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D. THE ESTIMATION OF METANEPHRINE, NORMETANEPHRINE, AND 3,4-DIHYDROXYMANDELIC ACID IN URINE

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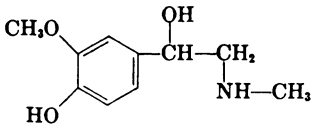
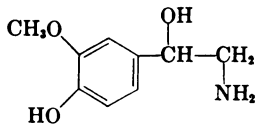
A. THE ESTIMATION OF METANEPHRINE AND NORMETANEPHRINE

Earlier methods for the estimation of metanephrine and normetanephrine in urine were based on their separation by paper chromatography or paper electrophoresis. The competence of these techniques is undeniable and they are still being used successfully (*e.g.*, 6, 15). However, their limitations soon led to a search for methods more suitable for the routine estimation of low concentrations in large series of samples. We intend briefly to review four such methods in all of which the final assay is fluorimetric. The fluorophors are oxidation products of metanephrine and normetanephrine; their spectra of excitation and emission are identical with those of adrenolutin and noradrenolutin and it is generally assumed that metanephrine and normetanephrine are converted to adrenolutin and noradrenolutin after oxidative demethylation. The reaction was first demonstrated by Bertler *et al.* (2) and their method was subsequently adapted for urine analysis by Häggendal (7). Smith and Weil-Malherbe (14) developed a method based on the same principle. More recently two more methods were published by Brunjes *et al.* (4) and Taniguchi *et al.* (16).



metanephrine



normetanephrine

While these four methods thus have essential features in common, they differ in important details. The several stages of the procedures have been compared in table 1. Since presumably the fluorophors formed from the catecholamines are identical with those formed from their O-methylated metabolites, catecholamines have to be eliminated at some stage. Frequently, a simultaneous estimation of both groups of amines would be of advantage; their separation is easily accomplished by passing the urine through a column of alumina. Metanephrine and normetanephrine, which are contained in the filtrate, are then extracted by passage through a cation exchange resin of either the carboxylic or the sulfonic acid type. The sulfonic acid resins, such as Dowex 50 or Amberlite CG 120, have the advantage of coping with a higher salt concentration, but they also require larger volumes or higher ionic concentrations for elution, either of which may be troublesome in fluorimetry. Conversely, elution from carboxylic acid resin is usually achieved with ease, but the retention of amines is liable to be incomplete, unless the salt concentration is low.

TABLE 1

Comparison of fluorimetric methods for the estimation of metanephrine (M) and normetanephrine (NM) in urine

	Smith and Weil-Malherbe (14)	Brunjes, Wybenga and Johns (4)	Taniguchi, Kakimoto and Armstrong (16)	Häggendal (7)
Removal of catecholamines	Alumina	Alumina	Decomposition in alkaline medium	Oxidation with ferricyanide
Extraction of amines	Amberlite CG 50	Amberlite CG 50	1) Dowex 50; 2) Amberlite CG 50. Fractional elution of M and NM from Amberlite	Amberlite CG 120
Oxidation (final concentrations)	*M* 1.36×10^{-4} M $Fe(CN)_6^{3+}$, 1.56×10^{-3} M Zn^{2+}, pH 3.0 *NM* 3.28×10^{-4} M I_2, pH 7.9	*M* + *NM*[a] First stage: 5.7×10^{-4} M $Fe(CN)_6^{3+}$, 4.36×10^{-3} M Zn^{2+}, pH 4.1 Second stage: 4.56×10^{-4} M $Fe(CN)_6^{3+}$, 3.48×10^{-3} M Zn^{2+}, pH 12.5 with buffer as published, pH 7.8 if 1 N NaOH is used instead of 10 N	*M* 0.58×10^{-3} M KIO_4, pH 4.5 *NM* 0.58×10^{-3} M KIO_4, pH 5.0	*M* + *NM* 1.1×10^{-3} M I_2, pH 6.5
Tautomerization	Alkaline ascorbate	Alkaline ascorbate	Alkaline ascorbate	Alkaline sulfite (pH adjusted to 5.3 for fluorimetry)
Differentiation of M and NM	Oxidation at different pH levels	Differential spectrofluorimetry	Chromatographic separation	Differential spectrofluorimetry
Milliliters of urine equivalent in 3 ml fluorescent solution	6.1 (M) 5.7 (NM)	0.1[a]	1.3–3.2	0.72
Relative fluorescence intensities[b]	M: 100 NM: 100	55 215 (oxidation at pH 7.8)	6 39	33 103
Remarks	Electrodialysis equally reduces varying salt concentrations of different urines and allows larger volumes to be used per column. Fluorophors stable for 1 hr	M and NM not independently measured. Because of low readings, small differences produce large differences in results. Fluorophors unstable	Chromatographic separation could not be reproduced in our hands. Very slow Low sensitivity High blank Fluorophors very unstable	M and NM not independently measured

[a] According to conditions specified in the addendum.

