

# Urinary Vanillyl-mandelic Acid (VMA) Excretion by Chronically Anaemic Children

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Hypoxia has been regarded as one of the many factors stimulating augmented catecholamine biosynthesis (Vogt, 1960). Plasma levels of epinephrine and norepinephrine were found to be increased in newborns with the respiratory distress syndrome or placental insufficiency (Cheek, Malinek, and Fraillon, 1963). Infants with severe heart failure eliminate in the urine twice as much metanephrine, normetanephrine, and 3-methoxy-4-hydroxymandelic acid (VMA) as do normal infants (Lees, 1966), and it has been suggested that increased catecholamine production may contribute to the relative hypermetabolism observed in some infants with congenital heart disease and undernutrition (Lees *et al.*, 1965).

Reports about the effects of heart failure on the urinary excretion of catecholamines or their metabolites in adults have been conflicting. They have included inconsistent changes (Raab and Gige, 1954; Pekkarinen *et al.*, 1960), important increases in norepinephrine and epinephrine (Tomatsu *et al.*, 1963), or in norepinephrine, but not in epinephrine or in VMA (Chidsey, Braunwald, and Morrow, 1965).

An increase in the urinary output of VMA has been demonstrated in some patients with severe pulmonary insufficiency (Gitlow *et al.*, 1961), but a normal urinary VMA has been reported in children with various conditions which may cause hypoxia, such as lobar pneumonia, atelectasis, acute asthmatic attack, or cyanotic heart disease (Young *et al.*, 1963).

The urinary excretion of catecholamines or their metabolites in patients with chronic anaemia has not been studied, and the present report deals with the daily urinary excretion of VMA, the major final product of catecholamine metabolism (Armstrong,

McMillan, and Shaw, 1957), by children with severe chronic anaemia.

## Materials and Methods

Studies were made of 50 children, aged 2 to 12½ years. Of these, 30, 17 boys and 13 girls, were healthy children in hospital awaiting discharge after recovery from mild upper respiratory of gastro-intestinal infection. The remaining 20, 8 boys and 12 girls, suffered from chronic anaemia: thalassaemia major (16); sickle-cell anaemia (1); iron-deficiency anaemia (1); megaloblastic anaemia (1); and acquired haemolytic anaemia (1). The Hb level ranged from 3.1 to 6.5 g./100 ml. Apart from anaemia no other illness was detected in any of the children.

During the collection period no restriction of activity was imposed on either the healthy or the anaemic subjects. Vanilla-containing foods and bananas were omitted from the diet 3 days before and during the collection. No medication was given. Children using sympathomimetic drugs, such as nasal drops, were strictly excluded from the study.

24-hour urine specimens were collected. Samples were acidified with 6 N HCl to pH 2 immediately after voiding, and stored at 4° C. After each 24-hour collection was completed, samples were measured and stored at -25° C.

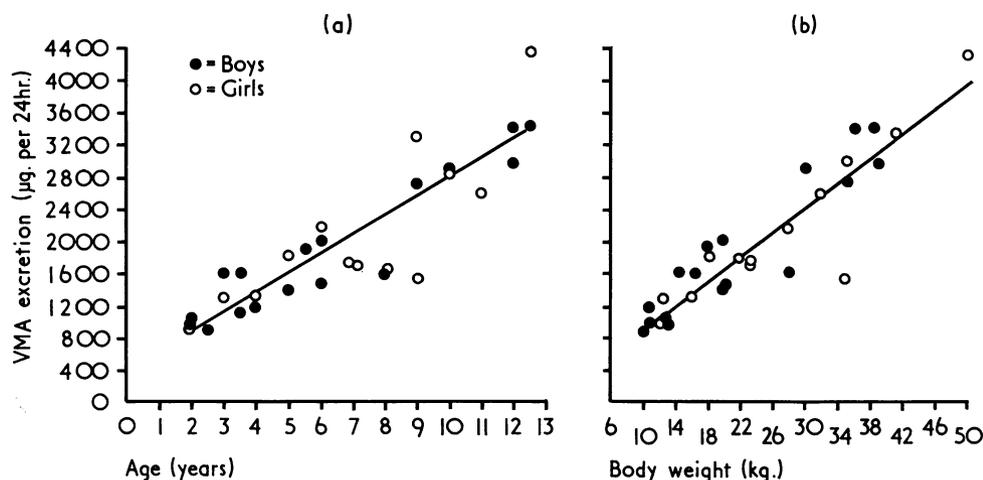
VMA was determined (Pisano, Crout, and Abraham, 1962) by extraction in ethyl acetate, re-extraction with potassium carbonate solution, oxidation with sodium periodate, extraction of vanillin in toluene, re-extraction with potassium carbonate, and spectrophotometry at 360 μm.

