445. *The Use of Deactivated Charcoals for the Isolation of Aromatic Substances.*

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Charcoal which has been deactivated by pre-treatment with various long-chain aliphatic compounds will selectively adsorb aromatic substances from aqueous solution, allowing a simple separation from aliphatic and inorganic contaminants even if the latter are present in large excess. Aromatic adsorbates can be displaced from deactivated charcoals by aqueous phenol, and this phenol is removed (by steam-distillation) on subsequent concentration. Recoveries depend on the degree of deactivation of the charcoal, but are usually good, and the method is rapid and simple. With stearic acid as deactivating agent, the method is applicable to benzene derivatives, pyrroles, pyridine derivatives, indoles, purines, and pyrimidines, but not to glyoxalines. With aqueous phenol as eluant, and charcoal deactivated with stearic acid, octadecane, or paraffin wax as adsorbent, there is only low recovery of substances with basic amino-groups, though high recoveries can be obtained by using acidified phenol as eluant. On the other hand, basic substances can be readily displaced by aqueous phenol if the charcoal has been deactivated with octadecylamine. Alumina is a specific adsorbent for *ortho*-dihydric phenols, with unsubstituted phenolic groups.

CHARCOAL has frequently been used for the isolation of natural products, by adsorption and subsequent elution. In many cases, however, adsorption is largely irreversible and the adsorbate can be recovered in only poor yield. Pre-treatment of the charcoal with a deactivating agent [1] such as stearic acid [2] blocks the most active positions on the charcoal surface and leaves available sites from which an adsorbate can be more readily displaced. Thus Synge and Tiselius [2] showed that charcoal deactivated with stearic acid allowed separation of aromatic substances into groups determined primarily by the number of aromatic systems present in a molecule. The Scandinavian school has done much work on the modification of charcoal to allow resolution of mixtures of aromatic substances.[3, 4, 5] To obtain good separations by such methods, it is necessary to adhere to strictly defined conditions.

Deactivated charcoals can, however, be used in a much less specific way for the rapid and simple separation of aromatic from aliphatic and inorganic substances in aqueous solution. An appreciable proportion of the metabolic effort of the living organism is concerned with the metabolism of aromatic compounds, but the amounts of most aromatic substances excreted are very small compared with non-aromatic substances, such as salts, urea, creatinine, and sugars. Even with modern analytical techniques identification may only become practicable after concentration and elimination of interfering substances. A method for separating aromatic from non-aromatic substances thus has potential value in both biochemical and synthetic fields. It has been found [6] that the aromatic substances of urine can be adsorbed on charcoal which has been deactivated with 4% by weight of stearic acid, and can subsequently be eluted with aqueous phenol. On concentration of the eluate in a vacuum both water and phenol are removed, leaving a mixture of aromatic excretory products. The method has been used for isolation of aromatic substances from urine [7, 8, 9] and from chemical reaction mixtures.[10] The degree of deactivation of the charcoals used in these experiments was selected empirically. It was desirable to determine the range of applicability and the effect of different amounts and types of deactivating

[1] Tiselius and Hahn, *Kolloid Z.*, 1943, **105**, 177.
[2] Synge and Tiselius, *Acta Chem. Scand.*, 1949, **3**, 231.
[3] Hagdahl, Williams, and Tiselius, *Arkiv Kemi*, 1952, **4**, No. 10.
[4] Williams, Hagdahl, and Tiselius, *ibid.*, 1954, **7**, 1.
[5] Porath, *ibid.*, p. 535.
[6] Dalgliesh, *J. Clin. Path.*, 1955, **8**, 73.
[7] Dalgliesh and Tekman, *Biochem. J.*, 1954, **56**, 458.
[8] Dalgliesh, *ibid.*, 1955, **61**, 328.
[9] *Idem, ibid.*, p. 334.
[10] *Idem, Arch. Biochem. Biophys.*, 1955, **58**, 214.

agent. This paper describes a preliminary survey from the practical standpoint of finding adsorbents which will allow a high degree of selective reversible asdorption of aromatic substances.