[b] The scale of the different methods was adjusted to a total volume of about 3 ml in every case.

In unhydrolyzed urine, only the free bases are retained on cation exchange resin, whereas the conjugates pass into the filtrate. By repeating the cycle of adsorption and elution after hydrolysis of the filtrate, it is possible to estimate the free and conjugated amines separately. Another way of separating the conjugated from the free amines is by adsorption of the former on the anion exchange resin Dowex 1, according to LaBrosse and Mann (10). As Armstrong and his co-workers (8, 16) have shown, the unconjugated fraction of the amines accounts for 10 to 20% of the total. In many situations the estimation of the sum total of the free and conjugated forms will suffice. The main conjugate in man appears to be the sulfate (10, 14) which is hydrolyzable at 100°C and a pH of about 1.5.

The method of Häggendal (7) is alone in using an eluate from a sulfonic acid resin directly for fluorimetry. We have no personal experience of this method, but according to Randrup (12) such eluates are frequently colored and have high blanks. In the other three methods an eluate from a carboxylic acid resin is used in the fluorimetric assay. The required degree of desalination is achieved by dilution in the method of Brunjes *et al.* (4), by a prior adsorption-elution cycle on Dowex 50 in the method of Taniguchi *et al.* (16), and by electrodialysis in the method of Smith and Weil-Malherbe (14). Owing to the extensive dilution prescribed by Brunjes *et al.*, the final fluorescence in this method represents a urine equivalent which is about one-sixtieth of that in the method of Smith and Weil-Malherbe and down to one-thirtieth of that in the method of Taniguchi *et al.* Readings are consequently very low in relation to the blank and are difficult to reproduce.

The design of the electrodialyzer originally described by us has been modified by the incorporation of a heat-exchanger within the central compartment so as to keep the temperature of the sample below 40°C (fig. 1, ref. 20). The heat exchanger consists of two Lucite jackets screwed onto the sides of the sample chamber and a number of thin glass tubes traversing the chamber from one jacket to the other.

Electrodialysis appears to be a somewhat critical procedure. If the correct degree of desalination is not reached, retention on the resin may be inadequate; undue prolongation, on the other hand, may result in losses of amines. It has been our practice to start the procedure with a current of 1 amp and to stop it when the current had fallen by about 50%. Drops of concentrated ammonia solution were added at intervals to keep the pH at about 10.5. We feel, however, that the conditions ought to be more rigorously defined, both in terms of conductivity and pH, so as to balance initial differences in the salt concentration and buffering power of different urines. Experiments in this direction are now underway in our laboratory.

Taniguchi *et al.* (16) succeeded in separating metanephrine and normetanephrine by fractional elution from the Amberlite column. Repeated and careful attempts were made in our laboratory to reproduce this separation but without success. The elution curves of the authentic substances showed considerable overlap, and when recovery experiments were conducted with samples of urine,

