4-Hydroxy-3-methoxymandelic acid (HMMA) excretion in retinoblastoma

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SUMMARY 4-Hydroxy-3-methoxymandelic acid (HMMA) concentrations in aqueous humour, urine, and serum were simultaneously estimated to determine if these levels were raised in cases of retinoblastoma. The catecholamine content of aqueous humour was significantly higher than that of urine and serum, but as there was no significant difference in the HMMA concentration in retinoblastoma and other non-malignant conditions it seems likely that retinoblastoma is not a catecholamine-secreting tumour.

Conflicting reports have appeared on catecholamine excretion in cases of retinoblastoma. While Frezzotti *et al.* (1967) found increased urinary vanillylmandelic acid (VMA) excretion in retinoblastoma, Armstrong and McMillan (1957) found normal catecholamine excretion in cases of this disease.

Catecholamines are produced by the adrenal medulla, the adrenergic part of the sympathetic nervous system, and by tumours arising from the neural crest cells, for example, neuroblastoma, ganglioneuroma, ganglioneuroblastoma, and pheochromocytoma. These neural crest tumours are capable of synthesising noradrenaline, its precursors, and its metabolites and are hence true functional neoplasms. Abnormally high urinary levels of these compounds are found in most patients with these tumours (Voorhess, 1966). 3-Methoxy-4-hydroxyphenyl acetic acid (HVA) and 4-hydroxy-3-methoxymandelic acid (HMMA) are 2 important terminal urinary metabolites. Armstrong *et al.* (1957) showed that HMMA was the main end product of the metabolism of both adrenaline and noradrenaline. Being very stable and not being influenced by nutritional factors, it permits an estimation of endogeneous catecholamine production. The urinary excretion of HMMA exceeds the excretion of noradrenaline and adrenaline by 10- to 100-fold (Labrosse *et al.*, 1958), and about 45% of the total noradrenaline and adrenaline production in man is excreted as HMMA (Kopin, 1960). As HVA estimation is much more erratic and diet-dependent (Gjessing, 1966) it was decided to assess catechol-

amine excretion by estimation of HMMA.

As it has been shown earlier that metabolically active retinoblastoma releases LDH into its surrounding media with forward diffusion through the vitreous into the aqueous (Dias *et al.*, 1971) it appears likely that, if the retinoblastoma secretes catecholamines, high concentrations of HMMA could be detected in the aqueous. As there have been no reports of HMMA excretion in aqueous humour it was decided to determine whether increased HMMA levels occur in the aqueous in retinoblastoma.

Materials and methods

Specimens of aqueous humour, urine, and serum were collected from 12 patients coming for surgery to the Victoria Memorial Eye Hospital, Colombo. Of these, 3 patients had retinoblastoma while all the others had non-malignant intraocular disorders. These controls (cataract patients) were matched so far as possible for age, as it has been shown that although there is no sex difference in HMMA excretion (Mckendrick and Edwards, 1965) the excretion increases with increasing age through infancy and childhood, approaching adult levels at about 10 years of age (Young *et al.*, 1963; Karki, 1956; Voorhess, 1967). As a diurnal variation in catecholamine excretion has been shown to exist (Weise, McDonald and Labrosse, 1961), all specimens were collected between 8.00 a.m. and 10.00 a.m. There were no dietary restrictions before operation.

Since 24-hour collection of urine specimens is fraught with many errors in children (Gitlow *et al.*,

1968), random urine specimens taken before operation were tested within 2 hours of collection, no preservative being added. Blood samples and aqueous samples were taken during the operation. The serum, urine, and aqueous samples were analysed simultaneously by the method of Pisano *et al.* (1962). The maximum amount of aqueous collected fell far short of the 2 to 3 ml needed for HMMA estimation by the Pisano method, hence the total volume of aqueous was measured in a graduated micropipette and diluted to 5.5 ml with sterile distilled water.

Catecholamine excretion in children has been expressed as microgrammes (*a*) per pound body weight, (*b*) per square metre of skin surface, (*c*) per hour, (*d*) per day (24-hour urine collection), (*e*) per millilitre of urine, (*f*) per milligramme of creatinine + creatine, and (*g*) per milligramme of creatinine. In our cases it was decided to express catecholamine concentration in the 3 body fluids in units of microgramme per millilitres of body fluid.