## Results

**VMA excretion by healthy children.** Table I shows the 24-hour urinary excretion of VMA by 30 healthy children. The output was the same in both boys and girls ( $p > 0.1$ ). There was a good linear relation between the 24-hour output and both age and weight (Fig. 1a and b). The slope of the line drawn through the points calculated by the method of least squares shows that at 2 years of

TABLE I  
 Daily Urinary Excretion of VMA by 30 Healthy Children

Sex (no. of cases)		Age (yr.)	Body Weight (kg.)	VMA Excretion	
				( $\mu\text{g.}/24 \text{ hr.}$ )	( $\mu\text{g./kg. 24 hr.}$ )
Boys (17)	Mean Range	6.3 2.0-12.5	22.0 10.0-38.5	1900 900-3430	88.3 58.5-110.3
Girls (13)	Mean Range	7.2 4.0-12.5	26.7 12.5-50.0	2105 934-4360	80.0 44.5-103.2
Total (30)	Mean Range	6.7 2.0-12.5	24.0 10.0-50.0	1990 900-4360	84.7 44.5-110.3


 FIG. 1.—30 healthy children. Variations in the 24-hour urinary output of VMA (a) with age; (b) with body weight ( $r_a = 0.978$ ,  $r_b = 0.913$ ,  $p < 0.001$ .)

age healthy children put out 920  $\mu\text{g.}$  VMA/24 hours and that the output increased yearly by 230  $\mu\text{g.}$  (Fig. 1a). The relation with body weight was equally close (Fig. 1b), the 24-hour excretion in a 10 kg. child being 930  $\mu\text{g.}$  and increasing by 75  $\mu\text{g.}$  for every kg. body weight gained.

These results are comparable to those reported previously from this (Choremis *et al.*, 1967) and other laboratories (von Studnitz and Hanson, 1959; McKendrick and Edwards, 1965).

Young and his co-workers (1963) were the first to report an increase in urinary VMA excretion with age in healthy children, a finding that has been confirmed by other investigators (McKendrick and Edwards, 1965; Voorhess, 1967). Recently, the output of VMA in childhood was shown to be dependent mainly upon body surface area (Voorhess, 1967).

**VMA excretion by children with chronic anaemia.** Table II shows the 24-hour excretion

of VMA by 16 children with thalassaemia major, and 4 children with other types of chronic anaemia. It can be seen that children with thalassaemia did not differ from children with other chronic anaemias in their daily output of VMA ( $p > 0.1$ ). The output of anaemic children was of the same order as that of healthy children ( $p > 0.05$ ). However, when excretion was related to body weight, anaemic children excreted per kg. body weight almost twice as much VMA as did healthy children ( $p < 0.001$ ).

The output depended upon both age and body weight almost as closely as it did in healthy children (Fig. 2a and b).

Fig. 3 shows that anaemic children put out more VMA per kg. body weight than healthy children at all ages. It also indicates that in the presence of chronic anaemia the younger the child the more VMA he excretes per kg. body weight, whereas healthy children excrete the same VMA per kg. body weight at all ages.

