ADRENALINE AND NORADRENALINE METABOLISM AND VANILMANDELIC ACID

The metabolism of adrenaline (A) and noradrenaline (NA) may initially begin with o-methylation by catechol-o-methyltransferase (COMT) into methyladrenaline (MA) and methylnoradrenaline (MNA) (12, 13). Part of these methylated compounds are excreted in the urine (13) and a part are further deaminated by monoamine oxidase (MAO) into 3-methoxy-4-hydroxymandelic acid, or vanilmandelic acid (VMA) (7), or are transformed by reduction to 3-methoxy-4-hydroxyphenylglycol (MHPG) (15) and excreted in the urine. Alternatively A and NA can be deaminated in a primary process, resulting in 3,4-dihydroxymandelic acid (DHMA) (147), a part of which is excreted in the urine but a large part is o-methylated into VMA (Fig. 1). VMA is mainly excreted unchanged in the urine, but only a very small fraction is oxidated further into vanillic acid (VA) (63, 250, 298). A few other smaller metabolites of A and NA have been demonstrated.

Among the metabolites of endogenous adrenaline and noradrenaline VMA predominates, being about 70 times the amount of the free A and NA excreted into the urine, and about 10 times the amount of the total MA+MNA in the urine (26, 292).

Following an infusion of radioactive A or NA into man, about 4 per cent of the total dose is excreted into the urine as free hormone, slightly more than a third as VMA, and about the same amount as combined MA+MNA (93, 147, 156, 166).

The part of radioactive NA injected into the circulation or the endogenous NA released from the noradrenergic neurones is rapidly taken up by the tissues containing the noradrenergic innervation (15, 323) and is stored for the greater part as chemically unchanged

\[
\begin{array}{c}
\text{Adrenaline} \\
\text{Noradrenaline}
\end{array}
\]

\[
\begin{array}{c}
\text{Methyladrenaline} \\
\text{Vanilmandelic acid}
\end{array}
\]

\[
\begin{array}{c}
\text{Methylnoradrenaline}
\end{array}
\]

Fig. 1. O-methylation and deamination of adrenaline and noradrenaline into vanilmandelic acid: 1) catechol-o-methyltransferase, 2) monoamine oxidase (in schematic presentation).
NA in the noradrenergic neurones or granules (223). This uptake or re-uptake mechanism is the most important factor in inactivation of exogenous and endogenous NA and A in the organism (9,10) and is of greater importance as an inactivation mechanism than their enzymic degradation by methyl transferases or monoamino-oxidases. The binding into a noradrenergic neurone is a relatively more important mechanism for this inactivation of circulating NA, and enzymatic o-methylation again is more important for A (9, 10).

A small part of "active NA" is released spontaneously from the storage form of NA, but more is released after sympathetic nerve impulses (115, 248). A small fraction of the released active noradrenaline reacts with the adjacent receptors and has thus a physiological function. The remainder of NA is to a great extent re-bound to the noradrenergic nerve endings or partly inactivated rapidly by o-methylation, or is washed off by the blood stream. Little, if any, is deaminated (9, 115).

Kopin (156) studied the relative importance of o-methylation and deamination in A metabolism in man by the double isotope method. The metabolites were determined in the urine 40 hours after simultaneous administration of H^3-A and C^14-MA. About 70 per cent of the administered A was first o-methylated to MA and 20 per cent was deaminated to DHMA. About a half of the MA was excreted unchanged or as sulphate or glucuronide conjugate, and a half was deaminated and oxidized to VMA or reduced to MHPG. The relative importance of MAO and COMT in the enzymic degradation of A and NA depends upon the extra- and intraneuronal amounts of these transmitters in the sympathetic and adrenomedullary systems and upon the amount of noradrenergic innervation of tissues and on the animal species (10).

The action of COMT is directed mainly upon extraneuronal NA in the tissues or in the circulating blood (157, 159, 160, 189), whereas the degradation of storage NA is mainly effected by MAO in situ (159). The release of this deaminated material is probably independent of nerve impulses, and when released it may be excreted unchanged in the urine (DHMA and DHPG, i.e., 3,4-dihydroxyphenylglycol) or DHMA is o-methylated further and is transformed to VMA (158). The VMA thus formed is probably the main source of the VMA in the urine and represents noradrenaline that is metabolized before having had any physiological action (158).

In fact, it has been suggested that under normal conditions the amount of VMA in the urine should be regarded as an index of the synthesis of catecholamines, rather than that of the activity of the noradrenergic nervous system (331).

ADRENALINE AND NORADRENALINE METABOLISM DURING THE FETAL PERIOD

The heart, kidneys and lungs of the human fetus had approximately the same amounts of A and NA as those of the adults of other species, whereas the contents of these hormones were much lower in the fetal than in the adult brain (108). The content of A in the brains of newborn animals was the higher the more developed the species was at birth (139).

Both A and NA are present in human amniotic fluid in small amounts (42, 161). The A and NA contents of the amniotic fluid in toxemic patients did not markedly differ from that in the controls (42).

C^14-A and C^14-NA injected into the circulation of the mother was recovered in considerable amounts from the placental blood-flow to the fetus, whereas only traces were found in the blood returning to the mother (259). Schaepdryver [cited by Gitlow (78)] demonstrated in a pregnant woman with pheochromocytoma that catecholamines penetrate the placental barrier and that the placenta is capable of changing these hormones to VMA. After C^14-NA injection into the maternal spiral artery MNA, DHMA, VMA and VA were detected in the fetal perfusate together with unchanged NA, which indicated that both MAO and COMT were involved in its inactivation (186).

