ISOLATION AND EXAMINATION OF URINARY METABOLITES CONTAINING AN AROMATIC SYSTEM

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It is becoming increasingly evident that pathological states may lead to urinary excretion of abnormal metabolites, and that in such cases identification of these metabolites may be a valuable diagnostic aid and may give a clue to the fundamental lesion. Classical test-tube colour reactions are of little value owing to the enormous complexity of urine and the, in general, relatively unspecific nature of the reagents. The exceedingly high resolving power of chromatographic methods and their technical simplicity enables separation of metabolites to be carried out before identifying tests are applied and so makes it possible to derive far more information from much less material than was formerly possible.

The method described here allows qualitative examination of one group of urinary metabolites, those containing an aromatic, e.g., benzene or indole, ring by a rapid and simple procedure.

Experimental Procedure

Principle of the Method.—The aromatic urinary metabolites are adsorbed on deactivated charcoal, eluted with aqueous phenol, the eluate concentrated, fractionated on paper chromatograms, and suitable spray reagents applied.

Preparation of Deactivated Charcoal.—A charcoal deactivated with 4% by weight of stearic acid is convenient for routine use. To a solution of 16 g. stearic acid in 1,200 ml. ethanol is added slowly with stirring 400 g. charcoal (e.g., B.D.H. activated). The mixture is left one hour with occasional stirring, and then 10 l. of water is added slowly with stirring. After settling, the bulk of the supernatant is drawn off, and the deactivated charcoal filtered on a Buchner funnel, washed with a large quantity of distilled water, and air-dried.

Phenol for Elution.—"Liquid" phenol (phenol liquefactum, B.P.), 80 ml., is diluted to 1 litre with water.

Treatment of Urine.—The urine is acidified with acetic acid and centrifuged. Deactivated charcoal is added to the supernatant and the mixture shaken occasionally for about 5 min. The charcoal is filtered

off on a small Buchner funnel and washed with water to remove the remaining salts, urea, sugars, and aliphatic amino-acids. It is then eluted by slowly passing the phenol solution through it, followed by more water. The eluate is evaporated in a water-bath under reduced pressure. The phenol is eliminated by steam-distillation during this process. To ensure complete removal of the phenol further water can be added and the mixture again concentrated. The concentrate thus obtained is applied directly to paper chromatograms.

For routine use about 100 to 200 ml. of urine is suitable, but there is no difficulty in using as little as 5 ml. The amounts of charcoal and phenol to be used vary greatly because of variations in the metabolite content of the urine, but a suitable amount can easily be estimated after some experience has been gained. If sufficient charcoal has been used the urine filtrate after the initial adsorption will be almost or quite colourless. If too much has been used a large volume of phenol will have to be passed through the charcoal before any colour is eluted, as the phenol molecules will first adsorb on vacant spaces on the charcoal before displacing metabolite molecules already present. Phenol should be passed till the eluate is almost colourless, followed by distilled water to remove eluted material still in the body of the charcoal. As a rough guide, for 200 ml. of normal urine about 2 to 3 g. deactivated charcoal and 50 to 70 ml. of the aqueous phenol should be suitable.

If the phenol eluate is allowed to stand before evaporation, a precipitate of uric acid may settle out. If of no interest to the experiment this is best discarded. Similarly on concentration of the eluate uric acid separates. Its presence in the concentrate does not affect the chromatography.

Paper Chromatography.—For routine examinations one-dimensional chromatograms in butanol:acetic acid:water (4:1:5 v/v/v; Partridge, 1946) have been found most suitable. R_F values given in Table I refer to this solvent, using Whatman No. 1 paper and the descending technique. R_F values have been determined at room temperature without any attempt to standardize conditions, and are not therefore to be taken as more than an indication of the approximate position on the chromatogram. Chromatograms run under such unstandardized conditions are adequate for almost all purposes provided

reference standards are run simultaneously. R_F values can be modified, amongst other things, by a metabolite being displaced by other substances in a crowded region of the chromatogram, and by variations in the pH of the applied solution. For example, the R_F value of a base, such as adrenaline, may be different if applied as free base and as hydrochloride. In such cases a reference standard should be applied in the appropriate ionic form.

