A possible source of error in the diagnosis of phaeochromocytoma

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SYNOPSIS The finding is reported of an apparent high level of urinary catechol amines in a child with pyelonephritis. As a result of investigations the source of error was traced to tetracycline therapy.

The biochemical estimation of catechol amines in the urine is now an established method of diagnosing phaeochromocytoma (Engel and von Euler, 1950; Goldenberg, Serlin, Edwards, and Rapport, 1954). Though more accurate than tests involving the use of adrenolytic drugs such as dibenylamine, piperoxane (benzodioxane), and phentolamine (rogitine) false positive results have occasionally been reported. The recent description (Malbin, 1959) of a patient, whose urine contained 10 times the normal concentration of catechol amines but in whom no adrenal tumour could be found, prompts the description of the following case history and observations.

CASE REPORT

E.P., a girl aged 3 years 7 months, attended the Royal Belfast Hospital for Sick Children in April 1957, because of recurrent attacks of otitis media. There was no history of any other illness. Her blood pressure was 95/60 mm. Hg. Albuminuria was detected on routine testing. The urinary deposit showed a few pus cells only; culture was sterile on three occasions. The blood urea was 21 mg. per 100 ml.

Towards the end of May she developed polydipsia, cardiac enlargement (confirmed radiographically), and hypertension (blood pressure 180/130 mm. Hg). The fundi were normal in appearance. Femoral pulsation was felt.

The urine contained protein, 0.1 to 0.2 g. %, and pus cells 5 to 15 per high power field (centrifuged deposit). Two specimens were sterile; one specimen on culture yielded growth of *B. coli* sensitive to oxytetracycline.

Blood urea was 24 mg. per 100 ml.

Intravenous pyelography showed the soft tissue outline of the right kidney to be smaller than the left. Dye was well secreted on both sides. Neither the pelvis nor the calyces were distorted.

The patient was thought to have chronic pyelonephritis and on 6 June 1957 was started on a course of oxytetracycline 125 mg. six-hourly. The catechol amine content of urine collected on 20 June was reported as 470 micrograms per 24 hours (Hingerty's method, 1957; normal value = 40 µg./24 hours). This high level suggested a phaeochromocytoma as the cause of the hypertension. However, an intravenous phentolamine (rogitine) test produced no fall in blood pressure. This observation cast doubt on the validity of the urine catechol amine result and this was accordingly re-estimated. On this second occasion a value of 67 micrograms per 24 hours was obtained. (Antibiotic therapy had been discontinued one week previously.)

In early July the patient's condition deteriorated rapidly and cardiac failure developed. Improvement followed treatment with digoxin, mecamlamine, and a low-salt diet though hypertension persisted (blood pressure 170/120 to 200/140 mm. Hg).

On 31 July 1957 a right-sided nephrectomy was performed. The kidney, which was grossly scarred and contracted, showed on histological examination active and extensive chronic pyelonephritis and marked endarteritic vascular changes.

BIOCHEMICAL INVESTIGATIONS

A specimen of urine (about 200 ml.) from the child was analysed by the Hingerty (1957) modification of the method described by von Euler and Floding (1955). This technique involves the extraction of the amines on alumina and treatment of the eluate from the latter with alkaline potassium ferricyanide in the presence of ascorbic acid. The trihydroxyindole derivatives so formed (Lund, 1952) exhibit strong green fluorescence under ultra-violet light and the intensity of this is compared with that produced by urine to which known additions of noradrenaline have been made.

Using this test the child's urine showed a fluorescence well in excess of that obtained from urine to which had been added 10 micrograms of noradrenaline per 100 ml.

urine. The presumptive diagnosis of phaeochromocytoma suggested by this result was thought by the clinicians to be unlikely and a sample of the alumina extract from the urine was examined by the Department of Physiology of Queen's University, Belfast, using a rat uterus assay preparation. The apparent catechol amine activity was less than 0.001 micrograms per ml. of the original urine, *i.e.*, less than 1/100 of the value obtained by the Hingerty method, and this was confirmed by tests on a further urine specimen from the patient using the rabbit blood pressure assay.

