FURTHER OBSERVATIONS ON THE DETERMINATION OF CATECHOLAMINE METABOLITES IN TUMOURS OF SYMPATHETIC NERVOUS SYSTEM*†

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In 1961 one of us (M.B.) reported our first results (Bettex and Käser, 1962) of vanillyl-mandelic acid (= 3-methoxy-4-hydroxymandelic acid, MHMA) determination in the urine of children with tumours of the sympathetic nervous system. It was recognized a few years ago (Armstrong and McMillan, 1957, 1959; Armstrong, McMillan and Shaw, 1957; Axelrod, Senoh and Witkop, 1958; Axelrod, Inscoe, Senoh and Witkop, 1958; Kirshner, Goodall and Rosen, 1958; LaBrosse, Axelrod and Kety, 1958; Leeper, Weissbach and Udenfriend, 1958) that MHMA represents, as shown in Fig. 1, the main end-product of the metabolism both of adrenaline and of noradrenaline. Urinary excretion of MHMA is not influenced by nutritional factors (Armstrong *et al.*, 1957) and therefore permits an estimation of the endogenous catecholamine production.

Since catecholamines are produced in both the adrenal medulla and the adrenergic part of the vegetative nervous system, a raised MHMA excretion was expected and found not only in phaeochromocytomas (Armstrong *et al.*, 1957; Bettex and Käser, 1962; Käser, Schweisguth, Sellei and Spengler, 1963; Robinson, Ratcliffe and Smith, 1959; Sandler and Ruthven, 1959; von Studnitz, 1960; von Studnitz and Hanson, 1959; Sunderman, Cleveland, Law and Sunderman, 1960) but in other tumours as well (Greenberg and Gardner, 1959; Käser, 1961a, b; Käser and Studnitz, 1961; Robinson *et al.*, 1959; Sandler and Ruthven, 1959; Stickler, Hallenbeck and Flock, 1959; von Studnitz, 1960, 1961); it seemed promising to find out which tumours were associated with an increased MHMA excretion and how often the excretion in these tumours reached pathological levels.

We investigated about 150 children with a wide variety of tumours and found normal MHMA concentrations (3-9 µg./mg. creatinine) in all patients with non-neural malignant tumours—mainly Wilms' tumours, reticulosarcomas and lymphosarcomas, retinoblastomas and stem-cell leukaemias, and in the six cases of ganglioneuroma observed until now (Fig. 2). On the other hand we saw an increase of MHMA not only in seven cases clinically simulating phaeochromocytoma but also in 45 out of 48 histologically proven neuroblastomas.

The determination of MHMA excretion, therefore, is a method of distinguishing phaeochromocytomas and neuroblastomas from non-neural malignancies as well as from other types of neural tumours in which raised MHMA values were found, i.e. in only a very few cases of ganglioneuromas having diarrhoea (Greenberg and Gardner, 1959; B. J. Rosenstein, 1962, personal communication; von Studnitz, Käser and Sjoerdsma, 1963). The differentiation between phaeochromocytomas and neuroblastomas, however, is not possible on this basis, and until recently it depended on the clinical symptomatology, the classical hypertension tests (e.g. histamine, regitine, dibenzylamine) and the course. We now believe that, at least in certain cases, biochemical differences exist between neuroblastomas and phaeochromocytomas.

Fig. 3 shows three different chromatograms of urinary phenolic acids: Fig. 3a is that of a normal subject, 3b of a patient with neuroblastoma and 3c of a patient with phaeochromocytoma. If we compare 3a with 3c we find a larger spot of MHMA on 3c. If we compare 3a and 3b we find not only a larger MHMA spot in 3b, but in addition a second spot that is considerably larger in 3b than in 3a and 3c. This latter substance could be identified as homovanillic acid (HVA = 3-methoxy-4-hydroxy-phenylacetic acid), a common end-product of 'dopa'

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FIG. 1.—Pathways of catecholamine metabolism.

and 'dopamine' (Fig. 1), which is normally excreted in small amounts in the urine independently of dietary factors (Armstrong, Shaw and Wall, 1956; Axelrod *et al.*, 1958; Shaw, McMillan and Armstrong, 1957).