Detection of Metabolites.—The following reagents and methods are of particular value :

Fluorescence under Ultra-violet Light.—When examined in ultra-violet light which has passed through a Wood's glass filter many metabolites are revealed as characteristically coloured (see Table I) fluorescent spots. Ultra-violet light is also the most convenient way of revealing the solvent front. Normal urine contains traces of many unidentified fluorescent substances, some of which may be of plant origin (from the diet) rather than true metabolites.

Ninhydrin.—Ninhydrin is of value chiefly for the aromatic amino-acids and also, using a modified reagent and the resulting ultra-violet fluorescence (Jepson and Stevens, 1953), for tryptamine derivatives.

Ehrlich's Reagent.—This is of particular value for detecting (*a*) indole derivatives, (*b*) pyrrole derivatives, and (*c*) aromatic amines. It is convenient to have two reagents available, differing in acid strength, which will be referred to as the " normal " and " strong " Ehrlich's reagent respectively. The " normal " reagent is a 2% (w/v) solution of *p*-dimethylaminobenzaldehyde in approx 1.3 N HCl (concentrated HCl diluted with 6 parts of water). The " strong " reagent is a 2% (w/v) solution of the aldehyde in approx. 6 N HCl (3 parts concentrated HCl to 2 parts water). After spraying, the papers are allowed to dry at room temperature.

Indole and pyrrole derivatives in general give shades of red or blue, and the colours may change characteristically with time. Aromatic amines give a yellow colour, which may be modified to, e.g., orange by suitable neighbouring groups (cf. kynurenine, hydroxyanthranilic acid). Aliphatic amines may give a weak yellow after some time. Urea gives a bright yellow (hence an important reason for removing it). Indican, though an indole derivative, gives a brown colour ; the compound formed in this case is chemically related to indigo, and is of a different type from the compounds usually formed with indole derivatives (for further discussion of indican see below).

The use of two reagents differing in acid strength is advantageous, as the coloured compounds formed are indicators. At the pH of the " normal " reagent aromatic amines give a yellow colour immediately, whereas coloured compounds from indole derivatives may take some time to form. At the pH of the " strong " reagent derivatives of aromatic amines are formed but remain colourless, whereas indole derivatives, including indican, give a colour rapidly. It is thus possible to distinguish the two types of compound even if present in the same spot. Ultimately

the picture obtained with both reagents is the same, the indole compounds appearing slowly with the " normal " reagent and the aromatic amines appearing as the HCl evaporates from the paper after the " strong " reagent.

Pauly's Reagent.—The paper is sprayed with diazotized sulphanilic acid. Certain phenols develop a colour at this stage, whereas the majority only do so after a subsequent spray with alkali (aqueous Na_2CO_3 or ethanolic ammonia). Colours obtained are listed in Table I. Urinary phenols have been studied especially by Boscott, Bickel, and their colleagues, and reference should be made to their publications (e.g., Boscott and Bickel, 1953, 1954 ; Boscott, 1954). Pauly's reagent also detects glyoxaline derivatives, e.g., histamine, histidine, and urocanic acid. It is possible that the stabilized diazo compounds now being developed for the van den Bergh reaction will prove a useful substitute for diazotized sulphanilic acid when they are more readily available.

Ekman's (1948) Reagent.—Aromatic amines are diazotized either by spraying the paper with acidified nitrite or by exposing the damped paper to nitrous fumes, and coloured azo compounds are then formed by spraying with an ethanolic solution of 1-ethylaminonaphthalene. Much additional information can be obtained in the first stage (spraying with acidified nitrite) (cf. Dalgliesh, 1952a). Yellows or browns are given by *o*-aminophenols (due to benzoxdiazole formation) and indole derivatives (due to N-nitroso compounds). A blue (due to indigo) is given by indican. Pink (urorosein reaction) is given by indoleacetic and indoleaceturic acids and also by indole itself.

