and p-dinitrobenzene.* In the present work the nitration of benzene was carried out at room temperature (18–25°) with conc. $H_2SO_4$ and fuming $HNO_3$, and significant amounts of *o*- and *p*-dinitrobenzene are formed. From the work of Wyler (1931), it appears that about 93–94% of *m*-, 4–5% of *o*- and 2·0–2·5% of *p*-dinitrobenzene are produced under our conditions. We therefore investigated the colour produced by the latter two isomers with alkali in the presence of methyl ethyl ketone. Pure samples of each were prepared (*o*-, m.p. 116·5° and *p*-, m.p. 174°). Solutions of the three isomers were made up in various concentrations in methyl ethyl ketone, by adding 0·5–5·0 ml.

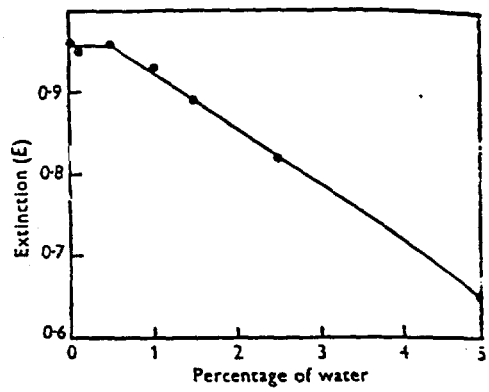


Fig. 4. Effect of water concentration. Varying amounts of a 10% solution of water in methyl ethyl ketone were added to 2 ml. of *m*-dinitrobenzene (40 μg./ml.) in the ketone. The colour was developed with 1 ml. of 0·05% ethanolic NaOH, volume made up to 10 ml. with the ketone and readings taken after 5 min.

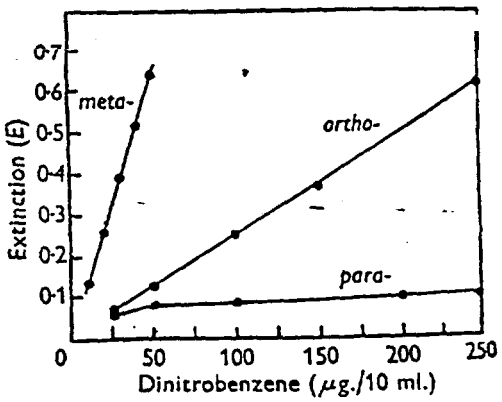


Fig. 5. A comparison of the colour intensities produced by the action of ethanolic NaOH on solutions of *o*-, *m*- and *p*-dinitrobenzene in methyl ethyl ketone.

of a 50 μg./ml. standard solution to 10 ml. flasks and diluting with the ketone. The colour was developed by adding 1 ml. of the 0·05% 'alkali reagent' (see below) and making up to 10 ml. with the ketone. The colours were read, after developing 5 min. in the dark, in a Spekker absorptiometer using a yellow-green filter (Ilford 605) and the heat filter (no. H 503). A blank was made up by diluting 1 ml. of the alkali reagent to 10 ml. with methyl ethyl ketone. The *o*-isomer gave a blue colour. The *p*-isomer also gave a blue colour which rapidly faded in 2–3 min. The relative intensities of the colours are shown in Fig. 5.

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...from this graph it can be ... likely to be encountered ... isomer present in the nitr... a colour equivalent to ...robenzene. The 2% of *para*- ... an equivalent of only 0·2% ... fore clear that the maxi... able as *m*-dinitrobenzene ... 95%. In direct nitration ... recoveries of nearly 95% ... of benzene were, the ... ation of *o*- and *p*-dinitrobe... found by 100/95. It should ... of benzene by passage w... by direct addition. There ... is lost as a result of its ... to the nitrating mixture.

