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The Pattern of Urinary Catecholamines and Their Metabolites in Duchenne Myopathy, in Relation to Disease Evolution

Y. Dalmaz*, L. Peyrin*, J. C. Mamelle, D. Tuil***, R. Gilly**, and J. F. Cier***

* Laboratoire de Physiologie, Faculté de Médecine Grange-Blanche, Lyon, ** Service de Pédiatrie, Hôpital Ste-Eugénie, St-Genis Laval, and *** Institut de Pathologie Moléculaire, Paris, France

With 3 Figures

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Summary

In this report we have tried to determine whether or not catecholamines are involved in the progressive muscular dystrophy. Catecholamines and their metabolites were studied in urines of children with Duchenne disease or other forms of myopathy (limb-girdle and facio-scapulo humeral myopathies). Catecholamine deaminated metabolites were normal in either form of myopathy; in contrast, Duchenne patients, contrarily to other children, eliminated excessive amounts of most amines (catecholamines and methoxylated amines) in relation to age and degree of disease evolution.

Our results indicate that catecholamines are not the primary factors involved in the pathogenesis of Duchenne myopathy, but are rather secondary to some disease effects. It is suggested that the high excretion of catecholamines and their methoxylated amine metabolites observed in severely affected Duchenne boys might be related to thermoregulatory processes or/and to alterations in some enzymatic systems.

Introduction

The pathogenesis of genetic human progressive muscular dystrophy (PMD) remains unclear, and three theories have been proposed:

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abnormal neural influence on muscle (*Coërs* and *Telerman-Toppet*, 1977; *McComas*, 1974; *McComas et al.*, 1971), abnormal microvascular supply of muscle (*Cazzato*, 1968; *Demos*, 1961, 1971; *Engel*, 1971), or a genetic fault of the muscle surface membrane (*Rowland*, 1976). In the neurogenic theory the number of surviving motor units in a dystrophic muscle would be lowered and the morphology of motor end-plates altered, including abnormal neural input on muscle. According to the vascular hypothesis, the muscular lesions of Duchenne myopathy would result from a defect in skeletal microcirculation. For the membrane theory, the genetic defect is in some components of the cell membranes (ATPase, protein kinase, phospholipids, adenylcyclase) (*Brown et al.*, 1967; *Iyer et al.*, 1977; *Mawatari et al.*, 1974; *Pearson*, 1978; *Souweine et al.*, 1978) resulting in altered transport properties: enhanced efflux of muscular protein toward serum or modification of energy-dependent monoamine uptake process (*Iversen*, 1973). As a whole, no major evidence has been accumulated in favour of either above theory.

Since catecholamines are strong vasoactive agents able to induce muscle ischaemia their role in human muscular dystrophy has been