Although there was some tendency for the more

TABLE II  
Daily Urinary Excretion of VMA by 20 Children with Chronic Anaemia

Type of Anaemia (no. of cases)		Age (yr.)	Body Weight (kg.)	VMA Excretion	
				( $\mu\text{g./24 hr.}$ )	( $\mu\text{g./kg. 24 hr.}$ )
Thalassaemia major (16)	Mean Range	6.2 2.0-11.0	18.0 10.0-32.0	2530 1570-4360	150.2 105.6-223.6
Sickle-cell anaemia (1)		3.5	18.0	2560	142.2
Iron-deficiency anaemia (1)		2.5	12.2	1500	122.9
Megaloblastic anaemia (1)		4.5	16.0	1716	107.2
Acquired haemolytic anaemia (1)		9.0	24.0	2860	119.2
Total (20)	Mean Range	5.95 2.0-11.0	17.9 10.0-32.0	2460 1500-4360	144.7 105.6-223.6

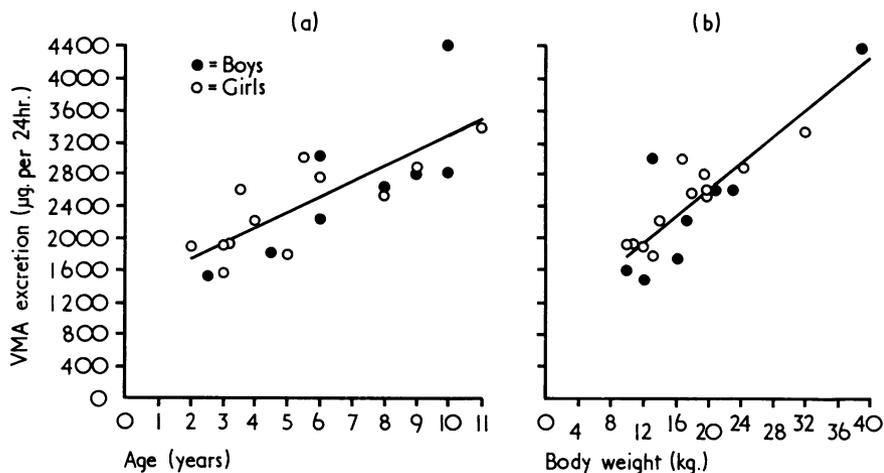


FIG. 2.—20 children with chronic anaemia. Variations in the 24-hour urinary output of VMA (a) with age; (b) with body weight. ( $r_a = 0.795$ ,  $r_b = 0.865$ ,  $p < 0.001$ .)

severely anaemic children to have a higher VMA excretion per kg. body weight, the relation between Hb levels and the 24-hour output of VMA per kg. was statistically insignificant (Fig. 4).

### Discussion

Several studies have been made on the effects of hypoxia upon the rate of catecholamine biosynthesis in the human, though the situation is still unclear. In those conditions causing hypoxia that have been studied, the hypoxia has generally been accompanied by other severe disturbances. In the neonatal respiratory distress syndrome, for example, hypoxia is associated with severe chemical and acid-base changes, and the situation is similarly complex in undernourished infants with severe heart disease, as well as in infants or adults with cardiac insufficiency. Furthermore, the results obtained from infants with heart disease in whom cyanosis rather

than cardiac decompensation is the major problem indicate only a small increase in catecholamine production (Lees, 1966).

Conclusions about the effects of hypoxia have been occasionally based on changes observed in the urinary excretion of VMA (Young *et al.*, 1963). Urinary VMA is a useful index of catecholamine production, for it is the major metabolite of both norepinephrine and epinephrine (Armstrong *et al.*, 1957), and its urinary excretion parallels that of norepinephrine (Chidsey *et al.*, 1965). However, when norepinephrine excretion was augmented by cardiac failure in adults, VMA excretion did not rise; when urinary norepinephrine rose further by the additional stress of cardiac surgery, the increase in VMA excretion lagged behind that of norepinephrine leading to an increase in the NE : VMA ratio (Chidsey *et al.*, 1965). Furthermore, since urinary VMA is derived from several sources, most