Standard conditions (see p. 2298) were chosen in which charcoal deactivated with 4% by weight of stearic acid [cf. 6] was the adsorbent and aqueous phenol the eluant. Recoveries from aqueous solution of a wide variety of substances occurring, or similar to those likely to occur, in urine, were determined, usually from both 0·005M- and 0·0005M-solution. Should a proportion of a substance be adsorbed irreversibly until a saturation point is reached, and the remainder then be adsorbed reversibly, this should be evident from comparison of the recoveries at the two different concentrations. In the case of non-aromatic substances, concentrations higher than 0·005M have in many cases been investigated.

In the various Tables the values under the headings " filtrate " and " eluate " represent the percentage of material respectively not held by the adsorbent and that adsorbed and recovered. The difference between these combined values and 100 represents the percentage of substance remaining on the adsorbent together with that lost in the working up, *e.g.*, by steam-distillation or mechanical losses. The figures for recovery in the eluate are not necessarily the maximum obtainable with the adsorbent. A standard amount of the displacing and eluting agent was used which, though probably adequate for most substances investigated, might not have been sufficient for those more firmly bound on the adsorbent surface.

Adsorption on deactivated charcoal is best carried out from acid solution. Table 1

TABLE 1. *Influence of* pH *of solution on adsorption of anthranilic acid by stearic acid-deactivated charcoal.**

	From 0·005M-solution:		From 0·0005M-solution:			From 0·005M-solution:		From 0·0005M-solution:	
pH	filtrate	eluate	filtrate	eluate	pH	filtrate	eluate	filtrate	eluate
1	2	86	0	82	6	36	57	0	88
2	1	86	0	88	7	40	51	35	57
3	1	91	0	88	8	92	4	86	2
4	0	86	0	83	9	92	6	92	3
5	6	82	0	86	10	91	3	93	2

* In this and other tables, the figures under " filtrate " and " eluate " represent percentages and have the significance described in the text (above).

shows the effect of pH on the adsorption of anthranilic acid. We have used pH 4 for adsorption under our standard conditions. This is not optimal, but was chosen as being less likely to lead to degradation of labile substances.

The non-adsorption of salts by stearic acid-deactivated charcoal is shown in Table 2.

TABLE 2. *Adsorption behaviour of inorganic ions and some aliphatic substances.*

Species	Concn.	Filtrate	Eluate	Species	Concn.	Filtrate	Eluate
Na^+	As 0·9% (w/v) NaCl	98	0	Urea	2 g./100 ml.	97	0
K^+	As 0·4% (w/v) KCl	99	0		0·003M	98	0
NH_4^+ ...	As 0·005M-NH_4Cl	101	0		0·0005M	98	0
	As 0·0005-M- ,,	96	0	Creatinine ...	0·005M	95	1
Ca^{2+}	As 0·005M-$CaCl_2$	99	0		0·0005M	100	0
Cl^-	As 0·9% (w/v) NaCl	99	0	Glucose	2 g./l.	98	0
SO_4^{2-} ...	As 0·014M-Na_2SO_4	102	0	Galactose ...	2 g./l.	97	0
PO_4^{2-} ...	As 0·037M-KH_2PO_4	103	0	Lactose	0·005M	97	3
I^-	As 0·005M-KI.....	80	12 *		0·0005M	100	0
				Glucosamine	0·005M	95	1
					0·0005M	94	0

* 9% remaining on charcoal.

Iodide ion differs from the others in appearing in the eluate to some extent, but little iodide normally occurs in biological fluids. Adsorption on deactivated charcoal and subsequent recovery thus afford a simple and effective method for desalting. The recoveries of various non-aromatic organic substances are also shown in Table 2, and it will be seen that these, too, are eliminated in the filtrate by our procedure.

Table 3 shows the behaviour of some naturally-occurring amino-acids: neutral, acidic, and basic aliphatic amino-acids are largely unadsorbed, whilst the aromatic amino-acids appear with relatively high recoveries in the eluate. In this respect histidine behaves like an aliphatic, rather than an aromatic, substance. Some recovery of the longer-chain aliphatic amino-acids in the eluate occurs, suggesting that deactivated charcoal is likely to adsorb long-chain substances (cf. the stearic acid used for deactivation) and branched-chain substances. A pH lower than usual is required for adsorption of tyrosine, suggesting that selective adsorption of components of a mixture might be possible with suitable pH changes.