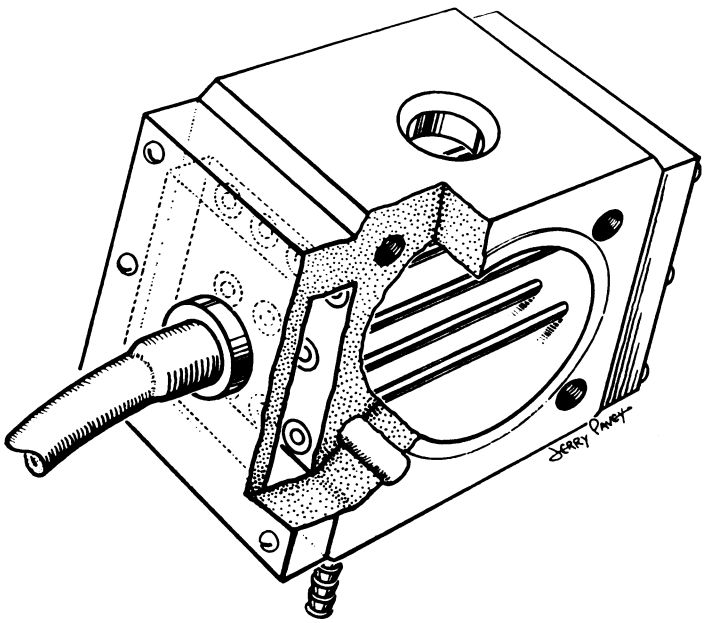


FIG. 1. Electrodialysis apparatus (center part). The cut-away shows one of the two water jackets and some of the cooling tubes connecting them. (From Z. Klin. Chem. 2: 161, 1964.)

normetanephrine was eluted in the fraction which was supposed to contain only metanephrine. We do not claim that the method of Taniguchi *et al.* cannot be made to work, but it seems to require a degree of skill and patience which is not readily available in the average laboratory.

In the other three methods under review metanephrine and normetanephrine are differentiated without actual separation, either optically as in the method of Brunjes *et al.* and in that of Häggendal or by oxidation at different levels of pH as in the method of Smith and Weil-Malherbe. The latter method seems to us to have advantages, since optical differentiation is more susceptible to interference from impurities which, by causing small differences in readings, may produce disproportionate effects on results.

As oxidizing agents ferricyanide, iodine, and periodate have been used. Of these, ferricyanide, which requires the presence of zinc ions at about 10-fold higher concentration, gives the highest fluorescence yield. The fluorescence yield of iodine is only about half that of ferricyanide, and the use of periodate, as described by Taniguchi *et al.*, gave even lower degrees of fluorescence in our hands. In the method of Smith and Weil-Malherbe, metanephrine alone is oxidized by ferricyanide at pH 3, whereas both metanephrine and normetanephrine are simultaneously oxidized at a pH near neutrality. For this step we use iodine because the fluorophors formed by ferricyanide at a pH of about 8 are unstable and decay at unequal rates; this would make the timing of readings

unduly critical. In the method of Brunjes *et al.* oxidation by ferricyanide is carried out in two steps. In the first stage of oxidation, presumably mainly aimed at metanephrine, the pH is 4.1. After 4 min the pH is raised by the addition of buffer to a value which, according to these authors, is 5.5. However, with the buffer specified we found the pH to be actually between 12 and 13 and the resulting fluorescence to be very weak. When the buffer was prepared with 1 N instead of 10 N NaOH, the pH was 7.8 and under these conditions metanephrine fluorescence was 55% of that obtained in our method at pH 3. Normetanephrine, on the other hand, gave about twice as much fluorescence as after iodine oxidation, although it decayed rapidly.

Häggendal, who uses iodine as oxidant, recommends pH adjustment to 5.3 after tautomerization in alkaline sulfite solution. The fluorescence obtained by this procedure is neither higher nor more stable than that obtained in our method.

Results obtained by different methods and authors have been assembled in table 2. It appears that our results tend to be somewhat lower than the rest and this tendency is particularly marked in the more recent results, shown in the last line, which were obtained in a study on patients hospitalized for depression. We were naturally concerned about the possibility that our recoveries had deteriorated. A recent series of recovery experiments is shown in table 3. These recoveries are indeed a little lower than those previously obtained, which were

TABLE 2

Normal excretion rates of total (free + conjugated) metanephrine and normetanephrine

Method	No. of observations	Metanephrine	Normetanephrine	Reference
Paper chromatography, absorptiometry	6	330 μg/24 hr	640 μg/24 hr	9
Paper electrophoresis, absorptiometry	?	100–300 μg/24 hr	100–300 μg/24 hr	22
Amberlite CG 50, paper chromatography, absorptiometry	50	112 μg/24 hr	116 μg/24 hr	15
Dowex 50, paper chromatography	55	28–84 μg/g creat.	32–100 μg/g creat.	8
Dowex 50, paper chromatography, absorptiometry	?	25–60 μg/g creat.	40–80 μg/g creat.	6
Dowex 50, Amberlite CG 50, fluorimetry	6 30	164 μg/24 hr 63.5 μg/g creat.	245 μg/24 hr 98.4 μg/g creat.	16
Amberlite CG 50, fluorimetry	47	96 mμg/min	198 mμg/min	3
Amberlite CG 50, fluorimetry	19	72.1 μg/24 hr	158.6 μg/24 hr	14
Amberlite CG 50, fluorimetry	372	39.6 μg/24 hr	56.7 μg/24 hr	21