Castrén et al.(38) studied the metabolism of i.v. administered H^3-NA in the human fetus and placenta. A large amount of H^3-MNA was demonstrated in the liver of 3-month-old fetuses, whereas H^3-DHMA and H^3-VMA were present in small amounts only. This points to the relative significance of
COMT in the metabolism of NA under these conditions. On the same basis, the brain of fetuses of the same age showed slight COMT activity, but MAO activity was not clearly demonstrable. Placentas examined in the 3rd to 10th months of pregnancy revealed both COMT and MAO, and with maturing of the placenta there was an increase in the metabolism of H-NA. Maternal diabetes and placental degeneration had no effect on the amount of NA metabolism, but in the degenerated placenta the metabolism appeared to occur to a relatively greater extent through the action of COMT.

URINARY EXCRETION OF VANIL-MANDELIC ACID IN NORMAL CONDITIONS

Effect of age

Neonatal period (with attention also to A and NA excretion)

VMA is excreted in the urine from the first day of life (23, 108, 191, 292, 332; Table 1). The VMA excretion increases daily and already during the first week attains about the same level, expressed in µg/kg, at which it remains during the later years of childhood (108, 332).

When the VMA excretion is expressed in µg/mg creatinine, the postnatal excretions are highest and the excretion decreases with increasing age (84, 89, 292). The VMA content in the urine (VMA in µg/ml) in full-term infants is also higher on the first day than on the following days (292).

In the premature newborn the VMA excretion in absolute terms is lower in the first week than in the fullterm newborn, but when calculated per kg body weight it corresponds to that of the latter (23, 108). However, in a study by Nicolopoulos et al. (191) the excretion of VMA in fullterm and premature infants was on the first day 4—5 times that observed by the present writer (108); on the 15th day of life the VMA excretion of the premature was extremely high, 2506 µg/24 hrs, whereas that of fullterm infants was even slightly lower than on the first day (191).

Table 1. Urinary UMA excretion of fullterm infants in the neonatal period as reported by various authors (I=UMA µg/24 hrs and µg/kg/24 hrs; II=UMA µg/mg creatinine)

| I Authors          | Age          | No. of cases | VMA excretion µg/24 h | VMA excretion µg/kg/24 h |
|--------------------|--------------|--------------|-----------------------|--------------------------|
| Zeisel, 1961 (332) | 1st day      | 5            | 60                    | 18                       |
|                    | 3rd          | 5            | 150                   | 50                       |
|                    | 6th—8th days | 8            | 270                   | 80                       |
|                    | 2nd—4th weeks| 13           | 170—190               | 50—54                    |
| Boehm & O'Brien, 1964 (23) | | | | |
| 1st day            | 4            |              | 47                    |                          |
| 3rd                | 5            |              | 71                    |                          |
| 5th                | 5            |              | 148                   |                          |
| Hakulinen, 1966 (108) | | | | |
| 1st day            | 30           |              | 85                    |                          |
| 2nd                | 29           |              | 54                    |                          |
| 4th                | 23           |              | 75                    |                          |
| 5th                | 16           |              | 84                    |                          |
| Nicolopoulos et al., 1968 (191) | | | | |
| 1st day            | 11           |              | 606±429               |                          |
| 15th               | 9            |              | 471±196               |                          |

| II Authors | Age          | No. of cases | VMA excretion, µg/mg creatinine Mean | Range |
|------------|--------------|--------------|---------------------------------------|-------|
| v. Studnitz, 1960 (292) | 1st day      | 6            | 10.6                                  | 5.0—18.3 |
|            | 3rd          | 6            | 5.4                                   | 5.0—7.3  |
| Gjessing, 1966 (89)     | 2nd week     | 6            | 6.2                                   | 4.0—9.0  |
| Lees, 1966 (169)        | 30±13 days   | 13           | 10.1±4.7                              |       |
| Nicolopoulos et al., 1968 (191) | | | | |
| 1st day            | 11           |              | 15.8±18.7                            |       |
| 15th               | 9            |              | 18.6±5.8                             |       |
Table 2. Urinary UMA excretion of children (I=UMA µg/24 hrs and µg/kg/24 hrs; II=UMA µg/mg creatinine; III=UMA µg/m²/24 hrs; IV=UMA µg/liter)

| Authors                        | Age 0—12 months | Age 1—10 years | Age over 10 years |
|--------------------------------|-----------------|----------------|-------------------|
|                                | Months          | Cases          | µg/24 h           | µg/kg/24 h       | Years          | No. of cases | µg/24 h      | µg/kg/24 h    | Years          | No. of cases | µg/24 h      | µg/kg/24 h    |
| v. Studnitz, 1960 (292)        | 2—5, 6         | 300, 60       | 2300 ± 250        | 11—15, 10       | 3200 ± 40     |
| Zeisel, 1961 (332)             | 6—12, 8        | 660, 83       | 600 ± 46          | 6—10, 8         | 3900 ± 40     |
| Voorhess & Gardner, 1962 (313) | 4—5, 2         | 612, 110      | 820 ± 70          | 1.2—1.7, 3      | 1272 ± 85     |
|                                | 9              | 440, 52       | 2041 ± 70         | 3—5.5, 8        | 2117 ± 70     |
| Young et al., 1963 (340)       | 1—7, 7         | 527, 170—1180 | (310—890)        | 1—2, 2          | 600 ± 1900    |
|                                |                |               | (1200—2800)      | 3—7, 6          | 2117 ± 1900   |
| Ritzel & Hunzinger, 1963 (240) | 0—2, 4         | 533, 165      | 1000 ± 900        | 0—10, 46        | 3600 ± 1900   |
| Cession-Fossion et al., 1964 (43) | 2—12, 29      | 592, 108      | (3.1)             | 10—30, 28       | 3050 ± 1050   |
| McKendrick & Edwards, 1965 (174) | 3, 12         | 470, ± 330    | 1200 ± 350        | 1—1.5, 10       | 1700 ± 350    |
| Hakulinen, 1966 (108)          | 2<4, 4         | 1610, 118     | 1930 ± 103        | 2<4, 15.9, 25   | 2350 ± 98     |
| Voorhess, 1967 (311)           | 0—12, 19       | 569, 126      | 1348 ± 88         | 6—15, 13        | 2373 ± 78     |
| Matsaniotis et al., 1968 (181) | 2—12.5, 30     | 1990, 84.7    | (900—4360) (44.5—110.5) | 2—12.5, 13.8 | 3192 ± 78     |
Table 2 (cont.)