Results

The HMMA content in aqueous humour, urine, and serum of the 12 patients studied is listed in Table 1.

The 3 cases of retinoblastoma presented with a white mass behind the pupil and subsequent

microscopic examination confirmed the diagnosis in all of them.

Discussion

If the retinoblastoma is a functioning tumour secreting catecholamines, it is likely that the highest concentration of HMMA will be present in the aqueous. The 3 cases of retinoblastoma show a mean HMMA concentration of 25.2 µg/ml in the aqueous (Table 2), while the 9 non-malignant cases

Table 2 Mean excretion of HMMA in aqueous humour, urine, and serum

		Aqueous humour	Urine	Serum
Retinoblastoma (N = 3)	Mean (µg/ml)	25.2	4.3	6.4
	SD	5.9	1.2	4.1
Non-malignant (N = 9)	Mean (µg/ml)	27.1	3.7	6.1
	SD	12.3	0.9	4.6
	P (t test)	0.2	0.6	0.1
(Not significant)				

show a mean HMMA level of 27.1 µg/ml in the aqueous. There is no significant difference ($P=0.2$) of the HMMA levels in aqueous humour in retinoblastoma and non-malignant conditions. Neither is there any significant difference ($P=0.6$) in the urinary HMMA concentration nor in serum HMMA levels ($P=0.1$) in retinoblastoma and other non-malignant conditions. A noticeable finding, however, is the much higher catecholamine concentration in the aqueous humour than in urine and serum, probably owing to transmitter release and forward diffusion from the retinal nerve plexuses.

Retinoblastoma, a malignant tumour arising from the nuclear layer of the retina, is not a tumour of the sympathetic nervous system nor does it originate from the neural crest ectoderm. Hence it is unlikely that a retinoblastoma can become a functional tumour-secreting catecholamines. Frezzotti *et al.* (1967) found an increased urinary catecholamine excretion in 2 patients with retinoblastoma, using only 4 controls. A high HMMA excretion in the urine may also occur owing to dietary constituents, the stress of hospital admission, any acute or urinary infection, endocrine or metabolic disorders (Mckendrick and Edwards, 1965), cold exposure (Schiff *et al.*, 1966), hypoglycaemia (Stern *et al.*, 1968), hypoxia (Vogt, 1960), heart failure (Lees, 1960), chronic anaemia (Matsaniotis *et al.*, 1968), and juvenile diabetes (Juelchristensen, 1970). Non-specific assay techniques may also account for sporadic reports of increased urinary catecholamine excretion.

Further, not all neuroblastomas or ganglio-

Table 1 HMMA excretion in aqueous humour, urine, and serum

Age (yr mth)	Sex	Diagnosis	HMMA in (µg/ml)		
			Aqueous humour	Urine	Serum
1 3	M	Leucoma	21.2	2.8	4.6
1 6	M	Retinoblastoma	32.0	3.2	3.6
2 2	M	Cong. cataract	28.4	4.0	2.8
3 1	F	Retinoblastoma	22.6	4.2	4.6
3 7	F	Buphthalmos	18.6	3.2	12.6
4 3	M	Retinoblastoma	31.2	5.6	11.2
9 6	M	Cong. cataract	24.8	5.8	15.4
12 2	M	Tr. cataract	9.2	4.2	2.2
45 2	F	Cataract	28.0	3.6	3.8
56 3	F	Senile cataract	56.4	4.2	6.2
75 6	M	Senile cataract	26.6	2.4	3.3
80 4	F	Senile cataract	33.0	3.8	4.3
		Mean (µg/ml)	24.3	3.9	6.2
		SD	12.5	1.1	4.3

neuroblastomas are functional. Voorhess and Gardner (1961), von Studintz *et al.* (1963), Mckendrick and Edwards (1965), and Kaser (1966) have reported normal urinary catecholamine excretion even in patients with these tumours.

In view of all these factors, it is unlikely that HMMA estimation in the aqueous humour, serum, or urine could be of any significance as a diagnostic test for retinoblastoma.

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