Quantitative HVA determinations in a number of our tumour patients (Fig. 4) showed that the excretion remained normal (5-40 $\mu\text{g./mg. creatinine}$) in all non-neural, malignant tumours as well as in our six ganglioneuromas. In 13 out of 16 neuroblastomas HVA concentrations were remarkably increased. On the other hand, compared with MHMA values, only 1 of 7 clinically obvious cases of pheochromocytoma had a raised HVA excretion. This case showed other metabolic abnormalities and was finally found to have an atypical malignant hypernephroid and paraganglionic tumour of the adrenal gland. We may conclude, therefore, that there is frequently a significant increase in HVA excretion in neuroblastomas but never in pheochromocytomas. These findings are similar to the observations of Robinson and Smith (1960, 1962) and of von Studnitz (1962). Quantitative HVA determinations can, therefore, be used as a further

FIG. 2.—Urinary excretion of MHMA in various tumours.

TABLE
URINARY EXCRETION OF CATECHOLAMINE
METABOLITES IN 19 CASES OF NEUROBLASTOMA

Metabolites	Excretion	
	No. Normal	No. Increased
Dopa	3	16
Dopamine	1	18
Adrenaline/noradrenaline	2	17
Metanephrine/normetanephrine	1	18
Vanillyl-mandelic acid	0	19
Tyramine	14	5

chemical criterion for the differentiation between these two tumours.

In an attempt to clarify the metabolic abnormalities observed in neuroblastomas we determined simultaneously, in collaboration with Dr. A. Sjoerdsma (von Studnitz *et al.*, 1963), dopa, dopamine, adrenaline plus noradrenaline, metanephrine plus normetanephrine, vanillyl-mandelic acid and tyramine in some of our patients. Of 19 histologically

confirmed neuroblastomas (Table) only 3 had normal, whereas 16 had significantly increased dopa concentrations (expressed in $\mu\text{g./mg. creatinine}$) in their urines. In 18 dopamine was increased, in 17 adrenaline plus noradrenaline and in 18 metanephrine plus normetanephrine. All had increased MHMA excretions but only 5 showed an increase of tyramine that exceeded the upper normal limit.

This demonstrates that patients with neuroblastomas are not only excreting larger amounts of MHMA and HVA, the main end-products on the one hand of adrenaline and noradrenaline, and on the other of dopa and dopamine, but that they must be associated with more profound disturbances in catecholamine metabolism. Even if one considers individual variations in the quantity of the metabolites excreted, the spectrum of the excreted products in neuroblastomas is characteristic enough to permit a differentiation from other tumours, e.g. from phaeochromocytomas which usually only show increased excretion of MHMA, noradrenaline or adrenaline and normetanephrine and metanephrine. At present we have no explanation for the biochemical abnormalities; they certainly have a differential diagnostic value and they might even give new prognostic and therapeutic clues in the future.

Summary

Some products of catecholamine metabolism were determined quantitatively in the urine of patients

FIG. 3.—Chromatograms (schematically) of urinary phenolic acids of (a) a normal individual, (b) a patient with neuroblastoma and (c) a patient with phaeochromocytoma.

FIG. 4.—Urinary excretion of HVA in various tumours.

with various tumours. No abnormalities could be found in non-neural malignant tumours and in the 6 ganglioneuromas examined. On the other hand, not only the pheochromocytomas but 45 out of 48 neuroblastomas and a malignant hypernephroid and paraganglionic tumour of the adrenal gland showed increased vanillyl-mandelic acid excretion. In addition pathological homovanillic acid values were found in 13 of 16 neuroblastomas and in the atypical tumour, but never in the pheochromocytomas. Finally investigations of 19 neuroblastomas revealed that in addition to the anomalies mentioned above and in contrast to other tumours, increased amounts of dopa, dopamine, noradrenaline and normetanephrine were frequently excreted. This observation is not only of theoretical interest but also has differential diagnostic value.

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