Ammoniacal Silver Nitrate.—The papers are sprayed and allowed to dry at room temperature. The reagent is of especial value in detecting substances containing readily oxidizable groups such as *o*-aminophenol or catechol derivatives (cf. Dalgliesh, 1952a ; Dalgliesh and Tekman, 1954). These rapidly give black or brown-black spots. Many other substances will react, but more slowly.

Other Reagents.—For particular compounds or types of compound many other reagents are available. Some useful examples are the Altman reagent (Gaffney, Schreier, Di Ferrante, and Altman, 1954), which is the only simple way of detecting hippuric acid on chromatograms, and is also useful for substituted hippuric acids (e.g., *o*- and *p*-aminohippuric acids). In the author's experience it is more convenient to allow colours with this reagent to form at room temperature, rather than by heating. James' ferricyanide reagent (James, 1948 ; James and Kilbey, 1950), which is particularly useful for the catechol amines ; the urorosein reaction (see under Ekman's reagent above) ; for phenols the Folin-Ciocalteu reagent (the commercially available reagent diluted 1 in 4), and the reagent of Barton, Evans, and Gardner (1952) ; for nicotinic acid derivatives and related quaternary pyridinium compounds see Kodicek and Reddi (1951) and Holman (1954) ; for the Thormählen reaction (for melanogens) applied to paper see Leonhardi (1953).

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF SOME SUBSTANCES ENCOUNTERED IN URINE

