

# PHAECHROMOCYTOMA: LABORATORY DIAGNOSIS

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Phaeochromocytomata may arise in the adrenal glands or in extra-adrenal medullary tissue. Although the tumour is responsible for less than 1% of all cases of arterial hypertension, its early and accurate diagnosis is important because it is a cause of hypertension that is curable by surgery.<sup>1-3</sup> The hypertension may be sustained, but in 30% of cases it is paroxysmal in type. Hypertension is not invariably present, and tumours may occur without clinical evidence of hormone production.

Phaeochromocytomata can be detected from clinical data in about 50% of cases, but special tests are necessary to establish the diagnosis. The tests are of two kinds,<sup>3,4</sup> viz. clinical pharmacological tests and laboratory examination of the urine for catecholamines or other excretory products.

The clinical tests that may be performed on the patient are (a) provocative tests, in which histamine or methacholine is used to stimulate the tumour to produce a paroxysm of hypertension, and (b) adrenergic blockade tests, in which phentolamine (regitine) is administered to antagonize the action of circulating adrenaline and noradrenaline. The use of these drugs requires very careful observation of the proper indications and the details of technique. It must also be appreciated that false positive and false negative responses may occur.

## LABORATORY TESTS

Estimations of catecholamines or other adrenal medullary substances in the urine are a more reliable guide to the presence of adrenal medullary tumour than the clinical pharmacological tests. Various chemical, physical and biological methods are available for the measurement of adrenaline, noradrenaline, and related compounds in the urine.<sup>3,5,10,11</sup> The tumour tissue can also be assayed; large amounts of noradrenaline and adrenaline have been

demonstrated in these tumours.<sup>5-9</sup> The proportion of the two catecholamines excreted in the urine and the relative content of these substances in the tumour is well correlated.<sup>12</sup>

Most laboratory tests of the urine for this purpose are relatively difficult and time-consuming and therefore unsuitable for routine screening of a large number of urine samples. A fluorimetric test studied in this laboratory<sup>12</sup> was found to be less reliable for the screening of phaeochromocytoma than the 'cat' test described below. The recently published methods<sup>10,11</sup> for quantitative estimation of 3-methoxy-4-hydroxymandelic acid are not suitable for routine screening of urine samples. In special cases they would be of inestimable value, since they determine the amount of this major metabolite of adrenaline and noradrenaline, which is of great diagnostic importance; large amounts may be excreted in the urine of patients with phaeochromocytoma.

The simple method of biological assay described by Moulton and Willoughby<sup>14</sup> is useful as a screening and diagnostic test. Untreated urine is examined for pressor activity in the anaesthetized cat. Random samples of urine or aliquots of 24-hour specimens are required; 50 mg. of ascorbic acid is added to each sample as anti-oxidant, and the samples are stored at 0°C until the test can be performed. The cat is prepared for recording arterial blood pressure and for femoral intravenous injections. Certain modifications of the test procedure have been recommended by the original authors (personal communication). Thus, a ganglion-blocking agent such as hexamethonium bitartrate, about 25 mg., is given initially to lower the blood pressure and to sensitize the preparation for pressor responses. The pH of the urine samples is not adjusted initially, but only if a sample produces a significant rise in blood pressure. The standard dose (0.2 microgram in 1 ml.) of noradrenaline and of