| II Authors       | Age 0—12 months | Age 1—10 years | Age over 10 years |
|------------------|-----------------|----------------|-------------------|
|                  | Months          | VMA excretion, |                   |
|                  | No. of cases    | µg/mg creatinine |                   |
|                  |                 | Mean Range     |                   |
| v. Studnitz, 1960 (292) | <12 (**) | 7.0 1.4—15 |                   |
| Gitlow et al., 1965 (84) |  |  |                   |
| Gjessing, 1966 (89) |  |  |                   |
| III Author       | Age 0—12 months | Age 1—15 years | Age over 15 years |
|                  | Months          | VMA excretion, |                   |
|                  | No. of cases    | µg/m2/24 h     |                   |
|                  |                 | Mean Range     |                   |
| Voorhess, 1967 (311) | 0—12 19 | 2021 893—5211 |                   |
| IV Author        | Age over 15 years | VMA excretion, |                   |
|                  | Months          | µg/m2/24 h     |                   |
|                  | No. of cases    | Mean Range     |                   |
| Young et al., 1963 (340) |  |  |                   |

*) Values are taken from the mean regression line of the daily VMA excretion of 96 subjects (healthy children and active hospitalized children with non-specific minor ailments)

**) Series of 106 normal subjects from 3 weeks to 18 years of age

****) 65 children from 1 week to 14 years of age with non-specific minor ailments
The MA+MNA/VMA ratios on the first and third days of life were 22.5 ± 11 and 14.2 ± 19 in premature infants and 5.4 ± 3.2 and 4.5 ± 3.2 in fullterm newborn infants (27). The difference between the ratios in premature and fullterm infants was significant. The ratio values in both groups differed also from those in the adult group; this difference was ascribed by the authors to the absence of MAO in newborn infants, which results in increased o-methylation. However, Greenberg & Gardner (100) were of the opinion that A administered to newborn infants is metabolized in a manner similar to that in adults.

A rise of 25% in the VMA excretion was seen in 16 children 4—9 days of age when the environmental temperature was lowered from 29.4 to 23.9°C (258).

A and NA: In the urine of the newborn 0.6—0.8 μg/24 hrs of NA and 0.1—0.4 μg/24 hrs of A are excreted (39, 101, 333). While the A excretion per kg of body weight by the newborn was nearly the same as that by children 1.5—6 years of age, the excretion of NA was only one third of that in the latter (39, 138). During the first three days of life the highest mean excretion of A was seen on the first day, whereas the NA excretion gradually increased during this period (39). In premature infants the A excretion on the 1st and 15th days of life approximately corresponded to those of fullterm infants, whereas the NA excretion on both days was significantly lower than in the fullterm infants whether calculated per 24 hours or per kilogram per 24 hours (190).

Feet down tilting of children aged 1—7 days raised the NA excretion to 2.5-fold and the A excretion to 2.4-fold (104); in children 4—36 days of age the value was 3.7- and 2.8-fold, respectively (116). Greenberg et al. (104) demonstrated also a selective adrenaline response to insulin-induced hypoglycemia.

Older infants and children

The daily excretion of VMA per unit of body weight is highest in early infancy (43, 191, 311, 332; Table 2), after which there is a slight gradual decrease. On the other hand, the absolute excretion of VMA increases with age throughout childhood and there is a good linear correlation between VMA excretion and the age (174, 181, 304) and the weight of the child (181).

When VMA excretion is expressed on the basis of creatinine output, decreasing excretion readings are obtained with increasing age and the adult level is attained at the age of 15 years (84). In relation to body surface area the mean VMA excretion was nearly similar in all the age groups (311); however, these excretions showed a fairly wide dispersion.

Adults

In the quantitative determinations of VMA the mean urinary excretions in adults vary, owing to differences in technique, in the range 1.2—7.5 mg/24 hrs, the mean of all mean values given in the literature being 3.7 mg/24 hrs (6, 52, 59, 65, 70, 76, 158, 163, 176, 184, 197, 203, 205, 208, 209, 213, 221, 240, 243, 247, 255, 262, 270, 292, 301, 309, 328, 329). When expressed on the basis of the VMA/creatinine ratio, the range values for VMA excretion of adults have been 0.8—7.5, mean being 2.7 μg/mg creatinine (6, 76, 80, 148, 164, 184, 213, 240, 243, 247, 255, 262, 270, 292, 301, 309, 324, 325).