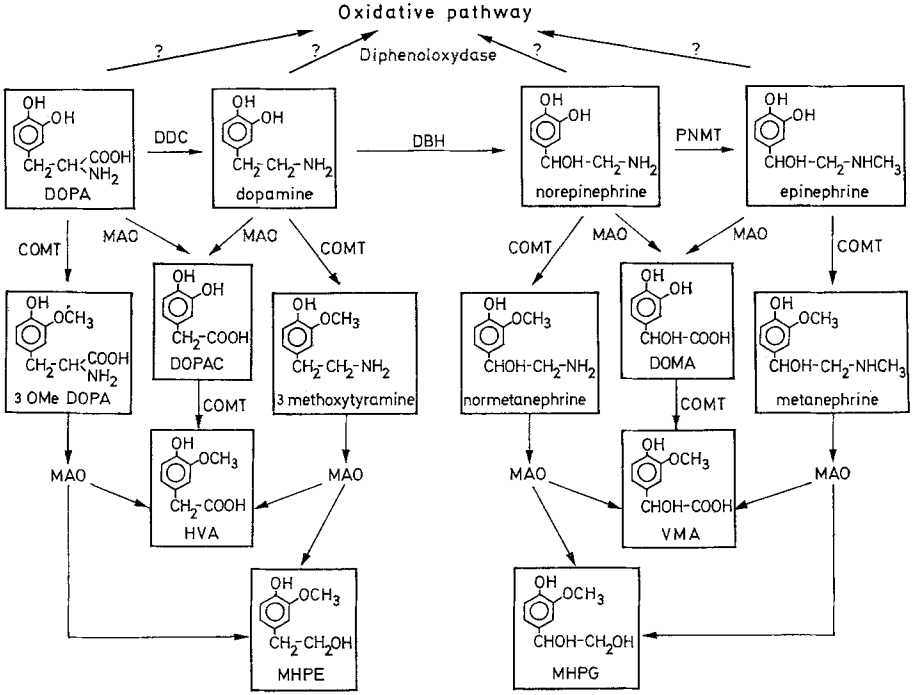

Fig. 1. The metabolism of catecholamines

questioned: indeed necrotic muscular lesions, reproducing the characteristic features of human PMD have been obtained experimentally in animals after injection of small dosis of vasoactive agents (as norepinephrine or serotonin) (*Engel* and *Derrer*, 1975). Accumulation of monoamine like materials and of catecholamines have been found in muscle of human Duchenne children (*Wright* and *Olson*, 1972) and of genetically dystrophic mice (*Gordon* and *Dowben*, 1966). High amounts of catecholamines have been reported in urine of these animals (*Gordon* and *Dowben*, 1966; *Kabara et al.*, 1976) but in human urinary samples previous results were conflicting (*Mendell et al.*, 1972; *Stern et al.*, 1956). In addition, no data on catecholamine metabolites are available.

The aim of the present study has been to investigate whether or not catecholamine metabolism is altered in PMD. Catecholamines (epinephrine [E], norepinephrine [NE], dopamine [DA]), methoxylated amines (metanephrine [MN], normetanephrine [NMN], 3-methoxytyramine [MT]), DOPA, and their acidic metabolites (vanillylmandelic acid [VMA], homovanillic acid [HVA], 3-4-dihydroxyphenylacetic acid [DOPAC] (see Fig. 1) have been estimated in urines from 20 patients with Duchenne muscular dystrophy and 7 patients with other forms of genetic myopathy.

The urinary data have been analyzed for their relation to the type of muscular dystrophy, age of patients and stage of disease evolution.

Materials and Methods

I. Subjects

All urine collections were done in hospitals in winter and spring. The ambient temperature was 22 ± 2 °C; the outdoor temperature varied from -2 °C to $+10$ °C.

All children received a vegetable-free diet in order to avoid dietary interference on the urinary metabolites of the catecholamines (*Claustre*, 1976). Medication was discontinued for a least eight days.

A. Patients

The patients were grouped in three classes taking into account their age, the form of dystrophy and rate of muscular impairment.

Groups D: children with ascending Duchenne de Boulogne myopathy of DDB I type (rapid evoluting myopathy) according to *Demos* and *Berthelon* (1972).

Group D_1: 2 boys, with DDB I, aged respectively 10 months and 3 years; mean age: 2.1 ± 0.5; 2 assays for each boy.

Group D_2: 18 boys, aged from 7 to 13 years, with DDB I, at different degrees of evolution; mean age: 9.6 ± 0.3; 27 assays. For some analytical purposes, this group was divided into 2 sub-groups D_2^+ and D_2^{+++}, corresponding respectively to mildly afflicted or severely afflicted myopathic boys.

Group M: is an heterogenous group, including 3 boys and 4 girls (5 to 14 years old; mean age: 10.5 ± 0.8; 13 assays) presenting other forms of myopathy (limb-girdle and facio-scapulo humeral myopathy).

B. Control Children

They were age and sex-matched convalescent children without pathology able to modify catecholamine metabolism (diarrhea, respiratory disease, endocrinological disorders). On the day of urine collection, only a moderate degree of physical activity was allowed. Children were classified as follows:

Group G_1: (7 boys, 10 months to 3 years old; mean age: 2.1 ± 0.1; 11 assays) was compared to the patients group D_1.

Group G_2: (14 boys, 7 to 13 years old; mean age: 9.3 ± 0.3; 21 assays) was compared to the patients group D_2.

Group G + F: (19 boys and 11 girls, 5 to 13 years old; mean age: 10.2 ± 0.3; 44 assays) was compared to the patients group M.

II. Methods

Daily urinary samples were collected over hydrochloric acid and kept at +4 °C until analysis. A single urinary sample was divided into four aliquots:

aliquot A (20 ml) for determination of DOPA, DA, NE, E and DOPAC;

aliquot B (20 ml) for methoxylated amines assays (MN, NMN, 3 MT);

aliquot C (5 ml) for HVA and VMA;

aliquot D (1 ml) for creatinine assay by automated colorimetry (*Paget et al.*, 1955).