Substance	R_F[1]	Fluorescence[2]	Ehrlich's Reagent[3]	Pauly's Reagent[4]	Ekman's Reagent[6] and Urorosein Test[9]	Other Reagents for Confirmatory Tests
Adrenaline	0·39	—[12]	—	Salmon-pink	—	Ammoniacal silver nitrate, James' ferricyanide reagent
p-Aminobenzoic acid ..	0·79	—	(n) Immediate yellow	—	Magenta	[10]
p-Aminosalicylic acid	0·84	—	(n) ,,	Brown	,,	[10]
o-Aminohippuric acid..	0·76	Purple	(n) ,,	—	,,	Altman reagent (pink)
p-Aminohippuric acid..	0·63	—	(n) ,,	—	,,	Altman reagent (orange-pink)
Anthranilic acid ..	0·90	Purple	(n) ,,	—	,,	[10]
Anthranilic acid glucuronide	0·52	Purple	(n) ,,	—	,,	[10]
2 : 5-Dihydroxyphenylalanine	0·32[8]	—	—	Brown-pink	—	Ammoniacal silver nitrate; James' ferricyanide reagent; ninhydrin
3 : 4-Dihydroxyphenylalanine	0·28	—	—	Magenta	—	Ammoniacal silver nitrate: James' ferricyanide reagent; ninhydrin
3 : 4-Dihydroxyphenylethylamine	0·44	—	—	,,	—	Ammoniacal silver nitrate : James' ferricyanide reagent
Hippuric acid	0·85	—	—	—	—	Altman reagent (orange)
Homogentisic acid ..	0·80	—	—	Brown-pink	—	Ammoniacal silver nitrate
3-Hydroxyanthranilic acid	0·84	Blue-purple	(n) Yellow or yellow-pink	Pink	(Yellow-brown)	Ammoniacal silver nitrate
5-Hydroxyanthranilic acid	0·54	Strong pale blue	(n) Immediate yellow	Brown	Blue (appears slowly)	Ammoniacal silver nitrate
m-Hydroxybenzoic acid	0·88	—	—	Orange	—	
p-Hydroxybenzoic acid	0 88	—	—	,,	—	
5-Hydroxyindoleacetic acid	0·75	—	(s) Blue	Brown-pink before alk., intensifying afterwards	(Brown, or red if strong acid)	Ammoniacal silver nitrate
3-Hydroxykynurenine	0·31[7] [8]	Green	(n) Pink or orange-pink	Pink-red	(Yellow-brown)	Ammoniacal silver nitrate
5-Hydroxytryptamine	0·33	—	(s) Grey-blue→ grey purple→ green	Brick-red	,,	Modified ninhydrin: ammoniacal silver nitrate
5-Hydroxytryptophan	0·17	—	(s) Grey-blue→ blue-purple-→ green	Red-magenta	,,	Ninhydrin: ammoniacal silver nitrate
Indican	0·41	—	(s) Brown	Slow brown to pink[11]	(Blue developing slowly)	(See text)
Indole	0·93	—	(s) Carmine	—[5]	(Pink)	
Indoleacetic acid ..	0·90	—	(s) Magenta-→ blue	—[5]	,,	
Indoleaceturic acid ..	0·82	—	(s) ,,	—[5]	,,	
Indolepropionic acid ..	0·91	—	(s) Blue-magenta →blue	—[5]	(Yellow-brown)	
Kynurenic acid ..	0·60	Weak blue	—	—	—	
Kynurenine	0·37[8]	Strong pale blue	(n) Orange	—	Magenta	Ninhydrin[10]
Noradrenaline ..	0 30	—[12]	--	Salmon-pink	—	Ammoniacal silver nitrate: James' ferricyanide reagent
Phenylalanine	0·55	—	—	—	—	Ninhydrin
Porphobilinogen ..	0·49	—	(s) Magenta→ weaker blue	—	—	
Riboflavin	0·21[7]	Yellow	—	—	—	
Salicylic acid	0·91	Purple	—	Orange-yellow	—	
Salicyluric acid ..	0·83	Blue-purple	—	Yellow	—	
Skatole	0·94	—	(s) Violet→blue	—[5]	(Yellow-brown)	
Tryptamine	0 60	—	(s) Red→violet→ grey-violet	—[5]	,,	Modified ninhydrin
Tryptophan	0·45	—	(s) Violet→green	—[5]	,,	Ninhydrin
Tyrosine	0·40	—	—	Pink	—	,,
Tyramine	0·57	—	—	,,	—	,,
Urea	0·50	—	(n) Immediate bright yellow	—	—	
Xanthurenic acid ..	0·60	Blue	—	Brick-red	—	

[1] On Whatman No. 1 paper, descending technique, using butanol : acetic acid : water, 4 : 1 : 5 v/v/v. See comments in text.
[2] In ultra-violet light which has been passed through a Wood's glass filter. Substances showing a very weak fluorescence are not noted, as this would almost certainly be masked in a chromatogram of a urine extract.
[3] (n) = normal reagent and (s) = strong reagent. For differences in the rates of appearances of colour with the two reagents see text. The reagent indicated is the most convenient for identification.
[4] Colours after the alkaline spray, unless otherwise stated.
[5] If the reagent contains an excess of nitrite the colours entered in brackets under Ekman's reagent may also be observed.
[6] The colours in brackets represent colours visible after diazotization (urorosein and related reactions. See text and note 9).
[7] If present in appreciable amount will be visible as a yellow spot on the untreated chromatogram.
[8] If D- and L-forms are present these may be resolved into their optical isomers (see Dalgliesh, 1952b).
[9] If it is the urorosein reaction which is of interest more intense colours are obtained by spraying with approx. 6N HCl to which sodium nitrite solution has been added.
[10] These substances may show some degree of yellow with the Altman reagent.
[11] This is complicated by the ready oxidation of indoxyl formed on hydrolysis of the indican. Especially in presence of excess nitrite only a blue (indigo) spot may be obtained.
[12] Fluorescent oxidation products may be observed.