Using the present method, Pekkarinen and his co-workers determined the urinary VMA excretion in some conditions. In soldiers (n=50) during normal military service it was 5.2 ± 0.58 (S.E.M.) mg/24 hrs (203), in hospitalized psychiatric male patients (n = 18) 4.5 ± 0.32 mg/24 hrs (209), in hospitalized cerebrovascular patients (n=29, male and female) 4.5 ± 0.19 mg/24 hrs (208) and in surgical patients (n=67) on the pre-operative day 3.8 ± 0.1 mg/24 hrs (195, 205).

The mean VMA content of urine has been found to be 5.9 (range 1.4—10.5) μg/ml (292) and 4.4 (range 1.1—9.1) μg/ml (213).

The daily excretion of VMA is fairly constant in the same individual, but there is considerable variation between individuals (152, 210, 309).

The intrapair variance of VMA excretion in monozygotic twins was smaller than in dizygotic twins and it was especially small among those monozygotic pairs who had been living together for more than 28 years (164). Race and the nutritional state influence the absolute VMA excretion values. So
for instance indigent and emaciated hindus excreted less VMA than well-nourished individuals (118).

Neither the premenstrual phase nor menstruation caused a rise in VMA excretion (88). In a series of 113 women with normal pregnancy the VMA excretion during the last trimester was $4.1 \pm 0.15$ (S.E.M.) mg/24 hrs, or the same as in the control series (197). Neither were any changes observed in another study in the excretion at the end of pregnancy (41).

**Diurnal rhythm**

The excretion of VMA of children has been found to be higher during the day than at night (108, 174). A similar daily rhythm has been observed in adults (185, 195, 213, 267, 292, 301, 321). The VMA excretion in adults was on the average $175 \pm 68 \mu g/hr$ in daytime and $85 \pm 31 \mu g/hr$ during sleep (267). Values up to $214 \pm 103$ and $124 \pm 54 \mu g/hr$, respectively, have also been reported (27). The difference between nocturnal and daytime excretions was mostly dependent on the state of wakefulness, while the effect of standing position was hardly noticeable (182). No significant day/night variation in the VMA excretion was, however, observed in Korean adults (152).

Tautz *et al.* (303) distributed the persons into three groups according to their VMA excretions during 24 hrs. The first group had a high morning and a relatively high night excretion, which was typical for sympathectonic individuals; the second had low morning and night excretions, which was typical of vagotonic individuals, and the third, a non-characteristic group, comprised most other persons.

**Sex difference**

No difference in the 24-hour VMA excretion was demonstrable between boys and girls 1—11.9 years of age, but in those aged 12—15.9 years there was a notable difference (174). The investigators considered, however, the possibility that the urine samples from the latter group of girls were not complete 24-hour excretions in all of the cases. No marked sex difference was seen by Terslew (304) in the excretion of VMA in 60 children (2 months to 14 years) nor by Matsaniotis *et al.* (181) in 30 children (2 to 12.5 years).

Weis *et al.* (321) found in adults that the total VMA excreted in 24-hour urine samples was markedly higher in men than in women ($p < 0.001$), but when the VMA excretion was expressed in either mg/kg of body weight per 24 hrs or $\mu g/mg$ creatinine there was no marked difference in the values for men and women. Likewise, according to Georges & Whitby (76) the urinary excretion of VMA was 21% higher in men than in women when calculated in mg/24 hrs, but when expressed in $\mu g/mg$ creatinine it was 19% lower in men than in women. Some other authors, too, have observed no marked sex difference in the urinary VMA excretion per 24 hrs (128, 213, 240, 292, 328).

**Effect of psychic tension and physical exercise**

In healthy air force men under a test series of actual and imagined forward accelerations there was a marked rise in the urinary VMA excretion both in true and imagined situations (20). At the time of conservative dental care the VMA excretion increased significantly in 35 patients out of 38 (267, 336). During *heart catheterization* 22 patients showed a significant elevation of the VMA excretion as a result of emotional stress (268). Inexperienced *underwater swimmers* had a higher VMA excretion before diving than trained divers, evidently due to emotional stress (261).

Analogous results have also been obtained in animal experiments. After being moved to a strange cage 8 rats out of 12 had an increased excretion of VMA for 10 days (123). Handling of the rats also caused a marked increase in this excretion until the animals became accustomed to it within a week. Withdrawal of food for 12 hours raised the VMA excretion during the following days (123). In the opinion of the authors, the amount of VMA excreted in the urine is a reliable index of fundamental changes during emotional stress. However, the emotional tension in connection with 6 hours' *maturation* examinations, which can be con-
sidered to be remarkable, did not cause an increase in the VMA excretion as compared to the corresponding control period (203).

Miyake et al. (185) examined the post-delivery VMA excretion from repeated samples of catheterized urine taken every 30 minutes and observed a marked rise in the VMA excretion 150—180 minutes after delivery. The VMA excretion of 45 women doing routine household work at the end of pregnancy (4.4 ± 0.30 S.E.M. mg/24 hrs) was only slightly higher than that of 68 mothers who were in bed-rest in the obstetric unit (3.9 ± 0.20 mg/24 hrs) (197). The VMA excretion during hospitalization was on the average 123 ± 106 μg/hr in 25 patients with liver disease and 126 ± 71 μg/hr in 25 patients with a healthy liver but suffering from intestinal and circulatory diseases, while the VMA excretion of 30 healthy test subjects was 176 ± 68 μg/hr. The difference in the mean values of the excretions was significant between patient groups and healthy persons (p <0.03 and p < 0.02, respectively) (272).