Table 1. *The recovery of b... to a nitrating*

Method *A*. Benzene slowly a... at 20°. The dinitrobenze... alized mixture with meth... and colorimetrically as descri... aerated from a 250 m... ating mixture at 20° during

B

| | Benzene added (mg.) | (mg.) |
|---|---|---|
| ...ded | 465 | 435 |
| ...d | 455 | 413 |
| ...d | 328 | 308 |
| ...d | 328 | 303 |
| ...d | 492 | 466 |
| ...d | 953 | 902 |
| ...d | 937 | 882 |

*Construction of the standar... curve*

*Reagents. Methyl ethyl keto...* $CaCl_2$ and distilled, the fractio...

*Dinitrobenzene.* A comme... recrystallized from etha... white needles, m.p. 89°. A ... methyl ethyl ketone appeared ... were prepared by di... benzene. Thiophene-free b... $CaCl_2$ and distilled, ar...

*...alkali reagent.* The stoc... dissolving 0·5 g. of NaO... ...ing and making up to 1... ...reagent' was freshly p... ...ng the stock solution (f... ...methyl ethyl ketone. Thi... ...burning brown and th... *...nitrating mixture* cons... ...(A.R.) and fuming H... *...centrated alkali.* For ne... ...(w/v) aqueous solutio...

From this graph it can be calculated that, in the concentrations likely to be encountered in this work, the 4–5% of *ortho* isomer present in the nitrated benzene mixture will contribute a colour equivalent to approximately 1% of *m*-dinitrobenzene. The 2% of *para* isomer present will contribute an equivalent of only 0·2% of *m*-dinitrobenzene. It is therefore clear that the maximum amount of benzene recoverable as *m*-dinitrobenzene using this colour reaction is 94–95%. In direct nitration experiments we obtained maximum recoveries of nearly 95% (Table 1). All values for recovery of benzene were, therefore, corrected for the formation of *o*- and *p*-dinitrobenzene by multiplying the values found by 100/95. It should be noted from Table 1 that recovery of benzene by passage with a stream of air is better than by direct addition. There is little doubt that some benzene is lost as a result of its volatility when it is added directly to the nitrating mixture unless great care is taken.

Table 1. *The recovery of benzene added directly to a nitrating mixture*

(Method *A*. Benzene slowly added to 20 ml. of nitrating mixture at 20°. The dinitrobenzenes were extracted from the neutralized mixture with methyl ethyl ketone and estimated colorimetrically as described in the text. Method *B*. Benzene aerated from a 250 ml. Dreschel bottle into the nitrating mixture at 20° during 4 hr.)

| Method | Benzene added (mg.) | Benzene recovered (mg.) | Benzene recovered (%) | Corrected for *o*- and *p*-dinitrobenzene (%) |
|---|---|---|---|---|
| *A* | 465 | 435 | 93·5 | 98·4 |
| *A* | 455 | 415 | 91·3 | 96·1 |
| *A* | 328 | 308 | 93·9 | 98·8 |
| *A* | 328 | 305 | 93·0 | 97·9 |
| *A* | 492 | 466 | 94·7 | 99·7 |
| *B* | 953 | 902 | 94·6 | 99·6 |
| *B* | 937 | 882 | 94·1 | 99·1 |

*Construction of the standard curve for m-dinitrobenzene*

*Reagents. Methyl ethyl ketone* was dried over anhydrous $CaCl_2$ and distilled, the fraction of b.p. 79–80° being used. *m-Dinitrobenzene*. A commercial sample, m.p. 75–78°, was recrystallized from ethanol. This material formed long needles, m.p. 89°. A stock 1% (w/v) solution in methyl ethyl ketone appeared to keep indefinitely. Standard solutions were prepared by diluting this. *Benzene*. Thiophene-free benzene was dried over anhydrous $CaCl_2$ and distilled, and the fraction of b.p. 80–81° used. *Alkali reagent*. The stock alkali solution was prepared by dissolving 0·5 g. of NaOH (A.R.) in absolute ethanol, cooling and making up to 100 ml. with ethanol. The 0·05% 'alkali reagent' was freshly prepared for each experiment by diluting the stock solution (filtered if necessary) with 9 vol. of methyl ethyl ketone. This reagent deteriorates in a few days, turning brown and thus producing a high blank. *Nitrating mixture* consisted of equal volumes of conc. $H_2SO_4$ (A.R.) and fuming $HNO_3$ (sp.gr. 1·5; A.R.). *Concentrated alkali*. For neutralizing the nitrating mixture a 40% (w/v) aqueous solution of NaOH (B.P.) was used.