Other methods which can give useful information are to elute a substance from the chromatogram and compare its behaviour before and after hydrolysis; to determine the migration on paper electrophoresis at varying *p*H values; and to determine the ultra-violet absorption spectrum (e.g., Dalgliesh, 1952a).

Results

Table I summarizes the reactions of many compounds encountered in urine examinations. Various points of interest of some of these compounds are noted below.

Drugs and Drug Metabolites.—It is desirable to withdraw all drugs for 24, or preferably 48, hours before urine collection. If this is not possible, allowance should be made for drug metabolites which might be present. Many of the popular drugs and their metabolites are readily isolated from urine and detected by the present method. For example, sulphonamides can produce urines so rich in metabolites as to obscure the normal constituents. Aspirin gives rise to salicyluric acid (see below), the excretion of which can last for a considerable time after the dose. *iso*Nicotinic acid hydrazide, *p*-aminosalicylic acid, and chloromycetin are other examples of drugs giving rise to readily detectable metabolites which might obscure the normal metabolic picture. Conversely, of course, the method is useful for studying the metabolism of suitable drugs.

Anthranilic acid is excreted in congenital hypoplastic anaemia (Altman and Miller, 1953).

3-Hydroxyanthranilic acid may be excreted in acute tuberculosis (e.g., Musajo, Spada, and Coppini, 1952; Makino, Satoh, Fujiki, and Kawaguchi, 1952).

5-Hydroxyindoleacetic acid is a normal constituent of human urine (Titus and Udenfriend, 1954) and is probably derived from tryptophan by way of 5-hydroxytryptamine. The excretion may be increased in certain types of cancer (Clerc-Bory, Pachéco, and Mentzer, 1954).

3-Hydroxykynurenine may be excreted, sometimes with kynurenine, in many cases of fever of varying origin (Dalgliesh and Tekman, 1954).

5-Hydroxytryptamine (enteramine, serotonin) probably does not occur in urine but may be encountered in other body fluids, e.g., blood, or tissue extracts (cf. Dalgliesh, Toh, and Work, 1953; for detection see this paper and Jepson and Stevens, 1953).

5-Hydroxytryptophan. is a normal urinary constituent (Titus and Udenfriend, 1954).

Indican.—Much controversy has occurred in the past as to whether or not indican is a normal constituent of urine. In the author's experience it is always present in a fresh normal urine. Negative results previously reported have probably been due to insufficiently sensitive tests. The present methods are far more sensitive than, for example, the frequently used Obermayer test. The indicator properties of the compound formed between indoxyl (from hydrolysis of the indican) and Ehrlich's reagent allow a test sensitive to a fraction of a microgram. The normal brown is allowed to develop on the chromatogram and the paper is then sprayed with saturated sodium acetate. The brown changes to an intense cherry-red, whereas the colour given by most other substances with Ehrlich's reagent disappears. Indican sometimes appears as multiple spots, but, as authentic potassium indoxyl sulphate added to urine can behave in the same way, these multiple spots are probably due to the one substance. If large amounts of indican are present the untreated chromatograms may show fast-running pink and blue spots. These are due to indirubin and indigo respectively, formed by oxidation of indoxyl.

Indole and skatole do not normally occur in urine but may be encountered if there has been faecal contamination.

Indoleacetic and Indoleaceturic Acids.—Indoleacetic acid is a normal urinary constituent. The excretion of indoleaceturic acid may be related to the metabolic defects in phenylketonuria (Armstrong and Robinson, 1954).

Noradrenaline.—The presence of abnormal amounts in the urine may allow diagnosis of phaeochromocytoma when other methods fail (von Euler, Lund, Olsson, and Sandblom, 1953).

Riboflavin somewhat resembles hydroxykynurenine in its fluorescence, but is readily distinguished by other tests.

Salicyluric acid is frequently encountered due to the widespread use of aspirin. In R_F value and fluorescence it resembles hydroxyanthranilic acid but is readily distinguished by other tests.