Both in obese persons and in female individuals of normal weight, the resting condition decreased and muscular work increased the urinary excretion of VMA (283). The VMA excretion of a basket ball team rose during a two hours’ training period from 250 μg/hr to 480 μg/hr and during contest games from 360 to 860 μg/hr. In the latter case the initial values already were high (mean excretion of control subjects was 175 ± 68 μg/hr), undoubtedly because of tension during waiting (267). In 20 young soldiers a march of 13 kilometers caused only a very slight increase in the VMA excretion per 24 hrs in comparison to the excretion in the 24-hour control period (from 5.2 ± 0.38 mg to 5.9 mg), while heavy physical stress, a skiing tour of 60 km, caused during 12 daytime hours a more distinct rise from the control values (from 2.7 mg to 3.9 mg) (203). During the 12 nocturnal hours following the skiing tour the excretion returned to 2.1 mg, i.e., to the control level (203).

According to another investigation (316), made in connection with 60—90 km of skiing by skiers of all ages, the mean excretion of VMA in 12-hour urine was 5.3 ± 0.27 (S.E.M.) mg per 12 hrs, significantly above the control value of 2.8 ± 0.13 mg per 12 hrs (p<0.01). After skiing the immediately following nocturnal VMA excretion, 3.2 ± 0.24 mg per 12 hrs, was also significantly increased above the control value of 2.6 ± 0.17 mg per 12 hrs (p < 0.05).

**URINARY EXCRETION OF VANIL-MANDELIC ACID IN PATHOLOGICAL CONDITIONS**

**Catecholamine-secreting tumors**

Pheochromocytoma, neuroblastoma and ganglioneuroma are neoplasms that produce, in addition to the adrenomedullary hormones A and NA, also their precursors dopa and dopamine and derivatives of these. These tumors develop from primitive sympathetic neuroblasts (sympathogones), which normally differentiate either to chromaffin cells or to neuroblasts and ganglion cells. Pheochromocytoma develops from chromaffin cells and, like its host cells, secretes both A and NA. Neuroblastoma develops from the primitive neuroblasts, and ganglioneuroma corresponds to mature ganglion cells. The latter tumors excrete NA and dopa as well as dopamine and metabolites of these.

In cases of pheochromocytoma, which occurs in both children and adults and generally is benign, increased urinary excretions of A and NA were first demonstrated by Engel & v. Euler (66) by a biological method and by Pekkarinen & Pitkänen (206, 207) by a chemical method, and an increased VMA excretion by Armstrong & McMillan (7). In addition to the elevated excretion of VMA, higher excretions of also MNA and MA have later been confirmed in numerous studies and are presented in, among others, many surveys of clinical and laboratory aspects (22, 60, 77, 114, 119, 285, 307, 310).

In contrast to the neuroblastoma, the excretion of dopamine or HVA is generally not increased in cases of benign pheochromocytoma (290), whereas increased excretions of dopamine and HVA seem to point to malignancy of this neoplasm (64, 82, 141, 175, 245, 260). On the other hand, a normal excretion of dopa, dopamine or HVA does not necessarily exclude the possibility of malignant pheochromocytoma (82, 144).

The excretions of A, NA and their metabolites may show considerable individual variation in patients with pheochromocytoma.
(53, 54, 55, 56, 81). For example, the ratio VMA/NA+A in the urine usually is lower in the presence of small tumors than of large ones (53, 54).

Although the rise in the excretions of A, NA, MNA and MA from the normal levels is relatively greater than that of VMA in patients with pheochromocytoma (55, 143), the determination of VMA seems to be the most widely used method in the diagnosis of this neoplasm (254, 322). To attain reliable results it is well to determine the VMA on several successive days, particularly in the presence of paroxysmal hypertension. In certain borderline cases determinations of MNA, MA and/or A and NA as well as other pharmacological tests are also needed in addition to determination of VMA (53, 67, 77, 226, 253, 322).

Mason et al. (180) were the first to observe increased excretions of A and NA in cases of neuroblastoma, and Sandler & Ruthven (257) demonstrated an elevated excretion of VMA in two cases of neuroblastoma, Greenberg & Gardner (102) in ganglioneuroma and Stickler et al. (282) in ganglioneuroblastoma.

Due to diagnostic difficulties and the relatively rare occurrence of these types of tumor it is not yet exactly known how often the VMA excretion is increased in their presence. Many authors have observed normal excretions in patients with neuroblastoma (19, 24, 105, 131, 133, 155, 293, 300, 312, 314), but the proportion of such cases is clearly under 25% in most series. So for instance only one of 25 patients had the VMA excretion within normal limits in the neuroblastoma series of v. Studnitz (293), 5 out of 26 in that of Voorhess et al. (315), one of 16 in Robinson’s (242) and only 4 of 73 in Käser’s (180) series.

In ganglioneuroma, a benign tumor encountered mostly in adults but occasionally also in children, increased VMA excretions have been comparatively seldom described (86, 100, 102, 151, 251, 279, 340). In Käser’s (180) series 6 out of 8 had normal values, as also had all the 5 patients with ganglioneuroma examined by v. Studnitz (295).

Ganglioneuroblastoma is histopathologically an intermediate form of neuroblastoma and ganglioneuroma, reflected also in the ganglioneuroblastoma’s hormonal excretion pattern of the metabolites of catecholamines. Thus, for instance, these tumors excrete frequently clearly higher amounts of VMA than the ganglioneuromas, but less than the neuroblastomas (86, 130, 151, 287, 297, 300, 313, 314, 340).