The standard curve (Fig. 6) for *m*-dinitrobenzene was constructed by adding to a series of 10 ml. graduated flasks 1–8 ml. of a 10 μg./ml. solution of *m*-dinitrobenzene in methyl ethyl ketone. These solutions were diluted to nearly 9 ml. with methyl ethyl ketone and then treated with 1 ml. of the 0·05% 'alkali reagent'. After making up to 10 ml. with

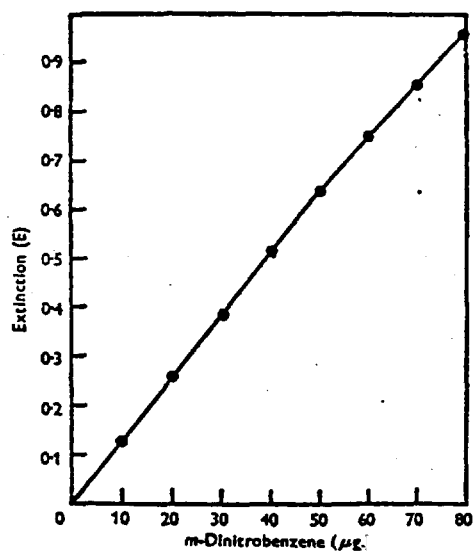



Fig. 6. Calibration curve for *m*-dinitrobenzene. Constructed as described in the text, p. 239.

the ketone and mixing, the colour was read, after 5 min. standing, in the Spekker absorptiometer using the colour filter Ilford no. 605 and heat filter no. E 503 and a 1 cm. cell. A standard blank was prepared by mixing 1 ml. 'alkali reagent' with 9 ml. methyl ethyl ketone. The curve obtained is linear from 0 to 40 μg./10 ml. and approximately linear up to 80 μg./10 ml.

*Recovery of m-dinitrobenzene from the nitrating mixture*

Various amounts of pure *m*-dinitrobenzene were added to 20 ml. of the nitrating mixture and left for 2 hr. The mixture was diluted with an equal volume of water and

Table 2. *Recovery of added m-dinitrobenzene from a nitrating mixture*

| Added to 20 ml. of nitrating mixture (mg.) | *m*-Dinitrobenzene Recovered (mg.) | Recovered (%) |
|---|---|---|
| 20 | 19·8 | 99 |
| 20 | 20·0 | 100 |
| 50 | 50·1 | 100·2 |
| 50 | 49·9 | 99·8 |
| 100 | 100·2 | 100·2 |
| 500 | 502·0 | 100·4 |

neutralized with the 40% aqueous NaOH, the temperature being kept below 30° under the tap. A few drops of the alkali were then added in excess. The mixture, which had a volume of about 100 ml., was extracted with 2 × 15 ml. and

2 × 10 ml. of methyl ethyl ketone and the extract made up to 50 ml. Suitable portions were then taken and the colours developed with the 'alkali reagent' as above. The recovery of *m*-dinitrobenzene in the range 20–500 mg. was accurate to 1% (Table 2).

### B. *Experiments on the elimination of unchanged benzene*

*Animals.* Chinchilla rabbits, 2–3 kg. in weight, were used.