Xanthurenic Acid.—This may be separated from kynurenic acid on chromatograms run with inorganic salt solutions, e.g., the 5% sodium formate or 20% KCl solutions used by Boscott (1952).

Not all types of compounds which are probably extractable from urine by the present method have been examined. It is likely that the method could, for example, be extended to some of the steroids, and to purines and pyrimidines and their metabolites. As uric acid is easily isolated by this method it is probable that xanthine would be too; xanthinuria (Dent and Philpot, 1954) should there-

fore be readily detectable. It is hoped in the future to investigate some of these possibilities.

Quantitative Aspects of Metabolite Recovery.— The recovery of many substances has been checked at various times both from pure solution and for substances added to urine. In no case has quantitative recovery been observed, but in the majority of cases recoveries have been in the region of 60 to 90%. Such a recovery is quite adequate for qualitative work. Lower recoveries have been observed for porphobilinogen (35–40%) and for certain hydroxylated metabolites, e.g., 5-hydroxytryptamine, where recoveries are variable but on the whole unsatisfactory. Methods for improving the recovery of such substances are being examined.

Results with two substances are recorded in Tables II and III.

TABLE II

RECOVERY OF TRYPTOPHAN BY CHARCOAL ADSORPTION PROCEDURE

Weight of Tryptophan Taken	Percentage Recovery		
	(a)	(b)	(c)
125 μg.	65	73	62
250 „	71	68	69
1,250 „	88	84	84
2,500 „	80	—	—
10 mg.	90	84	89
30 „	87	78	78
50 „	78	69	64
100 „	48	—	—

Column (a), tryptophan dissolved in 10 ml., column (b), 20 ml., column (c), 50 ml. of solution.

TABLE III

RECOVERY OF ANTHRANILIC ACID BY CHARCOAL PROCEDURE

Weight of Anthranilic Acid Taken	Percentage Recovery
380 μg.	95
1·7 mg.	85
3·7 „	92·5
17·7 „	88·5
45·0 „	90

Tryptophan Recovery.—The stated amount of tryptophan was dissolved in water (10 ml., 20 ml., or 50 ml.; solution was effected with acid if necessary) and each solution treated by the above procedure with 500 mg. deactivated charcoal, followed by elution with 35 ml. of aqueous phenol. The phenol was removed by concentration as described above, the eluate made up to a known volume, and the tryptophan estimated by the procedure of Horn and Jones (1945).

It will be seen from the table that a satisfactory recovery (taken as greater than 50%) is obtainable over a range of concentration greater than 4,000-fold. This is an important advantage when working with urine, where metabolites may be present in widely differing concentrations. The figures also suggest that small amounts of a substance such as tryptophan could be satisfactorily detected. For example, if 200 ml. of urine is assumed to be available and tryptophan is excreted at the rate of 1 mg./day, at least 50 μg. should be present in the urine concentrate, which would give a detectable level of tryptophan even if divided between several chromatograms. In the experiments referred to in Table II tryptophan was observed in the filtrate from the charcoal adsorption when 30, 50, or 100 mg. of tryptophan was used. From this one can deduce that for isolation purposes a level of about 20 mg. tryptophan/500 mg. deactivated charcoal would be most advantageous.

Anthranilic Acid Recovery.—A similar procedure was used, the anthranilic acid being dissolved in 25 ml. of water before charcoal treatment. Recovery was estimated by the Bratton and Marshall (1939) procedure as used by Knox (1953). The results are shown in Table III.

Discussion

The method is based on the work of Synge and Tiselius (1949), who found that on deactivated charcoal columns the elution of aromatic metabolites depended on the number of aromatic systems rather than on other functional groups present. Thus compounds with one aromatic system tended to be eluted together, and were quite separate from aliphatic compounds or compounds with two aromatic systems. The present technique is essentially a simplified form of displacement analysis. The most active positions of the charcoal, on which metabolites would be adsorbed irreversibly, are blocked with stearic acid. This deactivated charcoal can then adsorb aromatic metabolites reversibly. On treatment with a more strongly adsorbed substance the metabolite molecules are displaced and emerge in the eluate. Phenol is an excellent displacing agent, as it is eliminated from the eluate by steam distillation during concentration.