It is recognized that using the Hingerty method a positive result can occasionally be given by substances in the urine other than catechol amines (Frazer, 1957). Tetracycline (achromycin) has been incriminated in this respect (Glenn, 1959) and the fact that oxytetracycline (terramycin) was being given to our patient at the time of the initial catechol amine estimation suggested that this antibiotic might be the substance responsible for the observed false positive result. The fact that the catechol amine value had fallen to near normal levels when repeated one week after discontinuing antibiotic therapy gave extra support to this belief. Accordingly, the following experiment was set up *in vitro*. Noradrenaline, reserpine (serpasil), digoxin, mecamlamine (inversine), and oxytetracycline (terramycin) were added to separate urine specimens obtained from healthy individuals. These were then carried through the following biochemical extraction.

Each urine sample was evaporated under vacuum after bringing to pH 4 and the dried extracts treated with acid acetone. The insoluble matter was discarded and after evaporation to dryness the residue was again taken up in acid acetone. The evaporation was repeated and the final residue was dissolved in acetone and applied to paper strips for chromatography. This was carried out in the dark using the butanol-acetic-acid-water system. The dried strips were sprayed with potassium ferricyanide and alkaline ascorbic acid and examined under ultra-violet light for the appearance of fluorescence.

The urine samples containing noradrenaline and oxytetracycline each gave a bright fluorescence (Table I).

TABLE I

Substance Added to Urine	Colour of Fluorescence under Ultraviolet Stimulus after Ferricyanide Oxidation
Noradrenaline	Bright green
Reserpine	White
Digoxin	Non-specific
Mecamlamine	Non-specific
Oxytetracycline	Bright yellow-green

Since the estimation of catechol amines is based on the intensity of their fluorescence it is clear from these observations that false positive results could be obtained in patients on oxytetracycline should this antibiotic be excreted in the urine in sufficient concentration. To test this possibility catechol amine estimations were carried out by the Hingerty (1957) method on urine collected from 11 children who were confined to bed and receiving treatment with antibiotics of the tetracycline group. Specimens were collected one and seven days after starting treatment.

The results, which are set out in Table II, show in many instances a significant rise in the urine catechol

TABLE II

Patient Number	Antibiotic	Daily Dose (mg./kg. body weight)	¹ Catechol Amines as Noradrenaline in 24 Hours (μg.)	
			After One Day	After Seven Days
1	Tetracycline	20	98	65
2	Tetracycline	14	54	106
3	Tetracycline	21	93.	124
4	Chlortetracycline	21.5	83	102
5	Chlortetracycline	16	73	135
6	Chlortetracycline	11	106	104
7	Erythromycin	24	65	135
8	Erythromycin	27	53	79
9	Erythromycin	24	39	69
10	Oxytetracycline	22.5	86	57
11	Oxytetracycline	29	26	76

¹Estimated by the method of Hingerty (1957)

amine levels (normal values for children = 40 μg./24 hours). There was no obvious relationship between the catechol amine values and either the dose, method of administration, or type of antibiotic tested. On the other hand, the values recorded after a seven-day period of treatment tended to be higher than those noted after only 24 hours of therapy.

DISCUSSION

The investigations described show that when estimated by the Hingerty method a high urine catechol amine value can be obtained if the patient is receiving treatment with one of the tetracycline group of antibiotics. Although raised, the levels noted in the group of children receiving treatment with antibiotics of the tetracycline group were not as high as the initial value recorded in the patient E.P. Two factors probably responsible for this discrepancy would seem to be the more prolonged period of treatment (two weeks) and the much higher dosage schedule used in the case of E.P. (47 mg./kg.). Nevertheless, the urine catechol amine values recorded were some two to three times in excess of the normal standards for children accepted in our laboratory using this method. Since results of this order have been noted in patients with phaeochromocytoma (McMillan, 1957), it is clearly essential that patients should be taken off all tetracycline antibiotics some days before collecting urine for the biochemical estimation of catechol amines.

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