In view of the malignancy of neuroblastoma an early diagnosis is important. By determining also HVA and/or dopamine in addition to VMA, a correct diagnosis can be reached in nearly every case (53, 130, 326). Simultaneous determinations of VMA and total catecholamines will also lead to a diagnosis of neuroblastoma with a high degree of accuracy. Using Gitlow’s screening test (85) for the determination of VMA and his own total catecholamine screening determination, Bell (18) was able to make in his series a diagnosis of neuroblastoma with the accuracy of 100 per cent.

The relationship between the clinical symptoms of the neoplasms of neural origin, such as chronic diarrhea and hypertension, and the catecholamines and their metabolites produced by these neoplasms is not clear (4, 130, 310).Obviously these symptoms are associated more frequently with differentiated than with undifferentiated tumors and result from the tumor, since they disappear after removal of the neoplasm (99, 100, 124, 134, 151, 154, 180, 251, 279, 282, 289, 297, 314).

As other special clinical characteristics Chatten & Voorhess (44) reported 6 known instances of familial occurrence of neuroblastoma. Griffin & Bolande (107) followed up two sisters among these patients, both of whom showed progression of their retroperitoneal tumors to ganglioneuroma. In one case, metastatic tumor nodules of the skin which had matured to ganglioneuroma came to closely resemble neurofibroma through continued loss of ganglion cells. A 3-year-old child with ganglioneuroblastoma exhibited the clinical features of myasthenia gravis, which disappeared after excision of the tumor (241).

**Surgical operations**

Three children showed postoperatively no rise in the urinary excretion of VMA according to McKendrick & Edwards (174).

In adults, however, increased VMA excretions have been seen after surgical operations
(195, 200, 203, 205, 216, 244, 299, 318). In operations using extracorporeal circulation there was a correlation between the duration of perfusion and the excretion of VMA, and the highest excretions occurred in connection with fatal surgical complications (299). After gastric and lung surgery more VMA was excreted than after operations on the gallbladder, which involve a less major procedure with respect to both duration and extent (200). The mean VMA excretion reached the maximum on the 2nd postoperative day, being more than twice the average basal excretion before operation, and the highest VMA excretion was 24.6 mg/24 hrs in a patient who had undergone gastric operation (195, 200).

**Burns**

The excretion of VMA increases also in burns (244, 292). Seven patients 3 to 62 years old who had 11—111 degree burns and a burned area of 15—45 % had distinctly increased VMA excretions of up to 25 mg/24 hrs. These patients had two peaks in the VMA excretion curve, one during the acute phase and the other in the second week (292).

**Traumatic shock**

Greatly increased VMA excretions, 12.3—16.9 mg/24 hrs (normal 2—8 mg/24 hrs), were found in 18 patients in a state of traumatic shock (218). These patients also excreted increased amounts of A and NA. The percentage of increase of A was higher than that of NA. Activation of the sympathetic nervous system in skull injuries was observed also by Kassil (142), according to whose study the urinary excretion of A and NA rises more in severe cranial traumas than in mild ones.

**Cardiovascular diseases**

In patients with hypertension the urinary excretion of VMA seems according to many investigators to be within normal limits independent of the etiology (51, 79, 83, 137, 152, 168, 176, 214, 240, 243, 262, 301, 305, 309). Some authors, however, have reported elevated VMA excretions in essential hypertension (178, 246, 298), in climacteric hypertension (225, 246), in renal hypertension (52, 246) and also in toxemic pregnancy (36). In contrast to the above investigators, Schmid & Henning (270) found reduced VMA excretions in renal hypertension, and Brunjes (26), Georges & Whitby (76), Calandre et al. (32) and Piliego et al. (219) also in essential hypertension.

There were no significant mean differences from day to day in the VMA excretions of normal, labile hypertensive and fixed hypertensive subjects and no consistent relationship between the blood pressure level and the VMA excretion (309).

Brunjes (1964) found the MA+MNA/VMA ratio to be distinctly increased in 19 per cent of patients with essential hypertension. A significant rise in the A and VMA excretions into the urine was induced with histamine in healthy control persons but not in patients with hypertension (284, 305). The authors regarded this observation as evidence supporting the opinion of Brunjes et al. (30) that the metabolic transformation of adrenaline and noradrenaline is deficient in hypertension. The metabolism of catecholamines in hypertension does not, however, differ from the normal according to most investigations (193).

Persons who, because of their living habits, belonged to the risk group of coronary diseases had higher VMA excretions than the controls (31). An increased VMA excretion has been observed in patients with myocardial infarction (1, 117, 216, 252). According to Ambanelli & Starcich (3) the changes appearing in the VMA excretion of patients with coronary disease are inconstant and transitory.

In severe heart failure in infancy the MA+MNA and VMA excretions were twofold in comparison to the control values; on the other hand, cyanotic children without heart failure had no rise in VMA excretion and only a slight rise in MA+MNA excretion (169).

The VMA excretion rises also in heart failure in adults (117, 201). In the study by Pekkarinen & Fisalo (201) the mean excretion of VMA was slightly elevated in patients with heart failure during the first to five successive days of their hospitalization, being between 4.9 ± 0.45 and 6.3 ± 0.92 mg/24 hrs, and only one fourth of these patients had a mean excretion of more than 6 mg. The degree of severity of the heart
failure had no statistically significant effect on the mean VMA excretion. Unlike the series mentioned, that of Chidsey et al. (48) showed no significant rise in the excretion of VMA in patients with heart failure.