Table 4 shows the behaviour of a variety of aromatic substances. Good recoveries are

TABLE 3. *Adsorption behaviour of naturally occurring amino-acids.*

	From 0·005M-solution:		From 0·005M-solution:			From 0·005M-solution:		From 0·005M-solution:	
Amino-acid	filtrate	eluate	filtrate	eluate	Amino-acid	filtrate	eluate	filtrate	eluate
Glycine	96	0	95	0	Phenylalanine	7	90	1	96
Alanine	93	0	100	0	Tyrosine	12	81	14	80
Valine	96	2	96	0		(2 *)	(87 *)	(0 *)	(91 *)
Leucine	92	5	98	1	3 : 4-Dihydroxy-phenylalanine	4	81	0	70
Isoleucine	90	7	93	0	Tryptophan	0	87	0	88
Glutamic acid	98	0	92	0	Kynurenine	0	81	0	85
Arginine	69	4	49	6	3-Hydroxykynurenine	8	90	0	83
Histidine	90	4	96	0					
Lysine	90	4	96	0					

* Values obtained on adsorption at pH 2.

TABLE 4. *Miscellaneous aromatic substances recoverable in good yield after adsorption on stearic acid-deactivated charcoal.*

	From 0·005M-solution:		From 0·0005M-solution:	
Substance	filtrate	eluate	filtrate	eluate
Benzene derivatives				
N-Acetylsalicylohydrazide glucuronide	0	95	0	92
N-Acetyl-(2 : 3 : 5 : 6-tetrachlorophenyl)cysteine	not determined		0	73
p-Aminobenzoic acid	1	84	0	81
p-Aminohippuric acid	3	77	0	81
m-Aminophenylglucuronide	0	95	0	85
m-Aminophenyl *O*-sulphate	5	50	3	60
Hippuric acid	15	79	0	82
3-Hydroxyanthranilic acid	4	74	0	79
p-Hydroxyphenylacetic acid	0	90	0	90
m-Nitrophenyl *O*-sulphate	0	71	0	65
Salicyluric acid	0	90	0	97
Tyrosine *O*-sulphate	0	75	0	81
Indole derivatives				
Indican (indoxyl *O*-sulphate)	2	73	0	68
Indolylacetic acid	0	80	0	82
Indolylpropionic acid	0	83	0	93
Indolylbutyric acid	0	33 (89 *)	0	59 (83 *)
Purines				
Uric acid	0	96	0	91
Guanine	2	73	0	83
Xanthine	8	88	1	88
Pyrimidines				
Thymine	4	96	0	99
Uracil	6	75	6	70
Pyrroles				
2-Carboxypyrrole	14	61	14	51
Porphobilinogen	0	82 (88 *)	0	65 (73 *)

* Amount eluted by use of aqueous phenol–ammonia.

obtained with benzene derivatives (including phenols), indole derivatives, purines, and pyrimidines. Conjugates (*e.g.*, *O*-sulphates, glucuronides, glycine conjugates) are in general recovered as satisfactorily as unconjugated substances. However, in some cases recovery

after simple phenol elution is poor. Table 5 shows examples. It will be seen that substances with basic amino-groups tend to remain adsorbed after treatment with aqueous phenol, but are eluted in good yield when either aqueous phenol–hydrochloric acid or aqueous phenol–acetic acid is used. This suggested an ion-exchange reaction involving the carboxyl group of the stearic acid used for deactivation. However, when charcoal was deactivated with octadecane in place of stearic acid, comparable recoveries (Table 5) were obtained. Possibly there are acidic groups on the charcoal surface, though if so it is surprising that they do not bind histamine (cf. Table 6). If an acid eluant is used, acetic is preferred to hydrochloric acid because of the smaller amount of degradation it is likely to cause during subsequent concentration of the eluate, and because traces of hydrochloric acid in the concentrate can interfere in subsequent paper chromatography.