TABLE 3

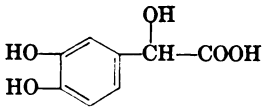
Recovery of metanephrine and normetanephrine from urine (additions were made before hydrolysis)

Metanephrine			Normetanephrine		
Added	Found	Recovery	Added	Found	Recovery
μg/25 ml		%	*μg/25 ml*		%
—	0.160		—	0.600	
0.5	0.555	79.0	—	0.623	
—	0.152		1.0	1.300	70.0
—	0.180		—	0.880	
0.5	0.512	66.5	—	0.912	
—	0.172		1.0	1.600	72.0
—	0.160		—	0.600	
0.2	0.322	81.0	—	0.584	
	0.166		0.5	0.878	55.6
—	0.160		—	0.600	
0.2	0.326	83.0	0.5	0.932	66.4
0.2	0.300	70.0	0.5	0.880	56.0
0.5	0.508	69.6	1.0	1.348	74.8
—	0.214		—	0.436	
0.2	0.320	53.0	0.5	0.684	49.6
—	0.570		—	0.760	
0.5	1.070	100.0	1.0	1.480	72.0
—	0.200		—	0.667	
0.2	0.380	90.0	0.5	0.900	46.6
—	0.220		—	0.560	
0.2	0.370	75.0	0.5	1.040	96.0
—	0.122		—	0.425	
0.2	0.254	66.0	0.5	0.750	65.0
—	0.146		—	0.450	
0.5	0.388	48.4	1.0	1.100	65.0
—	0.122		—	0.425	
0.2	0.206	42.0	0.5	0.600	35.0
—	0.292		—	0.900	
0.2	0.432	70.0	0.5	1.224	64.8
Mean ± S. E. M.		71.0±4.23			63.5±3.92

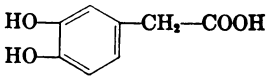
92% for metanephrine and 73% for normetanephrine (14), but our recent results remain low even when they are corrected according to our latest recovery figures. At present we have no explanation for this. We have now started a series of recovery experiments employing tracer amounts of radioactive amines and hope that these will throw more light on the problem.

B. THE ESTIMATION OF 3,4-DIHYDROXYMANDELIC ACID

3,4-Dihydroxymandelic acid (doma) may be converted *in vitro* to protocatechuic aldehyde in a reaction analogous to the oxidation of vanilylmandelic acid (VMA) to vanillin. In fact, the reaction occurs much more readily and under milder conditions with doma than with VMA. Miyake *et al.* (11) recommend heating at 100° in 10 M acetic acid. A more detailed study of this reaction in our laboratory (18) showed that it depends on the presence of traces of heavy metals, particularly copper. It is completely inhibited by the exclusion of oxygen or by the addition of chelators. In the presence of 2.7×10^{-4} M cupric ions the reaction rate increases and the stability of the reaction product decreases with increasing pH (fig. 2). An acceptable compromise is reached if the reaction is carried out at pH 4 and 75°.



3,4-dihydroxymandelic acid (doma)



3,4-dihydroxyphenylacetic acid (dopac)

The analytical usefulness of this reaction is however marred by interference from 3,4-dihydroxyphenylacetic acid (dopac) which forms the same end product although at a slower rate. The oxidation of the α-CH_2-group may appear surprising, but the identity of the reaction product with protocatechuic aldehyde has been demonstrated by the identity of the absorption spectra at various levels of pH, by paper chromatography, paper electrophoresis, and by color reactions with indole and with thiobarbituric acid. It is interesting to note that homovanillic acid is similarly oxidized to vanillin by cupric salt in an alkaline medium (19). In the absence of copper the oxidation of both doma and dopac was negligible (fig. 3). In its presence doma was oxidized much more rapidly than dopac, which, it should be noted, was added in 2.5 times the concentration of doma. In view of this difference in reaction rate attempts were made to differentiate the two acids by kinetic analysis. However, the oxidation of mixtures of the two acids always gave smooth curves without obvious break. It seemed as if the oxidation of dopac was accelerated by the simultaneous oxidation of doma.

