Advanced Screening Test for Pheochromocytoma and Argentaffinoma

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INTRODUCTION

Previously we reported a very simple screening test for pheochromocytoma which consists of a characteristic color reaction of catecholamine metabolites, metadrenaline (MA), normetadrenaline (NMA) and vanillylmandelic acid (VMA), to diazotized p-nitroaniline1). Namely, when urine samples from patients with this tumor who excrete abnormally large amounts of these compounds are plotted on filter paper, a distinct violet color is developed by spraying this reagent. And the urine in test tube shows a wine color with addition of the reagent. However, if urine contains these metabolites in slightly increased level above the normal upper limit, the result may be equivocal. In such a borderline case, the development of color on raw urine spot or in test tube is disturbed by the interference of other urinary compounds. Diazotized p-nitroaniline is not a special reagent for catecholamine metabolites, and the numerous other urinary phenols also give color reaction with this reagent. Thus, it will be very convenient when these metabolites are separated by a simple procedure from other components before color development.

For this purpose, we employed Curzon’s method2) which is based on single dimensional paper chromatography using NaCl-acetic acid solution, and developed an advanced test method for pheochromocytoma. In addition, we found that this test enables detection of argentaffinoma also.

REAGENTS AND PROCEDURES

No special apparatus was employed (Fig. 1). The chromatographic solvent for separation of MA, NMA, VMA and 5-hydroxyindoleacetic acid (5-HIAA) consisted of 8 g. of NaCl dissolved in 100 ml. of water + 1.0 ml. of glacial acetic acid. 0.05 ml. of fresh raw urine was plotted on filter paper (Toyo Roshi
No 51) with a micro-pipette and dried by a warm-air current. The sheet was stood for 30 to 45 min. in the solvent. During this period, the solvent traveled about 13 cm. at room temperature. The chromatogram was then dried in an oven, and was sprayed with diazotized p-nitroaniline. This reagent was prepared as follows; (1) 0.1 g. of p-nitroaniline dissolved in 2 ml. of conc. HCl and diluted with water to 100 ml. (2) 0.2% aqueous solution of sodium nitrite. (3) 10% aqueous solution of potassium carbonate. All these solutions were kept in a refrigerator. Just before use, one volume of (1), one volume of (2) and two volumes of (3) were mixed. The mixture must be used within 120 seconds.

RESULTS

Using this solvent, catecholamine metabolites and 5-HIAA traveled separately from each other but former compounds ran to almost the same Rf. The approximate Rf values obtained with pure substances are summarized in Tab. I. Although distilled water and 8% aqueous solution of NaCl can be used as chromatographic solvent, a satisfactory separation of these compounds was obtained with NaCl-acetic acid solution.

Diazotized p-nitroaniline gave violet color to MA, NMA and VMA and red to 5-HIAA. Moreover, its sensitivity was considerably high. Ehrlich's reagent (p-dimethylaminobenzaldehyde in HCl) showed only 5-HIAA in blue grey color and required additional procedure, heating at 60°C for 15 to 20 min. to develop color.

Normal urine chromatograms usually show red, orange and yellow spots
TABLE I. Rf values of Catecholamine Metabolites and 5-HIAA.

<table>
<thead>
<tr>
<th>Substance</th>
<th>NaCl-acetic acid</th>
<th>Aqueous NaCl</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>0.82</td>
<td>0.81</td>
<td>0.17</td>
</tr>
<tr>
<td>NMA</td>
<td>0.78</td>
<td>0.78</td>
<td>0.17</td>
</tr>
<tr>
<td>VMA</td>
<td>0.86</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>0.41</td>
<td>0.55</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Fig. 2. Diagrammatical reproduction of chromatogram. 
I. and II.: Pheochromocytoma, III.: Argentaffinoma, 
IV. and V.: Normal control.
at the different Rfs from catecholamine metabolites or 5-HIAA. This pattern was shown by 120 control urine samples. Occasionally, a light violet spot at Rf of 0.80 was observed. In addition, it was noteworthy that eating of vanilla-containing staff leads to false-positive result. In four patients with pheochromocytoma, the chromatogram showed a strong violet spot at the Rf of catecholamine metabolites. In one case of argentaffinoma, it showed a distinct red spot at the same Rf of 5-HIAA. 0.02 to 0.005 ml. of urine from these patients were also chromatographed and apparent color spots corresponding to these metabolites or 5-HIAA were observed. These chromatograms are illustrated in Fig. 2.

DISCUSSION

As same as Curzon's report, the result was obtainable in a little more than one hour without any special instruments and techniques. Inorganic salt solutions have previously been used by Boscott as chromatographic solvent, mainly for the separation of phenolic acids. As shown in Fig. 2., a fairly good separation was obtained by this simple solvent. There was, however, a little difference in Rf values between pure substances and urinary substances. It may be caused by the interference of other compounds in urine. At any rate, it was very convenient, for the purpose of screening test, that catecholamine metabolites travel as one group and separate from 5-HIAA with a perceptible distance. From the data presented here, it is obvious that pheochromocytoma as well as argentaffinoma may be readily screened by the application of diazotized p-nitroaniline as reagent.

Many urine samples from suspected cases of pheochromocytoma or argentaffinoma may be rapidly screened by our previously reported simple test (one spot or test tube method) as the first step. If the result obtained by the first test is still questionable, the method presented here as the second step may give further confirmation. Because of more increased specificity and accuracy than the first method, the second step test may be useful for the detection of pheochromocytoma as well as argentaffinoma in any clinical laboratory. It is our opinion that the combination of both tests can lead to a more reliable result. Of course, for the establishment of final diagnosis, it would be necessary to determine these metabolites in urine quantitatively and to analyse the patient's history or clinical features.

SUMMARY

A method of screening test for pheochromocytoma and argentaffinoma, which consists of a rapid paper chromatography using NaCl-acetic acid-water as solvent and spraying diazotized p-nitroaniline as color reagent, was described. Using this method, highly specific and accurate results may be obtained within one hour.
Acknowledgment

The authors are indebted to Prof. Dr. T. Torikai for giving us the opportunity of carrying out this study, and his continued direction.

References

3) Boscott, R.J., Chem. & Ind., 1952, p. 472.

Addendum

While this report was in press, we found that N-methylmetadrenaline gives also violet color with diazotized p-nitroaniline and its Rf value is almost similar to those of other catecholamine metabolites. We also observed the increased urinary output of this compound in some cases with pheochromocytoma (Nature, 193, 477, 1962.). We wish to thank Dr. J. Axelrod for giving us synthetic N-methylmetadrenaline.