The low recovery of indolylbutyric acid on elution from stearic acid-deactivated char-

TABLE 5. *Aromatic substances recoverable in good yield from stearic acid-deactivated or octadecane-deactivated charcoal using modified conditions.*

(Values under " S " refer to stearic acid-deactivated charcoal and under " O " to octadecane-deactivated charcoal.)

		Filtrate :		Eluate using aq. phenol :		Eluate using aq. phenol–HCl *		Eluate using aq. phenol–HOAc *	
Substance	Concn. (M)	S	O	S	O	S	O	S	O
Tyramine	0·005	0	1	10	14	74	79	70	76
	0·0005	0	1	3	10	82	88	82	82
Tryptamine	0·005	0	0	20	14	88	86	84	89
	0·0005	0	0	21	16	78	89	83	85
5-Hydroxy-tryptamine	0·005	0	1	8	11	31	47	44	52
	0·0005	0	0	13	10	31	24	32	25
Adrenaline	0·005	7	6	18	15	67	63	63	57
	0·0005	9	0	31	18	62	60	64	62
Adenine	0·005	0	3	25	33	90	87	86	95
	0·0005	0	0	18	19	95	94	89	94
Cytosine	0·005	7	—	40	—	79	—	—	—
	0·0005	0	—	40	—	77	—	—	—

* For composition see p. 2298.

TABLE 6. *Adsorption behaviour of some substances only poorly recovered in the stearic acid-deactivated charcoal procedure.*

	From 0·005M-solution		From 0·0005M-solution :	
Substance [1]	filtrate	eluate	filtrate	eluate
Salicylic acid	5	0 (57 †)	0	0 (80 †)
Nicotinic acid	9	62	9	51
Nicotinamide	9	45	9	40
Histamine	96	0	85	0
Riboflavin	0	40 *	0	38 *

* A further 10% (approx.) can be eluted with aqueous phenol–HCl.
† Values obtained when $NaHCO_3$ was added to the eluate before concentration.

coal with neutral phenol is surprising in view of the adequate recovery of its lower homologues (cf. Table 4). However, good recoveries are obtainable on elution with phenol–ammonia. The pyrrole derivatives examined (Table 4) are less well recovered than, *e.g.*, indole derivatives. The poor recovery of 5-hydroxytryptamine (Table 5) is due partly to degradation, but mainly to the firm binding of this substance on the charcoal. In Table 6 are summarised some miscellaneous substances which were poorly recovered. Salicylic acid, nicotinic acid, and nicotinamide are satisfactorily eluted from deactivated charcoal, but are lost during the concentration step. This loss by steam-distillation was confirmed by distilling solutions not subjected to any adsorption procedure. The low recovery of riboflavin is due to the firm binding on the charcoal of a polynuclear molecule, whilst the non-recovery of histamine is due to non-adsorption, as was observed for histidine (Table 3).

All the substances in Tables 2—6 were examined for their adsorbability on alumina, but only adrenaline and 3 : 4-dihydroxyphenylalanine were adsorbed and recovered in

high yield. The adsorption of adrenaline on alumina is well known,[e.g., 11] and it was thought desirable to determine whether alumina was a selective adsorbent for dihydric phenols. The results obtained by using as adsorbents alumina or stearic acid-deactivated charcoal are shown in Table 7. It will be seen that alumina is excellent for adsorption and recovery of *ortho*-dihydric phenols with unsubstituted phenolic groups. *meta*- and *para*-Dihydric phenols are little adsorbed, as are *ortho*-dihydric phenols in which the phenolic groups are modified (*e.g.*, ferulic acid, 3 : 4-dimethoxyphenylalanine). On the other hand, recoveries of diphenolic substances from charcoal deactivated with 4% by weight of stearic acid are poorer than for most aromatic stubances (cf. Tables 3 and 4) possibly owing to the firm adsorption of substances with several hydrogen-bonding groups on the aromatic ring, and consequent inadequacy of phenol as a displacing agent.