**Respiratory diseases**

In 12 premature infants with the respiratory distress syndrome (RDS) the plasma A level was fourfold, whereas no significant rise was noted in the plasma NA (46). According to Cheek & Rove (47) A is of major significance in the pathogenesis of this disease. However, in the study of Boehm & O’Rien (23) no greater VMA excretions were found in the newborn with RDS than in the control children.

The A and NA excretions of asthmatic children were greatly increased in Kolesov’s study (153) and were proportionate to the severity of the paroxysm. The excretion of A by one of the patients during a severe attack was 100-fold the normal. With improvement of the condition the excretions were normalized (153). A similar observation was made earlier concerning asthmatic adults by Knauff et al. (149), in whom, however, the increase of NA was greater than that of A. In good agreement with these findings is the observation of increased VMA excretion in severely ill patients with respiratory insufficiency (81), and the same concerns some of the asthma patients of Hitzenberger & Bürklen (117).

**Hepatic diseases**

In portal cirrhosis of the liver the formation of VMA from i.v. administered radioactive NA and MNA was reduced from the normal and a significant rise was noted in the sulfate conjugates of NA and MNA (95). This study revealed a slight reduction in MAO activity. However, the oxidative deamination of biological amines has been normal in some other studies of patients with liver diseases (269, 271); also the VMA excretion of patients with cirrhosis of the liver has been normal (215). Likewise, the VMA excretion of hospitalized patients with liver diseases was similar to that in patients with intestinal or circulatory diseases but without liver disease (272).

**Renal diseases**

Reduced VMA excretion have been observed in adults with renal insufficiency (202, 203, 214, 270). Pekkarinen & Tisalo (202) found that the VMA excretion decreased with an increasing serum creatinine level and concluded that the reduced VMA excretion in kidney diseases was due to low renal clearance of VMA.

**Some hormonal diseases**

In hyperthyroidism the VMA excretion was either normal (125, 327) or to some extent lowered (171, 215). In hypothyroidism normal VMA excretions were observed (125, 327). In the study by Brunjes et al. (30), reduced VMA excretions were seen in both hypo- and hyperthyroidism and the MA+MNA/VMA ratio was increased in hyperthyroidism.

In diabetes Petrášek & Dubovský (215) demonstrated a normal VMA excretion in 10 patients. Ten diabetics out of 22 in Floch’s series (72) showed a rise in VMA excretion in clinical conditions in which there was very little or no glucose in the urine, five having a maximum VMA content of 10—25 μg/ml in the urine and five over 30 μg/ml.

Sulfonyl urea caused rises in the VMA and VA excretions in diabetics and both intravenously administered ACTH and endogenously released ACTH in the metyrapone test caused an increase of the VMA and VA excretions in diabetics as compared with healthy individuals or with persons with latent diabetes (335). On the basis of their results the authors suggested that the sympathoadrenomedullary system of diabetics reacts more strongly to both exogenous and endogenous ACTH than that of other individuals.

**Disorders of the autonomic nervous system**

In 11 children with dysautonomia (Riley-Day syndrome) the HVA excretion was two-fold the normal and the VMA excretion only a half of the normal (281). Similar findings have been published by Geluzov et al. (75) in a newborn patient and by Gitlow et al. (84) in 40 patients with dysautonomia.
In contrast to the above, the VMA excretion was within normal limits in two patients with dysautonomia in the series of Young et al. (340) and in four patients in the series of Greer & Williams (106).

A disturbed catecholamine metabolism has been demonstrated also in phenylketonuria, in which there were reduced urinary excretions of dopamine, A and NA (188). In phenylketonuria, as in dysautonomia, the plasma levels of endogenous A and NA are deficient and both have an increased sensitivity to injections of A and NA (40, 75, 129, 188, 280).

Elevated A, NA and VMA excretions have been demonstrated in infantile acrodynia (217, 239). The probable etiological base of this disease is mercury poisoning (69, 319). Mercury inhibits S-adenosyl methionine (14) and thus has an inhibitory effect on meta-oxidation and potentiates the effect of adrenaline (45).

In orthostatic hypotension a change in the NA metabolism has been noted in which the urinary excretions of methylated catabolites, for instance of VMA and conjugated MNA and MHPG, are increased and those of non-methylated catabolites, for example DHMA and 3,4-dihydroxyphenylglycol sulfate, are reduced (94). A patient of Geltzer et al. (75) with postural hypotension showed no alteration in the VMA excretion on change of position from supine to standing. Increase of VMA excretion has been observed in Bürg's disease and Raynaud's syndrome (126).

### Neurological and mental diseases

Patients having complete transverse lesion of the cervical cord or the upper thoracic cord (above Th V) have been observed to have a reduced blood pressure and a low urinary VMA excretion (122, 224). Orthostatic stress caused a rise in VMA excretion in healthy persons and in persons with lesions of the lower spinal cord (below Th V); this rise was absent in high lesions (122). Schmid et al. (272) found in their series the lowest VMA excretions (0.1–0.6 mg/24 hrs) in four patients with tetraplegia.

The urinary excretion of VMA was normal in 13 patients with extrapyramidal disorders in the form of, e.g., chorea and/or athetosis, but HVA was significantly decreased (235, 238).

In patients with Parkinson's disease or other hyperkinetic conditions the urinary VMA and HVA excretions were normal both before and 4–5 days after stereotactic thalamotomy (233, 283). HVA in the cerebrospinal fluid was decreased in patients with Parkinson's disease (234, 237), but increased in patients with chorea (Birkmayer & Hornykiewicz, cited by Birkmayer (21)).