More recently, De Quattro *et al.* (5) published a modification of the method of Miyake *et al.* in which doma is oxidized by shaking an ethyl acetate extract containing the acid with 4 N ammonia for 3 min. The aldehyde is stabilized by the addition of bisulfite. Under these conditions dopac is converted to a yellow compound with an absorption peak at 390 mμ and isosbestic points at 348 and 420

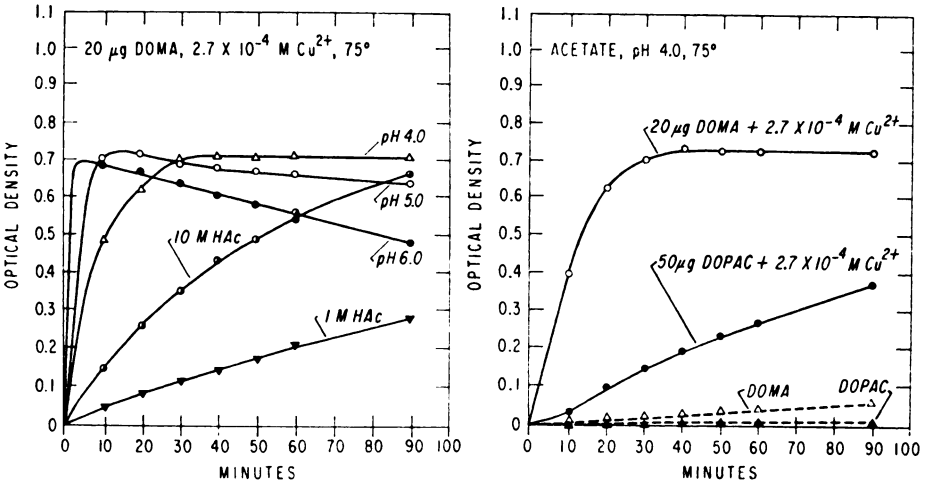


FIG. 2. Formation of protocatechuic aldehyde from doma at different levels of pH. Experiments at pH 4.0, 5.0 and 6.0 were carried out in 1 M acetate buffers. Optical density was read at 311 mμ.

FIG. 3. Formation of protocatechuic aldehyde from doma and dopac in the presence and absence of Cu^{2+}. 1 M Acetate buffer pH 4.0, previously treated with Dowex chelating resin A-1 (about 5% by volume), was used in the experiment.

mμ. The absorption maximum of protocatechuic aldehyde in alkaline medium is at 348 mμ; to correct for the presence of dopac, De Quattro *et al.* suggested subtracting the reading at 420 mμ from that at 348 mμ.

We have confirmed that, under the conditions used by De Quattro *et al.*, the oxidation of dopac yields a product different from protocatechuic aldehyde. However, in our experiments, a peak developed at 390 mμ only in the absence of bisulfite. Three absorption spectra obtained from dopac under different conditions of oxidation are shown in figure 4. Curve 1 shows the result of shaking a solution of 40 μg dopac in 30 ml of water-saturated ethyl acetate with 1.5 ml 4 N NH_3 for 3 min and running it into bisulfite solution, as described by De Quattro *et al.* No peak was observed at 390 mμ under these conditions and the correction of De Quattro *et al.* would have been grossly inadequate. In the experiment leading to curve 2 the ammonia extract was added to a solution of 0.1 N ammonia (without bisulfite); otherwise the conditions were identical with those of the preceding experiment. This curve comes closest to the description given by De Quattro *et al.* In the experiment which produced curve 3 the concentration of dopac was increased 25-fold and the ammonia extract was diluted in the same proportion with 0.1 N ammonia before absorptiometry. Here the secondary peak was much more marked and the correction of De Quattro *et al.* would have been excessive. Curve 3 is similar to, though probably not identical with the absorption spectrum of δ-carboxymethyl-α-hydroxymuconic semialdehyde, which is formed from dopac, with the opening of the benzene ring, by an adaptive enzyme from *Pseudomonas ovalis* (1). The oxidation product formed from dopac in the