In experiments on varying degrees of deactivation we studied the recoveries of (*a*) anthranilic acid, taken as typical of substances well recovered from 4% stearic acid-deactivated charcoal, (*b*) riboflavin, a polycyclic substance less readily displaced by phenol, (*c*) ferulic acid, a substance with hydrogen-bonding groups found to be less well recovered

TABLE 7. *Adsorption behaviour of dihydric phenols and derivatives.*

Substance	Concn. (M)	With stearic acid-deactivated charcoal: filtrate	With stearic acid-deactivated charcoal: eluate	With alumina: filtrate	With alumina: eluate
ortho-*Dihydric phenols*					
Catechol	0·0005	5	45	5	95
3 : 4-Dihydroxyphenylalanine	0·005	4	81	3	95
	0·0005	0	70	0	90
3 : 4-Dihydroxy-2-methylphenylalanine	0·0005	23	38	5	90
3 : 4-Dihydroxy-5-methylphenylalanine	0·0005	36	63	3	73
Adrenaline	0·005	7	18	1	92
	0·0005	9	31	3	95
3 : 4-Dihydroxyphenylethylamine	0·0005	0	0	0	96
Caffeic (3 : 4-dihydroxycinnamic) acid	0·005	0	43	0	81
	0·0005	0	58	0	76
O-*Substituted* ortho-*dihydric phenols*					
Ferulic (4-hydroxy-3-methoxycinnamic) acid	0·005	0	38	5	41
	0·0005	0	40	10	41
3 : 4-Dimethoxyphenylalanine	0·0005	19	75	93	6
meta-*Dihydric phenols*					
Resorcinol	0·0005	5	63	94	5
3 : 5-Dihydroxyphenylalanine	0·0005	0	33	95	5
2 : 4-Dihydroxyphenylalanine	0·0005	21	36	83	16
2 : 4-Dimethoxyphenylalanine	0·0005	10	70	98	3
para-*Dihydric phenols*					
Quinol	0·0005	11	88	53	11
2 : 5-Dihydroxyphenylalanine	0·0005	0	20	84	8
Homogentisic acid	0·002	0	49 *	not determined	
	0·0005	not determined		54	9

* A further 25% could be obtained by elution with phenol–ammonia and immediately concentrating the eluate under N_2.

(Table 7), and (*d*) substances with basic amino-groups such as adenine, tyramine, and tryptamine.

The recoveries in the eluate when using varying degrees of deactivation by stearic acid are summarised in Fig. 1. The superiority of deactivated over untreated charcoals as adsorbents is immediately evident. With untreated charcoal recovery even of anthranilic acid is low and the absence of material in the filtrate (cf. Table 8) shows that this low recovery is due to over-high affinity of the adsorbent. But whereas a comparatively low degree of deactivation suffices to make the adsorption of anthranilic acid in large degree reversible by aqueous phenol, yet in the case of both riboflavin and ferulic acid the degree of reversibility of the adsorption increases steadily with increasing degrees of deactivation.

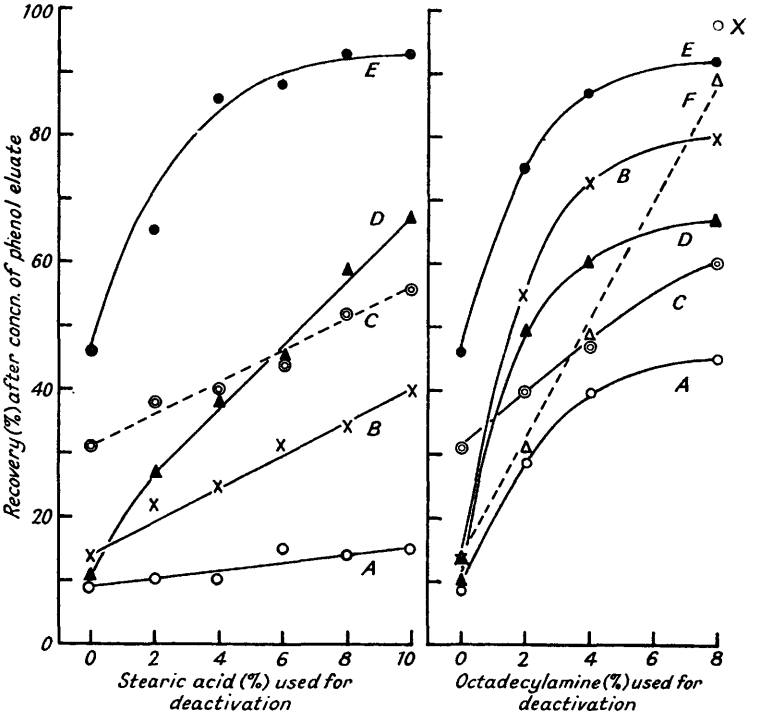
[11] Weil-Malherbe and Bone, *Biochem. J.*, 1952, **51**, 311.