The urinary excretion of VMA by schizophrenics was normal (177, 220) and the same was true according to Labrosse et al. (167) of the main metabolites of i.v. administered H3-A. In contrast, McDonald & Weise (173) found in 10 schizophrenics an elevated VMA excretion, and a similar observation was made by Pscheidt et al. (224) in acute psychotic exacerbations.

Histamine injection i.m. significantly raised the excretion of VMA in male but not in female schizophrenics (150).

Three patients with acute periodic catatonia showed greatly increased VMA, MA and MNA excretions during psychotom attacks; during clinically symptomless periods the excretions were mainly within normal limits (87). During attacks of migraine there was an increase in the excretions of 5-hydroxyindole-acetic acid (5-HIAA) and VMA (57, 58, 276). Siciteri et al. (276) noted VMA excretions of up to 12 mg per 24 hrs, which reverted to normal with convalescence.

### Other diseases

According to Matsaniotis et al. (181) chronically anemic children put out almost twice as much VMA as did healthy children when the excretion was related to body weight.

Decreased urinary excretion of VMA was seen in 19 children with rheumatic fever and other collagen diseases as compared to 16 controls; methylcatecholamines also were low, though in a less pronounced way (49). According to the authors these results suggest decreased mono-amino-oxidase activity in the patients.

Increased excretion of VMA has also been reported in association with a retinoblastoma (154), a carotid body tumor (90), and malignant carcinoid tumors (179, 294). In a case of malignant carcinoid tumor the highest value for VMA excretion was only twice that of the upper limit of normal, whereas the output of 5-H1AA was 70-fold the normal
level (179). The urinary VMA excretion of patients with pulmonary carcinoma metastases was significantly higher than that of other carcinoma patients, the latter being within the normal range (121).

In male patients with glaucoma the excretion of VMA was decreased (62).

Effect of drugs or diet

The results of investigations concerning the effect of certain drugs on the urinary excretion of VMA are shown in Table 3.

The intake of coffee, tea, citrus fruits and especially of food containing vanillin has generally not been permitted in connection with VMA determinations since they may lead to erroneous results in most methods. The same restriction has been made concerning bananas because of their high content of noradrenaline and dopamine (317). These foods and beverages do not, however, affect VMA determinations made by the method of Pisano et al. (221, 256, 320, 327). However, it has recently been demonstrated that an amount of instant coffee equivalent to 162 mg of caffeine raised the urinary VMA excretion to more than twofold during the following 4 hours (334).

STABILITY OF VANILMANDELIC ACID IN THE URINE

No loss of VMA was observed during 7 months in four urine samples stored in the deep-freeze at $-20^\circ$C (292). Neither was any significant change noted in our present study in the VMA content of urine samples (10 samples, pH 2—3) after storage in the refrigerator ($-5^\circ$) up to 4 months and freezing and melting of the samples three times (199). Gitlow et al. (80) observed that urinary VMA remained stable for several months at $+10^\circ$C when the pH of the samples was 3—4. Under the same conditions the standard VMA

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**Table 3. Effect of drugs on urinary VMA excretion**

| Increased VMA excretion | Decreased VMA excretion | No consistent influence |
|-------------------------|-------------------------|-------------------------|
| ACTH\(^a\)              | Alpha-methyl-p-tyrosine | Alloxan\(^a\)             |
| Adrenaline, oral        | Bretylium tosylate      | Amphetamine              |
| Adrenaline, parenteral  | Catapresan \(^b\)       | Angiotensin              |
|                         | Chlorpromazine, short-term | Ephedrine, nasal    |
|                         | Chlorpromazine, long-term\(^c\) | Guanethidine         |
| Caffeine                | Imipramine, long-term   | Isoprenaline, subl.    |
| Glucagon                | Iproniazid (11, 93, 98, 146, 231, 249, 264, 291, 338) | inhal. (80, 255) |
| Histamine               | Isocarboxazid (274)     | Mephobarbital (173)     |
| Hydrocortisone\(^d\)    | Methyldopa\(^e\)        | Morphine (173)          |
| Insulin (hypoglycemia)  | Nialamide (209)         | Naphazoline, nasal (80) |
|                         | N-isopropyl-p-nitrophenyl-ethanol amine (34) | Noradrenaline, oral (255) |
| Metyrapone\(^e\)        | Nitroglycerin, subl.    | Noradrenaline, i.v. (309) |
| Prenylamine, short-term | Oxycertine, long-term   | Oxedrine tartrate, oral (162) |
| Reserpine, short-term   | Phenelzine (88, 266)    | Pargyline (29)          |
| Tolbutamide             | Prenylamine, long-term  | Pentobarbital (173)     |
|                         | Psilocybin (278)        | Phenylephrine (80)      |
|                         | Reserpine, long-term\(^f\) | Thyroxin\(^d\)       |

\(^a\) In diabetics only
\(^b\) Experimental animals
\(^c\) In patients with hypertension; inconsistent results in other diseases (35, 74, 135, 136, 275, 326)
\(^d\) No effect according to one investigator (228)
\(^e\) Consistent changes not observed by some investigators (29, 330)
solution remained stable for over a year. According to v. Studnitz (292) the urinary VMA was unchanged during 8 days at +4 and +20°C also without the addition of acid. Jacobs et al. (128) report that there was no significant difference between samples stored for one week with or without preservative (bisulfite fluoride) in the refrigerator (+4°C) and at room temperature (+25°C). Sato et al. (262), again, observed that in urine stored without acid at +15°C the loss of VMA began on the 4th day and by the 7th day was in some cases as much as 70%. In an infected urine sample kept at +4 — +20°C no VMA whatsoever could be demonstrated by chromatography (273).