*The* animal chamber (Fig. 7) consisted of a Perspex tank of dimensions 60·5 x 33·5 x 22·5 cm. with an internal volume of 45·6 L The lid of the tank was removable and was secured by twelve wing-nuts to the tank walls with a rubber diaphragm in between to ensure airtightness. This diaphragm

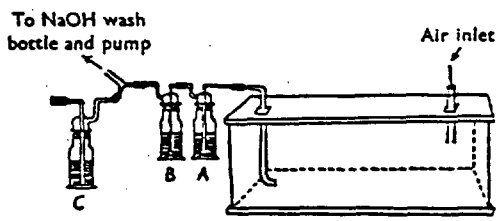



Fig. 7. Diagram of repiration tank and absorption train (see text, p. 240 for description). *A*, *B* and *C*, Dreschel bottles containing nitrating mixture. *C* is the control bottle which shows presence or absence of benzene in the atmosphere.

was painted with collodion so that no rubber surfaces were exposed to benzene. The lid was also equipped with three glass tubes fitted with ground glass stoppers lubricated with silicone high vacuum grease which is inert to benzene, and two copper air tubes, all these being affixed to the lid in rubber cushions whose exposed surfaces were painted with collodion. The tank also had a removable, perforated aluminium floor, enabling the animals to sit out of contact with any urine excreted during the experiment. To increase the draught at the air inlet and produce a current of air across the tank, air was admitted through a 3 mm. glass tube fitted into the glass tube diagonally opposite the copper outlet tube. The other copper air tube was sealed.

*The absorption train* (Fig. 7). This consisted of two 50 ml. Dreschel gas bottles in series with ground glass heads each containing 20 ml. of 2 mm. glass beads and 20 ml. of nitrating mixture. A third bottle, similarly filled, was connected in parallel and was used to check the in-going air for benzene.

The three bottles were connected by a glass Y-piece to a wash-bottle containing 20% (w/v) aqueous NaOH [illegible] acid vapours and thence to the pump. To prevent [illegible] benzene through connexions, the latter were made of 'Portex' polyvinyl plastic tubing, which also resisted attack by nitrous fumes. All ground joints were lubricated with silicone high vacuum grease which is not permeable to benzene.

During prolonged aeration, the nitrating mixture becomes depleted of the oxides of nitrogen and may become so weakened as to produce only mononitrobenzene. In all experiments, therefore, the Dreschel bottles were changed every 2 hr. by fresh ones containing a new charge of beads and nitrating mixture.

*The recovery of benzene from the tank.* Known [illegible] of benzene in a weighing bottle (attached to a thread [illegible] inside the tank) were introduced into the tank and the lid secured and connected to the absorption train. The benzene in the bottle was scattered inside the tank by pulling the attached thread, the end of which protruded through one of the orifices in the lid. The tank was now aerated at the rate of 20 l./hr. (measured with a flowmeter) until the benzene concentration reached a negligible value. This required approx. 12 hr. Since the capacity of the tank is about 45 l., aeration at 20 l./hr. removes 40% of the original atmosphere in 1 hr. and 99–100% in about 12 hr.

The contents of the Dreschel bottles were worked up as follows. After disconnecting they were allowed to stand for 1 hr. The contents were then poured into a beaker and the nitrating mixture decanted from the beads into a conical flask. The Dreschel bottle and head were repeatedly rinsed with water, the washings being added to the beads in the beaker to rinse them as well. The washings were now added to the nitrating mixture, the temperature being kept below 30°. The bottle and beads were finally rinsed with small volumes of 40% (w/v) aqueous NaOH which were added to the nitrating mixture with cooling. The mixture was then neutralized with 40% (w/v) NaOH and a few drops of the latter were added in excess. The final volume of the solution was about 100 ml. This was extracted (at 25–30° to prevent precipitation of salts) with 2 × 15 ml. and 2 × 10 ml. methyl ethyl ketone and the extract made up to 50 ml. Suitable portions were then taken and the colour developed as above.