This suggests that for routine use degrees of deactivation higher than the 4% previously used are desirable.

With basic substances the effect varies. Whereas recoveries of the weakly basic adenine improve with increasing degrees of deactivation, recoveries of the more strongly basic tyramine are hardly affected. As already mentioned, this is unlikely to be due to ion-exchange involving the stearic carboxyl group, as deactivation of the charcoal with octadecane (Tables 5 and 8) or paraffin-wax (Table 8) gives results similar to those obtained with stearic acid for all types of compound examined. Basic substances such as tyramine can be recovered in good yield from such adsorbents if an acid, instead of a neutral, eluant is used (cf. Table 5), but prolonged exposure to acid conditions is undesirable in isolation of natural products.

If the charcoal is deactivated with octadecylamine a different picture emerges (Fig. 2).

FIG. 1. FIG. 2.



A, Tyramine; B, adenine; C, riboflavin; D, ferulic acid; E, anthranilic acid; F, tryptamine.

X = Tyramine recovered on use of more adsorbent.

The effect of increasing degrees of deactivation on the recoveries of anthranilic acid, riboflavin, and ferulic acid are similar to those found with the acidic and neutral deactivating agents, but in the case of basic adsorbates increasing amounts of the basic deactivating agent result in markedly increased recoveries with a neutral eluant. This appears to be general for all basic substances investigated (cf. Tables 8 and 9), and the basic deactivating agent does not lead to lower recoverabilities of acidic substances such as indolylacetic acid and indoxyl sulphate (Table 9), compared with those when an acidic deactivating agent is used (cf. Table 4).

Increase in the degree of deactivation of the charcoal, of course, results in a decreased capacity for the adsorbate. *E.g.*, with a charcoal deactivated with 8% of octadecylamine much tyramine remains unadsorbed (Table 9) under the standard conditions of our experiments. But, as shown in Table 9, this difficulty can be overcome by increasing the amount

of the adsorbent, whereupon recoveries rise markedly. In other words, octadecylamine-deactivated charcoal permits reversible adsorption of a substance such as tyramine under essentially neutral conditions, whereas untreated or, *e.g.*, stearic acid-deactivated charcoals do not.

TABLE 8. *Recoveries (from 0·005M-solution) of some aromatic substances after various pre-treatments of the charcoal.*

Deactivating agent	Deactivator (%) *	Anthranilic acid : filtrate	Anthranilic acid : eluate	Ferulic acid : filtrate	Ferulic acid : eluate	Riboflavin : filtrate	Riboflavin : eluate	Adenine : filtrate	Adenine : eluate	Tyramine : filtrate	Tyramine : eluate
None	0	0	46	0	10	0	31	0	14	0	9
Stearic acid	2	0	65	0	27	0	38	0	22	0	10
,,	4	0	86	0	38	0	40	0	25	0	10
,, ...	6	1	88	1	45	0	44	0	31	15	15
,, ...	8	2	93	1	59	0	52	2	34	30	14
,, ...	10	4	93	6	67	0	56	4	40	33	15
Octadecane	2	0	80	0	23	0	33	0	24	0	13
,, ...	4	1	92	0	43	0	40	3	33	1	14
,, ...	8	7	95	4	63	0	52	2	49	13	20
Paraffin-wax ...	2	0	75	0	49	0	36	0	28	6	12
,, ...	4	0	91	0	65	0	44	2	55	15	29
,, ...	8	1	94	0	73	0	47	2	60	20	35
Octadecylamine	2	0	75	0	49	0	40	0	55	13	29
,,	4	0	87	0	60	0	47	2	73	25	40
,,	8	1	92	0	67	0	60	4	80	49	45

* Percentage by weight of deactivating agent relative to weight of charcoal.