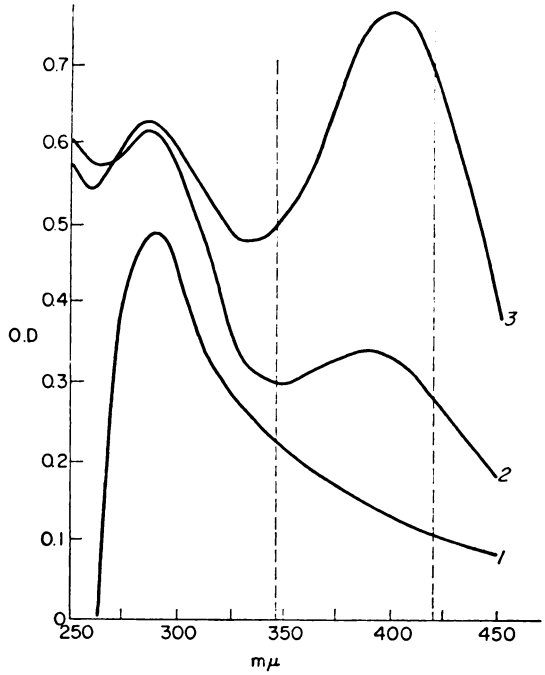


FIG. 4. Absorption of dopac after extraction with 4 N ammonia from ethyl acetate. Curve 1: 40 μg dopac extracted and extract added to bisulfite solution according to De Quattro *et al.* (5). Curve 2: 40 μg dopac extracted, extract diluted with 1 part 0.1 N NH_3. Curve 3: 1000 μg dopac extracted, extract diluted with 25 parts 0.1 N NH_3.

reaction of De Quattro *et al.* forms a dinitrophenylhydrazone which does not melt below 300°C. It is thus probably an aldehyde but it no longer gives the purple color reaction with indole.

The problem of oxidizing doma to protocatechuic aldehyde without interference from dopac has now been overcome with an enzymatic method. Following the example of Rosano (13), who described the estimation of VMA with the aid of an L-mandelic acid dehydrogenase from specially adapted cells of *Pseudomonas fluorescens*, we found that the same enzyme oxidizes doma but is quite inactive towards dopac. The reaction is allowed to proceed at pH 5.5, where the nonenzymatic oxidation of doma and dopac is negligible, especially when the medium contains EDTA; it is observed by following the increase of absorption at 311 mμ, the absorption maximum of the aldehyde at pH 5.5. After the addition of enzyme solution containing 0.1 mg of protein per ml of test solution the reaction is completed in 2 to 3 hr at room temperature. The enzyme appears to be specific for mandelic acids. VMA and *p*-hydroxymandelic acid are of course eliminated by the preliminary adsorption of doma on alumina. Some urine extracts have a high initial absorption and have to be diluted, but enzyme inhibition does not appear to be a problem.

C. THIN-LAYER CHROMATOGRAPHY OF NONBASIC CATECHOLIC CONSTITUENTS OF URINE

A procedure for the simultaneous estimation of catecholamines and several catecholamine metabolites in urine has recently been described (20). In it, urine, at pH 8.4, is passed through a column of alumina. The column is eluted first with 0.2 N acetic acid and then with 1 N sulfuric acid. The acetic acid eluate, at pH 6.0, is further passed through a column of cation exchange resin and the filtrate combined with the sulfuric acid eluate from the alumina column. This fraction which contains the nonbasic catechols of urine has been studied by thin-layer chromatography. After extraction with ethyl acetate and evaporation of the solvent, the residue was applied to plates covered with a 0.25 mm layer of polyamide powder (E. Merck, W. Germany) and developed in two directions, first with toluene-ethyl acetate-isopropanol-acetic acid, 40:35:20:5, and afterwards with methanol-formic acid-water, 37.5:2.5:60, as solvents. The chromatograms were sprayed with a mixture of equal volumes of ethylenediamine, concentrated ammonia, and water and inspected after 24 hr in ultraviolet light. Good resolution was obtained with a mixture of the following reference compounds: catechol, protocatechuic acid, protocatechuic aldehyde, 3,4-dihydroxyphenylacetic acid (dopac), caffeic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxyphenylpyruvic acid, 3,4-dihydroxyphenyllactic acid, and 3,4-dihydroxyphenylglycol. Chromatograms of urine eluates usually showed spots in the positions of protocatechuic acid, catechol, and dopac, as well as several unidentified spots, but doma was usually not detectable or present only in traces. The unidentified spots seemed to correspond to nonacidic compounds since they were not retained on a column of the anion exchanger DEAE-Sephadex A-25. Nor did they react with 2,4-dinitrophenylhydrazine, with the exception of a single spot in a single chromatogram.