Table 3 shows that 1 or 0·1 g. can be recovered almost quantitatively by aeration from the tank. The last figure in this table was obtained by adding benzene to the tank when it contained the warm intact body of a freshly killed rabbit. This experiment was carried out because it was possible that a considerable part of the exhaled benzene might be absorbed

Table 3. *Recovery of benzene from the respiration chamber*

(Temperature of nitrating mixture, 20–25°.)

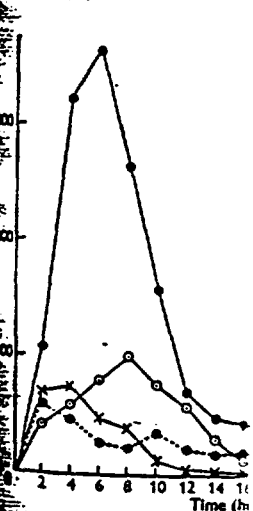
| Benzene in chamber (g.) | Rate of aeration (l./hr.) | Duration of aeration (hr.) | Benzene recovered (g.) | Benzene recovered (%) | Corrected for *o*- and *p*- isomers (%) |
|---|---|---|---|---|---|
| 1·023 | 20 | 14 | 0·967 | 94·5 | 99·5 |
| 0·989 | 20 | 14 | 0·936 | 94·6 | 99·6 |
| 0·958 | 30 | 10 | 0·913 | 95·3 | 100·3 |
| 0·1024 | 30 | 10 | 0·0965 | 94·2 | 99·2 |
| 0·0962 | 15 | 18 | 0·0905 | 93·9 | 98·9 |
| 0·0178 | 20 | 10 | 0·0152 | 85·5 | 89·9 |
| 0·946* | 20 | 14 | 0·857 | 90·6 | 95·3 |

* With a freshly killed rabbit in the chamber (see text, above).

Table

| Rabbit No. | Dose (mg./kg.) |
|---|---|
| 8 | 250 |
| 10 | 250 |
| 12 | 250 |
| 1 | 500 |
| 8 | 500 |
| 9 | 500 |
| 10 | 500 |
| 11 | 500 |
| 12 | 500 |
| 8 | 500* |
| 12 | 500* |
| 9 | 1000 |
| 10 | 1000 |
| 12 | 1000 |

[illegible] fur of the animal and re- [illegible] Lazarev, Brusilovskaya & [illegible] this experiment that, altho[illegible] the dead animal was prese[illegible] small one.




The elimination in the un[illegible] injected benzene by rabbit [illegible] zene eliminated (uncorrec[illegible] ed against time. ●——●, [illegible] kg. orally; ×——×, 0·25 [illegible] kg. injected.

[illegible] blank was always negligi[illegible] hr. aeration. Benzene a[illegible] laboratory during an experi[illegible] nearby was being polished [illegible] and on this occasion the bla[illegible] of benzene.

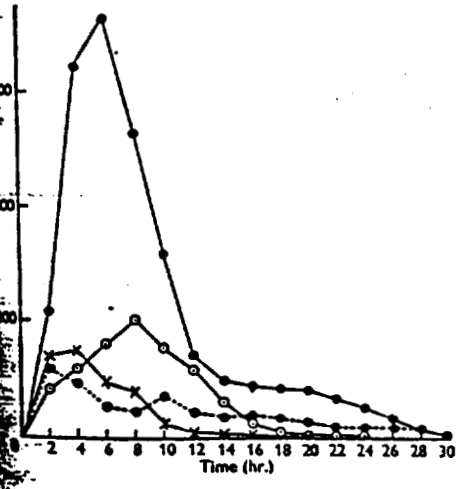
[illegible] chem. 1950, 46

Table 4. *The elimination of unchanged benzene in the expired air of rabbits receiving benzene orally*