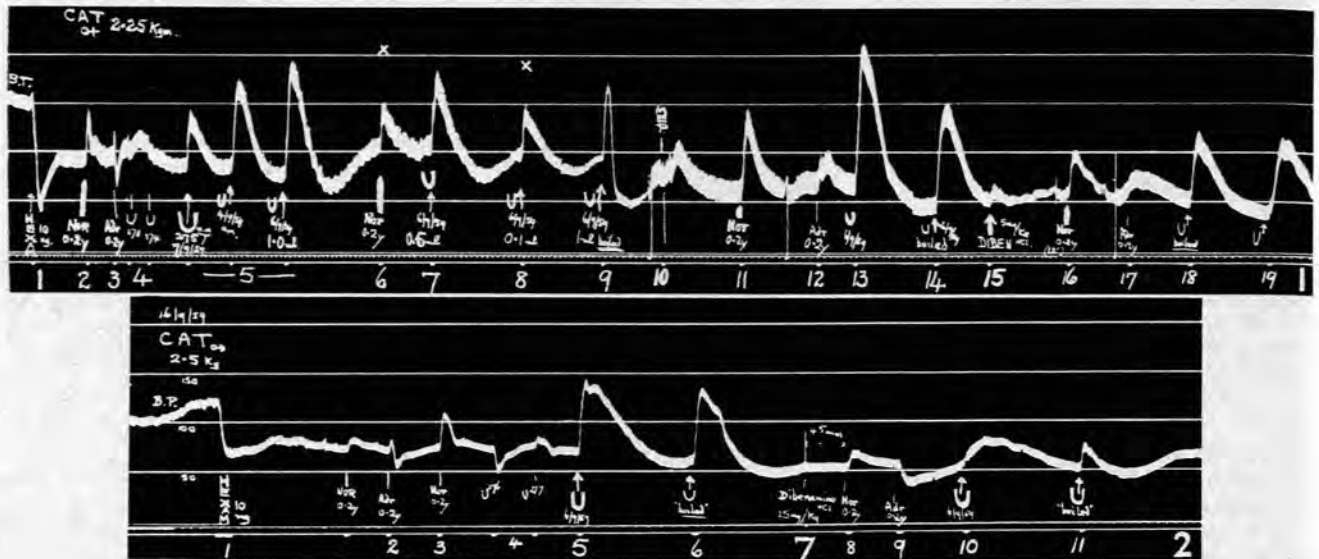


Fig. 1. Marked pressor action of 1 ml. of urine: at 5 and 7 (compared with 0.2 microgram of noradrenaline at 2 and 6): at 13, compared with noradrenaline at 11, after mepyramine given at 10; all effects reduced after dibenamine given at 15. Note: 0.1 ml. of urine at 8 produced an effect similar to that of noradrenaline at 6. At 18, 'boiled' urine still produced a marked pressor action.

Fig. 2. Marked pressor action of urine at 5, and of 'boiled' urine at 6, compared with noradrenaline at 3; all effects much reduced after dibenamine given at 7.

adrenaline is given initially and at suitable intervals during the test. All injections are given intravenously through the cannula in the femoral vein in constant volume (1 ml. of drug solution or 1 ml. of urine), each dose washed in at constant speed with a constant volume of saline (2 ml.). If when the urine is injected an alteration in blood pressure occurs equal to or greater than that produced by the standard dose of noradrenaline or adrenaline, a study is made of the modifying effect of an antihistamine drug and an adrenergic blocking drug on the vasomotor response. The heating of a suspect urine with sodium hydroxide and ferric chloride is no longer part of the procedure.

Of 330 samples of urine examined in this laboratory, evidence for the diagnosis of phaeochromocytoma has been found in 3 cases. Details of the clinical findings in the last of these cases are reported elsewhere\* by Dr. Rainier-Pope.<sup>15</sup> The present note deals with the bio-assay findings in this case.

#### CASE REPORT

Samples of urine from an Indian boy E.N., aged 10 years, were examined for pressor activity. As shown in Figs. 1 and 2 remarkable increase in the blood pressure was produced by a number of different urine samples. In one instance 0.1 ml. urine produced an effect equal to that produced by the standard dose of noradrenaline. Incidentally the pressor activity of the urine was not abolished by oxidation with ferric chloride. The effects produced by the adrenergic drugs and the urines were more marked after the antihistaminic drug (mepyramine) (Fig. 1), and were much reduced after the adrenergic blocking agent (dibenamine) (Figs. 1 and 2).

An aqueous extract prepared from the tumour tissue ground with sand was found to exert marked activity: 1 g. of tissue contained pressor activity equivalent approximately to 4 mg. of noradrenaline. (Some workers have extracted tumour tissue ground with sand and 10 ml. of N/10 HCl per g. of tumour, heating in a boiling water bath for 10 minutes, filtering, and adjusting the filtrate so that 1 ml. = 0.1 g. of tumour.)

\* See page 255 of this issue.

Samples of urine collected on 3 consecutive days in the post-operative period showed no pressor activity.

#### DISCUSSION

It is advisable to perform laboratory tests in all cases of suspected phaeochromocytoma before exploratory surgery. The 'cat' test for catecholamines is simple, and many samples of urine may easily be examined at one session. Although an increase in the excretion of catecholamines has been demonstrated in the majority of cases of phaeochromocytoma when the patient's blood pressure is elevated, an increase has occasionally also been demonstrated even when the blood pressure was normal at the time of collection of the urine. It must be noted that in the bio-assay of urine and of tumour extract the responses obtained are the resultant of competition between noradrenaline and adrenaline for equal cell receptors, and interference in their actions sometimes occurs. The pressor activity of the urine may also be modified by other substances that are present, so that small but significant increases in catecholamine excretion may therefore be missed. The standard doses of noradrenaline and adrenaline given singly produce effects that may be different from a urine sample because of their separate unimpeded action.

As an approximate guide to the total amount of noradrenaline that may be found in the urine in 24 hours the following values have been presented by Goldenberg:<sup>16</sup> 0-50 micrograms in normal subjects, 0-100 in essential hypertension, and over 100 in patients with phaeochromocytoma (usually 600-2,700 in persistent hypertension and 190-1,530 in paroxysmal hypertension). Sjoerdsma<sup>5</sup> states that the normal urinary excretion of adrenaline plus noradrenaline is usually less than 100 micrograms per day, whereas in phaeochromocytoma it is typically in the range 300-3,000 micrograms per day; most of the tumours contain 500-10,000 micrograms

per g. of total catecholamines. According to Robson and Keele<sup>3</sup> the total amount of the two amines in tumours is 5 - 15 mg. per g. (in normal human adrenal tissues about 1 mg. per g. of pressor substances).

#### SUMMARY

The simple screening 'cat' (blood pressure) test for detecting catecholamines in the urine of patients with a suspected phaeochromocytoma has a number of advantages. The cat preparation is easily set up, it is reliable, numerous urine samples can be investigated at one session, and samples can be repeatedly examined under fairly exact conditions. Some aspects of the technique of the assay and a positive result are reported. References are given to important general review articles on phaeochromocytoma.

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