TABLE 9. *Adsorption (from 0·005M-solutions) by octadecylamine-deactivated charcoal.*

Deactivation (%) *	Weight of adsorbent (g.) †	Tyramine : filtrate	Tyramine : eluate	Tryptamine : filtrate	Tryptamine : eluate	5-Hydroxytryptamine : filtrate	5-Hydroxytryptamine : eluate	Adrenaline : filtrate	Adrenaline : eluate
0	1	0	9	0	14	0	7	0	11
2	1	13	29	0	31	0	40	9	35
4	1	25	40	0	49	1	53	14	47
8	1	49	45	9	89	2	90	26	53
8	2	16	72	0	93	0	90	9	62
8	4	4	98	0	93	0	87	3	80

By use of 8% deactivated charcoal under the standard conditions the values obtained for indolylacetic acid were: filtrate, 0; eluate, 79%; for indican, filtrate, 5; eluate, 55%.

* See footnote to Table 7.

† Under the standard conditions of the previous tables, 1 g. of adsorbent was used in each case.

The percentage recoveries reported above were obtained by using simple aqueous solutions. They therefore take no account of the competition which is to be expected if adsorption is carried out from a complex mixture such as obtains in urine. This aspect is under investigation. However, competition should not prevent recovery of all aromatic substances, provided adsorption is reversible, and an adequate amount of adsorbent is used. The development of adsorbents on which metabolites can be reversibly adsorbed is being further investigated.

EXPERIMENTAL

Adsorbents.—Activated charcoal (British Drug Houses Ltd.) was used directly, or after deactivation. Charcoal was deactivated with stearic acid, octadecane, or octadecylamine by stirring it with an appropriate amount of a 1·5% (w/v) solution of the deactivating agent in ethanol for 1 hr., and diluting the mixture with water (9 l. per l. of ethanol) slowly with stirring. It was collected on a Buchner funnel, washed with water, and dried in air.

Deactivation with paraffin-wax was carried out by adding the charcoal (100 g.) to an appropriate amount of a 1% (w/v) solution of paraffin wax (m. p. 57°) in light petroleum (b. p. 60—80°) and periodically shaking the mixture. After 1 hr. the charcoal was filtered off, suspended in 400 ml. of ethyl acetate, again filtered off, suspended in 400 ml. of ethanol, again filtered off, suspended in water, filtered off, and air-dried.

Eluants.—Aqueous phenol was prepared by diluting 80 ml. of phenol liquefactum, B.P., to

1 l. with water (crystalline phenol is equally effective but dearer). The final phenol concentration is about 7·2%, but is not critical. Phenol–hydrochloric acid, phenol–acetic acid, and phenol–ammonia represent 5 vol. of the above aqueous phenol with 1 vol. of concentrated hydrochloric acid, glacial acetic acid, or concentrated ammonia (*d* 0·88) respectively.

Standard Experimental Procedure.—Before adsorption all solutions were adjusted with acetic acid to pH 4, unless otherwise stated. To 25 ml. of an appropriate concentration of solution of the substance being investigated was added 1 g. of deactivated or untreated charcoal, and the mixture shaken at intervals for 15 min. It was then filtered on a Buchner funnel (5 cm. diam.) and the charcoal washed with 75 ml. of water. The filtrate and washings were combined and concentrated, and their content of adsorbate determined. This value is entered under "filtrate" in the Tables. The charcoal was then eluted by slowly passing 50 ml. of aqueous phenol (or in the experiments with acid and ammoniacal phenol, with an appropriate mixture of 50 ml. of aqueous phenol and 10 ml. of acid or ammonia), followed by 30 ml. of water. The combined eluate and subsequent washings were evaporated to dryness under reduced pressure (water-pump) in a water-bath held below 80°. Water was then added, and the whole reconcentrated to dryness (the second evaporation with water ensured removal of phenol). The residue was made up to a known volume, and the adsorbate determined by an appropriate method. The value so obtained is given under "eluate" in the Tables. With conjugated substances chromatograms of the concentrated eluate were run to check that hydrolysis had not occurred.