| Rabbit no. | Dose (mg./kg.) | Duration of experiment (hr.) | Percentage of dose recovered (corrected for *o*- and *p*-dinitrobenzene) | Average (%) | Benzene catabolized (by difference) (%) | Benzene catabolized (by difference) (mg./kg.) |
|---|---|---|---|---|---|---|
| 8 | 250 | 16 | 27·6 | 37·3 | 62·7 | 157 |
| 10 | 250 | 18 | 45·8 | | | |
| 12 | 250 | 20 | 38·5 | | | |
| 1 | 500 | 30 | 38·7 | 39·1 | 60·9 | 305 |
| 8 | 500 | 26, 22 | 31·7, 40·4 | | | |
| 9 | 500 | 28 | 43·1 | | | |
| 10 | 500 | 22 | 55·9 | | | |
| 11 | 500 | 30 | 37·8 | | | |
| 12 | 500 | 24, 22 | 39·1, 26·3 | | | |
| 8 | 500* | 30 | 33·6 | 31·0 | 69·0 | 345 |
| 12 | 500* | 30 | 28·2 | | | |
| 8 | 1000 | 28 | 56·1 | 64·3 | 35·7 | 357 |
| 10 | 1000 | 28 | 75·1 | | | |
| 12 | 1000 | 30 | 61·7 | | | |

* Injected.

by the fur of the animal and re-absorbed through the skin (Lazarev, Brusilovskaya & Lavrov, 1931). It is clear from this experiment that, although recovery is a little less than the dead animal was present, the error introduced is only a small one.




The elimination in the unchanged state of ingested injected benzene by rabbit no. 12. The actual amount benzene eliminated (uncorrected) in each 2 hr. period is ted against time. ●——●, 1 g./kg. orally; ⊙——⊙, g./kg. orally; ×——×, 0·25 g./kg. orally; ●- - - -●, g./kg. injected.

blank was always negligibly low, being less than hr. aeration. Benzene and toluene were not used laboratory during an experiment. On one occasion a nearby was being polished with a mixture containing and on this occasion the blank reached the equivalent of benzene.

*Elimination of ingested and injected benzene by rabbits*

The experiments were carried out at three dose levels, 0·25, 0·5 and 1·0 g./kg. The rabbits were kept on a constant diet of 200 g. each of bran and cabbage a day prior to the experiments. For oral administration, the benzene was given suspended in 50 ml. water by a stomach tube made of 'Pertex' polyvinyl plastic which does not absorb benzene

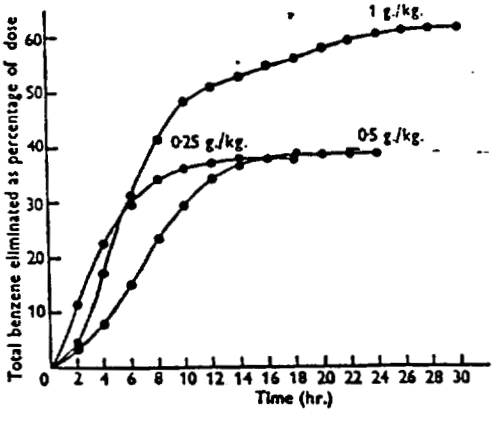



Fig. 9. The elimination in the unchanged state of orally administered benzene by rabbit no. 12. Total elimination, as percentage of dose (corrected), plotted against time.

as does rubber tubing. In the injection experiments only one dose level (0·5 g./kg.) was used and the benzene was injected intraperitoneally. Immediately after administration of benzene the rabbits were placed in the tank which was sealed and connected with the absorption train. The first absorption bottle was removed every 2 hr. and the contents analysed for *m*-dinitrobenzene, until only 0·1% of the dose was found during a 2 hr. period. Most experiments lasted 20–30 hr., and at the end the contents of the second absorption bottle

were analysed for dinitrobenzene. The second bottle rarely contained more than 0·2% of the dose of benzene. The results are summarized in Table 4 and in Figs. 8 and 9, the results for rabbit no. 18 are expressed graphically. It is to be noted that the elimination of unchanged benzene takes longer when it is injected than when fed by mouth.