DOPA, DA, NE, E and DOPAC

After hydrolysis of urinary conjugates, the extraction was performed by applying the double step-ion-exchange procedure previously described (*Dalmaz* and *Peyrin*, 1978) which ensures a high specificity for each compound and totally eliminates interference from methoxylated amines, HVA and VMA.

In the final eluates, DOPA, DA, NE and E were assayed by automated fluorimetry of hydroxyindoles derivatives: DOPA at pH 6 (*Cottet-Emard* and *Peyrin*, 1977), E at pH 2.85 and NE at pH 6 (*Peyrin* and *Cottet-Emard*, 1973) and DA at pH 5.2 (*Dalmaz* and *Peyrin*, 1976). DOPAC was assayed by continuous flow colorimetry (*Peyrin et al.*, 1978).

Methoxylated Amines

Aliquot B (20 ml) was first submitted to hot acidic hydrolysis and treated for extraction of total methoxylated amines by the double-step ion-exchange procedure previously developed (*Dalmaz* and *Peyrin,* 1976).

MN, NMN and MT were obtained in separate eluates, and assayed by the specific hydroxyindole fluorimetric methods, detailed earlier, MN and NMN after periodate oxidation (*Peyrin,* 1968) and MT after iodine effect (*Dalmaz* and *Peyrin,* 1976).

HVA and VMA

These compounds were extracted from acidified urines by means of ethylacetate. The organic extract was then chromatographied overnight on Whatmann 3MM paper in isopropanol ammonia water (42 : 1 : 8). The coloured spots, revealed by diazotized paranitroaniline, were analyzed for their HVA or VMA content, by comparing them in an automated densitometer (Chromoscan, Joyce Loebl) to those given by known amounts of authentic compounds, run together.

The sensitivities of all procedures were high and convenient for the estimation of above metabolites in urines of children.

Expression of Results

The results were expressed as μg/24 hours (mean $\pm$ S.E.); since the excretion of creatinine was highly altered in myopathic patients (except in D_1 group), the expression of urinary metabolites as μg/mg of creatinine was unacceptable. Similar observations were previously reported by *Mendell et al.* (1972).

In addition, to obtain further informations from the present metabolic study, individual data were pooled as follows:

the inaltered amines: DOPA + DA + NE + E;

the methoxylated amines: 3 MT + NMN + MN;

the whole deaminated pool: VMA + HVA + DOPAC;

the compounds more related to functional activity: DA + MT; NE + NMN; E + MN.

The mean data (m $\pm$ S.E.) from patient and control groups were compared using Student's t-test for unpaired data.

Results

I. Diuresis and Urinary Creatinine

For all dystrophic patients (D_1, D_2 and M groups) the daily urine output was lower than in control children of matched age (Table 1).

The creatinine excretion was lower than in corresponding controls in all groups of myopathy except in D_1 (Table 1). Body weights were normal for M groups but significantly lower than in controls for D_1 and D_2 boys (Table 1).

Table 1. *Daily urine output and urinary creatinine in healthy and myopathic children (m ± S.E.)*

		Group D_1 n = 2 d = 4	Group D_2 n = 18 d = 27	Group M n = 7 d = 13
Urine output (ml/24 hours)	Patients	230 ±44	472 ±31	437 ±84
	Controls	386 ±29	657 ±64	706 ±50
		(59.6±11.4)**	(71.8± 4.7)*	(61.9±11.9)**
Creatinine (mg/24 hours)	Patients	114 ±24	232 ±20	240 ±37
	Controls	117.7±11.8	526 ±51	540 ±30
		(96.9±20.4)	(44.1± 3.8)***	(44.4± 6.8)***
Body weight (kg)	Patients	10.1± 0.49	24.3± 2.1	31.6± 2.8
	Controls	12 ± 0.5	29 ± 0.97	32 ± 1.2
		(84.2± 4.08)*	(83.8± 7.2)*	(98.7± 8.7)

n = number of patients, d = number of assays. There were 7 controls for group D_1, 14 for group D_2 and 30 for group M.

The numbers under brackets refer to percent of patient values against corresponding controls.

Significative differences between patient and control values at * $0.01 < p < 0.05$; ** $0.005 < p < 0.01$; *** $p < 0.005$.

