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Gas–liquid chromatographic determination of benzene metabolites

The metabolism of benzene has been extensively studied by various investigators, including WILLIAMS[1] and NAKAJIMA AND TOMIDA[2]. However, determining benzene metabolites spectrophotometrically and radiochemically was so difficult that, after PARKE AND WILLIAMS[3], no one repeated the comprehensive experiment on benzene metabolism. Now, a gas–liquid chromatographic technique is available for determination of these metabolites.

Materials and methods

A male Japanese white rabbit, weighing 3.5 kg, was injected subcutaneously with benzene, 1.0 ml/kg, mixed with an equal volume of sesame oil. Urine was collected every 12 h for 3 days after the benzene injection. Urinary phenols were extracted into diethyl ether and subjected to GLC; if these solutions were dilute it was necessary first to concentrate them by freeze-drying. Conjugated phenols were extracted after hydrolysis in 6 *N* sulfuric acid at 100° for 1 h[4].

In agreement with PORTEUS AND WILLIAMS[5,6], phenol and catechol were found to be excreted early; hydroquinone was excreted later. A description of the other metabolites will be given in another paper.

Benzene and phenols (phenol, catechol, resorcinol, hydroquinone, pyrogallol, hydroxyhydroquinone, phloroglucine) were dissolved in diethyl ether in a concentration of 10 mg/ml; 1 μl of this solution was employed for the determination, using an Hitachi gas–liquid chromatograph, Model K 53. DL-Phenylmercapturic acid and *trans*, *trans*-muconic acid were also dissolved in hot ethyl alcohol and determined by GLC as well.

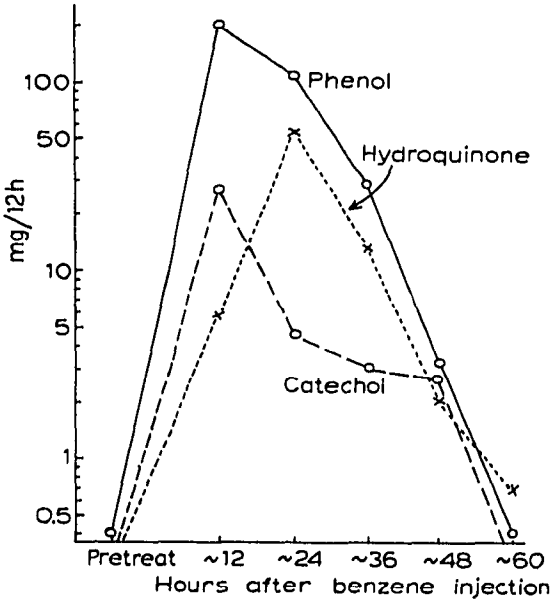

Fig. 1. Urinary excretion of main benzene metabolites in a rabbit after a subcutaneous 1.0 ml/kg benzene administration.

The apparatus consisted of a glass column (3 mm I.D., 2 m long) filled with Chromosorb G AW 80/100 mesh, coated with 3 % Silicone OV-17 and pretreated with dimethyldichlorosilane as support, had a hydrogen-flame ionization detector and used nitrogen, 60 ml/min, as carrier gas. The sample was run on the column treated with hexamethyldisilazane.

TABLE I

Compounds	*Retention time (min)*
Benzene	0.4
Phenol	0.7
Catechol	1.3
Resorcinol	1.9
Hydroquinone	1.9
Pyrogallol	3.0
Hydroxyhydroquinone	4.5
Phloroglucine	7.9
DL-Phenylmercapturic acid	0.7
trans,trans-Muconic acid	1.9

Benzene and phenols, but not resorcinol and hydroquinone, were clearly determined. Because resorcinol and hydroquinone have been difficult to fractionate by GLC[7,8] (see Table I), they should be separated by a chemical procedure[3]. After the initial determination of benzene and phenols in the sample by GLC, saturated lead acetate was added to the solution and the pH was adjusted to 9 with 2 *N* ammonia solution. Hydroquinone, just remaining in solution at pH 9, was determined when the supernatant was first centrifuged and then subjected to GLC. Resorcinol was calculated from the difference between the peaks of total resorcinol–hydroquinone and hydroquinone. Retention times were the same for *trans,trans*-muconic acid and hydroquinone and for DL-phenylmercapturic acid and phenol. Because *trans,trans*-muconic acid and DL-phenylmercapturic acid were barely soluble in diethyl ether, the resorcinol–hydroquinone and phenol peaks were practically uncontaminated. After crystallization, DL-phenylmercapturic acid was determined by GLC and *trans,trans*-muconic acid by spectrophotometry[5].

Discussion

Benzene metabolites have been determined by GLC, but this technique has only been applied to the identification of monophenol in an experiment *in vitro*[9]. Also the determination of phenols, with the exception of triphenol, has been attempted by this technique[7,10,11]. However, all the benzene metabolites are difficult to determine.

Our newly developed technique has enabled us to determine mono-, di- and triphenols with one column without destruction of phenols and to perform the experimental work on benzene metabolites with ease.

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A protection device for temporary power failure to automatic fraction collectors

One problem encountered in operating a conventional fraction collecting device is caused by a temporary power failure while the instrument is unattended. If a photoelectric or thermistor tripping mechanism is used in the volumetric device of the instrument and if the level of liquid passes this detection device during the power failure the fraction collector will never empty without manual resetting even if the power is restored. Such short term power failures that prevent the volumetric device from operating can be just as disastrous as long term power failure. However, it is possible to provide a safety mechanism for a fraction collector to overcome this problem of short term power interruption by modifying the instrument circuit so that the volumetric unit will automatically empty whenever the power is turned on.

The operation of the volumetric unit of many fraction collectors is such that a photocell or thermistor activates a small relay which in turn activates a mechanical emptying mechanism. If a time-delay relay which is opened by the same power source as the instrument is connected in parallel with this relay, it will trigger the emptying mechanism each time that power is turned on. After the time-delay period for the relay has passed, it will open and the fraction collector will function in the normal manner until the power is again interrupted.