### *Excretion of unchanged benzene in the urine*

First we required to know whether benzene added to urine could be recovered by aeration. Accordingly, 165 mg. of benzene were added to 100 ml. freshly voided rabbit urine in a Dreschel bottle and thoroughly mixed. A current of air was then passed for 6 hr. at 20 l./hr. through the urine into the absorption circuit and the *m*-dinitrobenzene estimated as before. The benzene recovered was 147 mg. or 94% (corrected value).

It was noticed on some occasions that when a rabbit in the tank voided urine there was a jump in the benzene elimination. This suggested that some unchanged benzene appears in the urine. Rabbits were therefore given 500 mg./kg. of benzene orally, placed in metabolism cages and the urine collected in Dreschel bottles placed beneath the funnels supporting the cage. As soon as urine was passed its benzene content was estimated by aeration. It was found that at this dose level no unchanged benzene appeared in the urine.

## DISCUSSION

The elimination of unchanged benzene by the rabbit at a dose level of 500 mg./kg. appears to be entirely via the lungs, no detectable benzene appearing in the urine. The elimination at different dose levels is summarized in Table 4. Since the animals were kept in an enclosed chamber it is possible that our estimates of the unchanged benzene are slightly low, because the animal will rebreathe and absorb some of the benzene it has eshaled. At the dose level of 500 mg./kg. the average concentratiou of benzene in the chamber is about 4 mg./l. at the period of maximum excretion. However, basing our calculations on the experimental findings of Lehmann *et al.* (1910), we have assessed the amount of benzene reabsorbed during an experiment as being not more than 5–10 mg., despite the fact that during maximum excretion the total amount of benzene breathed in would be 120–140 mg./hr. Our results on this basis would not be more than 0·5% low.

The striking feature of Table 4 is that at the high dose level of 1 g./kg. a much greater percentage of the dose is eliminated unchanged than at the lower levels. This indicates that there is a limit to the amount of benzene which the rabbit can metabolize. This shows more clearly in the last column of Table 4. The rabbit therefore deals with benzene by eliminating it unchanged and by catabolizing it, i.e. by oxidation. The second way is, however, quantitatively limited, and it appears from Table 4 that it does not exceed about 300–350 mg. benzene/kg. of rabbit in about 24 hr. Thus up to 500 mg./kg. about two-fifths of the benzene administered comes out unchanged and three-fifths is catabolized. At the higher dose level of 1 g./kg. the animal catabolizes benzene at about 350 mg./kg. but eliminates two-thirds is eliminated unchanged and one-third is catabolized. Thus, on average figures, 11·2% of benzene at 500 mg./kg. is oxidized and excreted as glucuronides, 9·5% as ethereal sulphates (Parke & Williams, 1949) and 39·1% is eliminated unchanged. On these figures some 60% of the benzene administered can be accounted for. It is felt that glucuronides, ethereal sulphates and unchanged benzene account for most of the benzene fed to rabbits, and, if all the estimations of these metabolites could be carried out simultaneously on the same animals, we have little doubt that they would account for 80–90% of the benzene administered. The problem of getting a balance sheet for the metabolism of benzene is, however, one of great difficulty owing to the scatter of the results: the extreme and average values obtained so far are as follows:

| | (%) | Average (%) |
|---|---|---|
| As glucuronides | 4·2–22·7 | 11·2 |
| As ethereal sulphates | 5·9–16·3 | 9·5 |
| As phenol | 6·3–17·0 | 9·2 |
| Unchanged benzene | 26·0–56·0 | 39·1 |

Other aspects of the metabolism of benzene, such as the formation of muconic acid and phenylmercapturic acid, are being actively pursued.

## SUMMARY

1. The method for the determination of phenol based on nitration to *m*-dinitrobenzene and development of a colour by the interaction of the dinitrobenzene and a ketone in the presence of alkali has been carefully investigated. The influence of alkali, ethanol, water, nature of the ketone and of *p*-dinitrobenzene on colour development has been studied, and a rapid and improved method has been developed.