REFERENCES

1. Adachi, K., Takeda, Y., Senoh, S. and Kita, H.: Metabolism of *p*-hydroxyphenylacetic acid in *Pseudomonas ovalis*. Biochim. biophys. Acta **93**: 483–493, 1964.
2. Bertler, A., Carlsson, A. and Rosengren E.: Fluorimetric method for differential estimation of the 3-O-methylated derivatives of adrenaline and noradrenaline (metanephrine and normetanephrine). Clin. chim. Acta **4**: 456–457, 1959.
3. Brunjes, S.: Catecholamine metabolism in essential hypertension. New Engl. J. Med. **271**: 120–121, 1964.
4. Brunjes, S., Wybenga, D. and Johns, V. J., Jr.: Fluorometric determination of urinary metanephrine and normetanephrine. Clin. Chem. **10**: 1–12, 1964.
5. De Quattro, V., Wybenga, D., Studnitz, W. von and Brunjes, S.: Determination of urinary 3,4-dihydroxymandelic acid. J. Lab. clin. Med. **63**: 864–878, 1964.
6. Gjessing, L. R.: Studies of periodic catatonia. II. The urinary excretion of phenolic amines and acids with and without loads of different drugs. J. psychiat. Res. **2**: 149–162, 1964.
7. Häggendal, J.: Fluorimetric determination of 3-O-methylated derivatives of adrenaline and noradrenaline in tissues and body fluids. Acta physiol. scand. **56**: 258–266, 1962.
8. Kakimoto, Y. and Armstrong, M. D.: The phenolic amines of human urine. J. biol. Chem. **237**: 208–214, 1962.
9. Kraupp, O., Bernheimer, H. and Papistas, D.: Isolierung und quantitative Bestimmung von 3-O-Methyladrenalin und 3-O-Methylnoradrenalin im Harn. Clin. chim. Acta **6**: 851–860, 1961.
10. La Brosse, E. H. and Mann, J. D.: Presence of metanephrine and normetanephrine in normal human urine. Nature, Lond. **185**: 40, 1960.
11. Miyake, H., Yoshida, H. and Imaizumi, R.: Determination methods for urinary 3-methoxy-4-hydroxymandelic acid and 3,4-dihydroxymandelic acid. Jap. J. Pharmacol. **12**: 79–92, 1962.
12. Randrup, A.: On the differential fluorimetric determination of metaadrenaline and normetadrenaline. Clin. chim. Acta **6**: 584–586, 1961.

13. Rosano, C. L.: Enzymatic method for determination of vanillylmandelic acid. Clin. Chem. **10**: 673–677, 1964.
14. Smith, E. R. B. and Weil-Malherbe, H.: Metanephrine and normetanephrine in human urine: method and results. J. Lab. clin. Med. **60**: 212–223, 1962.
15. Spengler, G. A., Käser, H. and Riva, G.: Die Bestimmung von Metanephrin und Normetanephrin im Urin. Schweiz. med. Wschr. **93**: 1684, 1963.
16. Taniguchi, K., Kakimoto, Y. and Armstrong, M. D.: Quantitative determination of metanephrine and normetanephrine in urine. J. Lab. clin. Med. **64**: 469–484, 1964.
17. Wada, Y.: Quantitative determination of 3,4-dihydroxymandelic acid (doma) in human urine. Tohoku J. exper. Med. **79**: 389–400, 1963.
18. Weil-Malherbe, H.: Studies on the estimation of acidic metabolites of catecholamines. Fed. Proc. **23**: 491, 1964.
19. Weil-Malherbe, H.: Studies on the estimation of 3-methoxy-4-hydroxymandelic acid (VMA) in human urine. Anal. Biochem. **7**: 485–494, 1964.
20. Weil-Malherbe, H.: The simultaneous estimation of catecholamines and their metabolites. Z. klin. Chem. **2**: 161–167, 1964.
21. Weil-Malherbe, H. and Smith, E. R. B.: Unpublished results.
22. Yoshinaga, K., Hoh, C., Ishida, N., Sato, T. and Wada, Y.: Quantitative determination of metadrenaline and normetadrenaline in normal human urine. Nature, Lond. **191**: 599, 1961.