The method as described above has proved most valuable for routine urine examinations (e.g. Dalgliesh, 1952a ; Charconnet-Harding, Dalgliesh, and Neuberger, 1953 ; Dalgliesh and Tekman, 1954). As can be seen from the figures quoted, the recoveries are not quantitative. The work of, for example, Hagdahl, Williams, and Tiselius (1952) and Porath and Li (1954) suggests that if column methods were used conditions might well be found for the isolation of a specific aromatic substance with a high recovery. But such conditions would vary for each substance, and the method described here,

though not quantitative, gives satisfactory qualitative recoveries for a wide range of metabolites. An unsatisfactory recovery is normally due to the metabolite being incompletely eluted, adsorption being in general complete. Simple alterations which might be found advantageous for isolation of a particular metabolite are: (a) Altering the degree by which the active positions of the charcoal are blocked, e.g., by using charcoal deactivated with 6% or 8% by weight of stearic acid. A metabolite which is strongly adsorbed is more easily removed from a charcoal in which only weakly active areas are available for adsorption. (b) The pH of the eluant may be altered, e.g., by using acidified phenol (though hydrolysis of conjugates may then occur during concentration). (c) A displacing agent other than phenol may be used, provided it can be removed from the eluate without also removing the metabolites under investigation.

The advantages of the method are the following: (1) It is cheap, simple, and rapid. The actual working time in preparing a sample for chromatography is well under half an hour, and the total time, if an efficient water pump is used for the evaporation, need be no more than an hour. (2) A high degree of concentration is readily obtained, and interfering substances, such as salts, urea, sugars, and aliphatic amino-acids, are eliminated. (3) The method is far simpler (and quicker) than solvent extraction procedures. The latter are frequently accompanied by difficulties due to emulsion formation. In general the urine must first be hydrolysed, as many metabolites are excreted mostly or entirely as hydrophilic conjugates which cannot be directly extracted. Moreover, hydrolysis can very largely destroy indolic compounds. By the charcoal adsorption procedure conjugates are isolated unchanged and the conjugation pattern can be readily assessed. This can be of considerable interest, e.g., as a guide to liver function, and is of course also highly desirable in, e.g., studies of the metabolic fate of drugs. (4) The method is far simpler and shorter than electrolytic desalting procedures. The latter result in loss of strongly charged metabolites, and the conditions are sufficiently vigorous to cause chemical changes in labile molecules. Moreover urea, which can cause interference in paper chromatography, is not removed. The charcoal method is largely carried out at neutral pH under mild conditions and isolates charged and uncharged molecules equally, with elimination of both salts and urea. (5) Ion-exchange resins, like charcoal, can be used to isolate a given type of metabolite, in this case based on net charge. Suitable combinations of both methods, allowing separation of aromatic metabolites with

a given type of charge, can be most useful in isolation studies.

The disadvantages of the method are:
(1) It is not quantitative. (2) If a metabolite is more strongly adsorbed than the eluant it will not be eluted to any appreciable extent, and will remain undetected.

Work attempting to overcome these disadvantages is at present in progress. In spite of them the method is considered to be of great value and worthy of wider use. It reveals, even in normal urine, a large number of unidentified substances. The identification of these and of metabolites abnormally excreted in pathological states offers a rich field for research.

Summary

A method is described for rapid isolation of the group of urinary metabolites containing a simple aromatic system. The method is applicable both to studies of metabolic defects, e.g., of the aromatic amino-acids, and to studies of drug metabolism.

The chromatographic behaviour of many substances likely to be encountered in urine is summarized.

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