A Paper Strip Test for Screening of Pheochromocytoma

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EVIDENCE has been demonstrated that metabolic products of catecholamines are elevated in the urine from the patients afflicted with pheochromocytoma. Technics for urinary assay of the major metabolites of catecholamines, metadrenaline, normetadrenaline and vanillylmandelic acid, have been reported from several laboratories and widely used to establish the diagnosis of pheochromocytoma. However, some of them may not find it convenient to carry out in all clinical laboratories, because of technical difficulties and need of expensive, specialized equipments. In addition, it is important in practice that a simple test for an adaptation of quantitative assay of these catecholamine metabolites is available, since pheochromocytoma should be suspected in many hypertensive patients.

In 1961, the authors reported a simple screening test for detection of pheochromocytoma¹. The test was based on the color reaction of these metabolites with diazotized *p*-nitroaniline. Namely, when one drop of urine from the patients with pheochromocytoma was plotted on filter-paper, characteristic violet color was given after spraying of the reagent. Contrariwise, only slightly red or purple color on the circumference of yellow spot was stained in normal or control urine. During the past 5 years, the test has been carried out in this country over 25 cases of pheochromocytoma in whom the diagnosis is verified at the time of surgery or autopsy, and positive results have been reported in 22 cases². Our experience revealed that the test can also be applicable for the detection of patients with neuroblastoma or with malignant carcinoid³.

However, it was our hope that a simplified procedure, such as paper strip test for urinary

protein, sugar, pH, etc., should be developed for routine laboratory work. The present paper describes a simple paper strip test for the screening of pheochromocytoma and discusses its utility.

METHODS

The reagents were: *p*-nitroaniline (0.1 g of *p*-nitroaniline dissolved in 2 ml of concentrated hydrochloric acid and diluted with water to 100 ml); 0.2 per cent sodium nitrite solution; and 10 per cent potassium carbonate. Preparation of the test paper was as follows: Whatman No. 1. filter-paper was dipped into the freshly prepared mixture of equal volume of *p*-nitroaniline solution and sodium nitrite solution and the paper dried over night. The paper was cut small pieces, about 0.5 by 4 cm in size, 5 or 10 pieces of them were stored per one dark bottle with tight stopper containing silica gel in the bottom. All procedures should be done in the dark cold room. The bottles were kept in refrigerator at 4°C before use.

Five cases with pheochromocytoma, 2 patients with neuroblastoma and one patient with malignant carcinoid were subjected in this study. Over one hundred urine samples from normal and other patients were examined as control. Twenty-four-hour urine was collected in a bottle containing 10 ml of concentrated HCl.

Following technics were adopted. One piece of the paper strip, which is now impregnated with diazotized *p*-nitroaniline reagent, was removed from the bottle with tweezers. The paper was dipped briefly into the urine specimen to half or two-thirds of its whole length, removed and wait for 15 seconds. Then the wet paper was dipped in 10 per cent potassium carbonate for 5 seconds. The paper was put on a white dish and the staining color was inspected with comparison of control urine. The color developed immediately and reached full intensity within a minute and faded only slowly.

RESULTS

Diffuse violet color was seen on the paper

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stirp in all 5 cases with pheochromocytoma and 2 cases with neuroblastoma. This was a positive result. No violet color was found in normal or control urine samples, though slight red or purple band may be noticed at the margin of immersion of urine (negative result). Fig. 1 illustrates the color reaction in some of these patients and Table I summarizes the clinical data and estimated values of amine-metabolites in these cases. As shown in Fig. 1, the difference in the coloration between the positive and negative result is clear. In a case of malignant carcinoid, reddish color due to excessive amounts of 5-hydroxyindoleacetic acid was given and was also quite distinctive for the differentiation of this tumor.

DISCUSSION

Since the simple qualitative one spot test of catecholamine metabolites has been reported from our laboratory, it has prevailed widely in this country and its accuracy has been confirmed, as mentioned previously^{1,2,3}. So far as tested, sustained type of pheochromocytoma gave consistently positive result.

However, technics for the preparation of reagent is still somewhat troublesome. Three kinds of reagent, *p*-nitroaniline, sodium nitrite and potassium carbonate, have to be prepared and kept separately in the cold. They are then sprayed immediately after mixing of each solutions in specified ratio and order. If there is a

paper which already contains the color reagent, such as pH paper, it makes possible to simplify the procedure. Diazonium salts are usually extremely unstable, particularly to heat, light and moisture. Preliminary studies in our laboratory showed that when the paper has been stored at room temperature, about 18°C, and under usual room light for more than 24 hours, color intensity of the paper decreased rapidly, less than half as compared with that of freshly prepared paper. Avoiding the exposure to the light, but keeping at room temperature, the paper did work for about one week. However, it could really work at half year later when the paper was kept in a tightly stoppered dark bottle containing silica gel and in the refrigerator until use. It may be preferable to keep the paper one by one in a sealed aluminum foil and in the cold.

Several stabilized diazonium compounds are now commercially available, but they are still not perfect and not suitable for our purpose. Cooperation with chemists should be necessary for an availability of adequate stabilizer of diazonium salts. At the present stage, therefore, caution must be taken for preparation and storage of the paper strip.

Color reaction of the urine from the patients with amine-producing tumors, pheochromocytoma, neuroblastoma and malignant carcinoid, is quite specific and sufficiently sensitive for screening use, as mentioned above. However,

TABLE I CLINICAL AND LABORATORY DATA OF PATIENTS WITH PHEOCHROMOCYTOMA AND MALIGNANT CARCINOID

<i>Pheochromocytoma;</i>							
<i>Patient**</i>	<i>Age</i>	<i>Sex</i>	<i>Type of hypertension</i>	<i>Urinary</i>			<i>Urine volume (ml/day)</i>
				<i>MA*</i>	<i>NMA*</i>	<i>VMA*</i>	
1 E. M.	12	M	Sustained			13.0	500
2 M. F.	45	F	Sustained	0.13	1.56	39.6	1350
3 T. I.	29	F	Sustained	9.52	2.43	43.1	1900
4 Y. S.	25	M	Sustained	0.92	6.20	26.5	1800
5 T. M.	31	M	Sustained	8.70	3.10	47.0	2000
<i>Malignant carcinoid;</i>							
<i>Patient**</i>	<i>Age</i>	<i>Sex</i>		<i>Urinary 5-HIAA*</i>			<i>Urine volume (ml/day)</i>
1 T. F.	47	M		52.0			1000

* Abbreviations: MA=metadrenaline; NMA=normetadrenaline; VMA=vanillylmandelic acid; 5-HIAA=5-hydroxyindoleacetic acid, mg/day.

** Patient number as in Fig. 1.