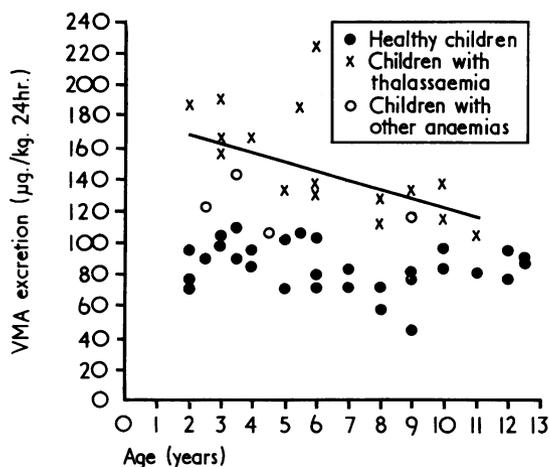


FIG. 3.—24-hour urinary output of VMA per kg. body weight in 30 healthy and 20 chronically anaemic children of various ages. (Healthy:  $r = 0.24$ ,  $p > 0.1$ ; anaemic:  $r = 0.513$ ,  $p < 0.02$ .)

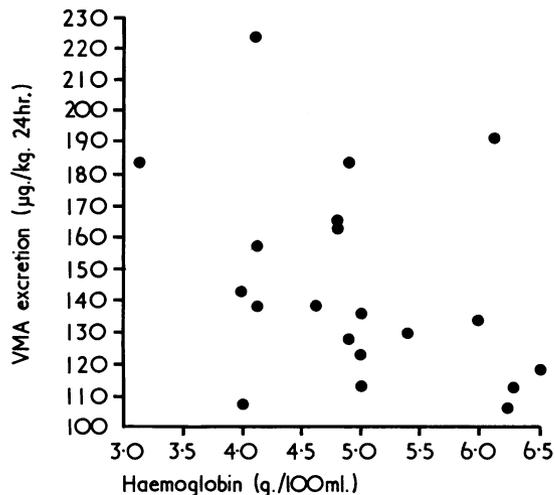


FIG. 4.—Relation between 24-hour urinary output of VMA per kg. body weight and Hb level in 20 chronically anaemic children. ( $r = 0.369$ ,  $p > 0.05$ .)

of which do not represent 'active' norepinephrine and epinephrine (that is, catecholamines with sympathomimetic activity (Wurtman, 1965)), the urinary output of VMA, though indicating the rate of catecholamine production, does not necessarily reflect the level of activity of the sympathetic nervous system (Wurtman, 1965).

The higher urinary VMA output in chronically anaemic children is consistent with the concept that hypoxia is indeed associated with catecholamine overproduction. That this was an effect of hypoxia *per se* is indicated by our failure to detect any difference in urinary VMA excretion between children with thalassaemia and those affected by other types of chronic anaemia, though the number of children with chronic anaemias other than thalassaemia studied was admittedly small. Tachycardia, which often accompanies chronic anaemia, suggests an overactive sympathetic nervous system; it fits well with the overproduction of catecholamines indicated by increased urinary VMA.

Norepinephrine excretion in heart failure tended to be higher in the most severely decompensated infants (Lees, 1966) or adults (Chidsey *et al.*, 1965). Our failure to demonstrate a significantly similar trend in the output of VMA may be explained by the rather narrow range of Hb levels in the chronically anaemic children investigated. It would be interesting to know whether the urinary excretion of catecholamines or their metabolites declines as the level of Hb rises, following blood transfusion, for example.

The results of the present study also suggest that the effect of hypoxia on catecholamine biosynthesis probably grows weaker with increasing age, a finding that is difficult to explain.

### Summary

The 24-hour excretion of VMA by healthy and by chronically anaemic children aged 2–12½ years, increased with both age and body weight. When the excretion was related to body weight anaemic children put out almost twice as much VMA as did healthy children. The latter excreted the same VMA per kg. body weight at all ages, but in the presence of chronic anaemia the younger the child the more VMA he excreted.

The results are consistent with the concept that hypoxia is associated with increased catecholamine biosynthesis.

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