2. The elimination of unchanged benzene by rabbits receiving benzene by mouth and by injection has been investigated. By placing rabbits, receiving benzene, in a respiration chamber, the amount of unchanged benzene eliminated was estimated by aeration into a nitrating mixture and subsequently determining the *m*-dinitrobenzene produced.

3. The benzene was eliminated during 15–30 hr., about 40% being recovered unchanged at the levels of 0·25 and 0·6 g./kg. and 64% at 1 g./kg.

4. The results have been discussed in relation to earlier studies on benzene and it has been shown that the ability of the rabbit to catabolize benzene is limited to roughly 300–400 mg./kg./day.

5. The benzene eliminated unchanged plus that oxidized and excreted as oxygen-conjugates accounts for at least 60% of the administered benzene.

We wish to express our thanks to Prof. J. M. Peterson and Dr John Pryde of University College, Cardiff, for the loan of the Perspex respiration tank used in this work. We are also grateful to Prof. Sir Alexander Fleming, F.R.S., for animal accommodation, and to Prof. A. St G. Huggett for the loan of a flowmeter.

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# Studies in Detoxication

## 31. THE ISOLATION OF *m*- AND *p*-CYANOPHENOLS AS METABOLITES OF CYANOBENZENE (BENZONITRILE) *AND* THE PROBLEM OF THE ORIENTATION OF HYDROXYL GROUPS FORMED *IN VIVO*

BY J. N. SMITH AND R. T. WILLIAMS
*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*

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When monosubstituted *benzenes* carrying the chemical *ortho-para*-directing groups (e.g. $NH_2$, $NHAc$, Cl, OH) are fed to animals, it is found that the urine contains conjugates of the corresponding *ortho*- or *para*-substituted phenols, or both, e.g. acetanilide (Smith & Williams, 1948), aniline (Smith & Williams, 1949), phenol (Garton & Williams, 1949) and chlorobenzene (Spencer & Williams, unpublished observations). Little definite information, however, is available about the position of biological hydroxylation of monosubstituted benzenes carrying the chemical *meta*-directing groups (e.g. $NO_2$, CN, COOH). According to Meyer (1905) small amounts of *m*-nitrophenol occur in the urine of rabbits receiving nitrobenzene, but this was not unequivocally confirmed. Baumann (1863) isolated small amounts of *m*- and *p*-hydroxybenzoic acids from the acid-hydrolysed urines of dogs dosed with cyanobenzene. Evans, Gyorgy & Evans (1949) have suggested that *p*-hydroxybenzoic acid may be the first product of the oxidation of benzoic acid by certain bacteria (see also Parr, Gyorgy & Evans, 1949). The information available suggests that monosubstituted benzenes, if oxidized to phenols in vivo, give rise to *ortho*- or *para*-substituted phenols, or both, irrespective of the nature of the substituent.

Now Smith & Williams (1948) pointed out that the orientation of hydroxylation of acetanilide and aniline in the rabbit was similar to that found during nitration, i.e. *para* in acetanilide and mainly *ortho* and *para* in aniline. This suggested to us that one possible interpretation of the orientation of biological hydroxylation could be *done* the lines used to explain aromatic substitution in pure organic chemistry, i.e. *that* substitution is either ionic or free-radical in nature (for discussion of free-radical substitution me Hey & Weka, 1937, 1948; Waters, 1948; Dewar, 1949). Whilst other interpretations of the orientation of biological oxidation are also possible, we *studied* the fate of cyanobenzene and benzoic acid with the purely chemical theory of aromatic substitution in mind Working on these assumptions we thought, that if the orientation *of* hydroxylation *in vivo* were known for both *ortho*-, *para*- and *meta*-directing group, then we could decide whether biological hydroxylation had the characteristics *of* ionic substitution or of free-radical substitution, assuming, of course, that the hydroxy-