Alumina Adsorption Procedure.—The solution (25 ml.), previously adjusted to pH 4 with acetic acid, was shaken with alumina (7 g.; Savory and Moore, Ltd., "for chromatographic analysis"). The alumina was filtered off on a Buchner funnel, washed with water, and then eluted with 70 ml. of 0·1N-hydrochloric acid, and the eluate concentrated to dryness as above before estimation.

Determination of Adsorbates.—Since the recovery of single substances was being determined, relatively unspecific methods could be used. In this general survey a high degree of accuracy was unnecessary to gain the overall picture and we therefore accepted methods accurate to within ± 5%, though we consider the accuracy of most of our determinations to be better than this. Most substances were determined colorimetrically, and in cases where the estimation procedure used was not known to be applicable to the particular compound, a standard curve was first prepared to ensure that only a linear region of the curve was used. The following were the methods employed :

Inorganic ions. Chloride ion was determined iodometrically as described by King.[12] Ammonium ion was determined by Nesslerisation as in the urea procedure described by King.[12] All other ions were determined by measuring the activity of isotopically labelled solutions. ^{24}Na and ^{131}I were determined by using a "Coronet" counter[13] (E.R.D. Engineering Co., Slough); $^{32}PO_4$ and ^{42}K were measured in a liquid counter;[14] $^{35}SO_4$ and ^{45}Ca were measured by "infinite thinness" counting of solutions distributed on lens tissue and dried.

Aliphatic substances. Urea, creatinine, glucose, and galactose were determined as by King.[12] King's glucose method[12] was found suitable for lactose. Glucosamine was determined with ninhydrin as for amino-acids.

Amino-acids. The ninhydrin procedure[15] was used except for tyrosine (Folin–Ciocalteu) and tryptophan (Ehrlich).

Phenols. The Folin–Ciocalteu procedure was used as described by King[12] for the determination of phenol in the King–Armstrong phosphatase procedure. The same procedure was used with dihydric phenols.

Aromatic amines. Bratton and Marshall's procedure[16] was used.

Indoles. The Ehrlich reaction was used as described by Horn and Jones[17] for tryptophan, except for indican,[18] indolylacetic acid,[19] and 5-hydroxytryptamine.[20]

Pyrroles. Watson and Schwartz's procedure[21] was used for porphobilinogen, and Ehrlich's reagent, as in the Horn and Jones[17] procedure, for 2-carboxypyrrole.

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Glyoxalines. Histamine was determined colorimetrically after coupling with diazotised sulphanilic acid and basification. Urea was added to remove excess of nitrite and prevent colour fading.

Purines and pyrimidines. Uric acid was determined as by King,[12] adenine according to Woodhouse,[22] guanine and xanthine by the Folin–Ciocalteu procedure (as for phenols), cytosine and uracil as by Svodak *et al.*,[23] and thymine as by Woodhouse.[24]

Conjugated substances. Those with a readily determinable group were determined directly, *e.g.*, *m*-aminophenylglucuronide by Bratton and Marshall's procedure.[16] Others were first hydrolysed, *e.g.*, phenolic sulphates were determined as phenols after hydrolysis. Hippuric acid was determined by the procedure of Gaffney *et al.*[25] The mercapturic acid, *N*-acetyl-(2 : 3 : 5 : 6-tetrachlorophenyl)cysteine, was determined by iodine titration after alkaline hydrolysis (method based on ref. 26).

Miscellaneous. Nicotinic acid, nicotinamide, and riboflavin were determined by their ultraviolet absorption at 262, 262, and 450 mμ respectively.

We thank Professors A. Neuberger, F.R.S., C. Rimington, F.R.S., and R. T. Williams and Drs. J. Harley-Mason, G. A. Stein, and W. V. Thorpe for gifts of substances.

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