II. Excretion of Catecholamine Metabolites

1. *Catecholamines, DOPA and Methoxyamines* (Table 2). As a whole, there was an increase of urinary amines in patients with Duchenne myopathy, whilst the levels of these compounds were normal or even decreased in children with other myopathies (M group).

In young Duchenne boys (D_1) a significant increase was observed only for NE and NMN, urinary DA had a trend to be higher than in controls; E, MN and DOPA were normal.

In older DDB I boys (D_2), there was a dramatic increase in urinary E, MN, NMN, and DA, whilst NE, DOPA and 3-MT remained normal.

In contrast, in children of M group, urinary DA and methoxyamines were normal, and E, NE and DOPA were significantly lower than in controls.

Comparing Duchenne patients of D_2 group to children with other forms of myopathy (group M) pointed out increased amounts of either

aminated compound (except DA and MT) in the D_2 group (see under Table 2).

Table 2. *Daily excretion (μg/24 hours) of catecholamines, DOPA and their corresponding methoxyamines in children with different forms of muscular dystrophy and matched-age controls (m ± S.E.)*

		Group D_1 n = 2 d = 4	Group D_2 n = 18 d = 27	Group M n = 7 d = 13
E	Patients	3.2 ± 1.3	55 ± 20.6	3.4 ± 0.8
	Controls	2.9 ± 0.7	7.5 ± 0.86	8.5 ± 0.8
		(109 ± 44	(733 ± 274)*	(40 ± 9)***
NE	Patients	23.7 ± 7.5	48.7 ± 5.2	29.7 ± 7.9
	Controls	14.6 ± 2	41.4 ± 4.2	45 ± 3.7
		(162 ± 51)*	(117.6 ± 12.5)	(66 ± 18)*
DA	Patients	315 ± 170	452 ± 90	287 ± 101
	Controls	168 ± 30	255 ± 37	312 ± 29
		(187 ± 100)	(177.2 ± 35.3)*	(92 ± 32)
DOPA	Patients	22.5 ± 8	30.8 ± 3.8	17.7 ± 4.3
	Controls	16.9 ± 2.2	35.5 ± 4.4	35.5 ± 2.6
		(133 ± 47)	(86.7 ± 10.7)	(50 ± 12)***
MN	Patients	52 ± 11	310 ± 96	82 ± 17.8
	Controls	53.7 ± 5.6	94.7 ± 13	101 ± 9.6
		(97 ± 20)	(327 ± 101)*	(81 ± 18)
NMN	Patients	92.5 ± 13	133.3 ± 17.8	79.4 ± 17.3
	Controls	54.5 ± 7	81 ± 10.8	95.5 ± 12
		(170 ± 14)**	(164.5 ± 21.9)*	(83 ± 18)
MT	Patients	40 ± 12	64.6 ± 8.2	58.7 ± 15.6
	Controls	32 ± 4	54.5 ± 9.5	66 ± 6.9
		(126 ± 38)	(118.5 ± 15)	(89 ± 24)

n = number of patients, d = number of assays. There were 7 controls for group D_1, 14 for group D_2 and 30 for group M.

The numbers under brackets refer to percent of patient values against corresponding controls.

Significative differences between patient and control values at * $0.01 < p < 0.05$; ** $0.005 < p < 0.01$; *** $p < 0.005$.

Comparison of urinary data between D_2 and M groups: E *; NE *; DA: NS; DOPA *; MN *; NMN * and MT: NS.

2. *Deaminated Compounds* (Table 3). Neither in D_1 nor in D_2 Duchenne patient groups the urinary excretion of VMA, HVA and DOPAC was modified. Myopathic children of M group eliminated normal VMA and HVA amounts but lower DOPAC levels than corresponding controls. No difference was observed between D_2 and M groups.

Table 3. *Daily excretion (μg/24 hours) of deaminated metabolites (VMA, HVA, DOPAC) in children with different forms of muscular dystrophy and matched-age controls (m ± S.E.)*

		Group D_1 n = 2, d = 4	Group D_2 n = 18, d = 27	Group M n = 7, d = 13
VMA	Patients	830 ±250	2775 ±477	2040±400
	Controls	1128 ±232	2945 ±465	2952±319
		(73.5± 22)	(94.2± 16.2)	(69± 14)
HVA	Patients	1400 ±600	1933 ±338	1793±424
	Controls	778 ± 60	1283 ±146	1443±110
		(180 ± 77)	(151 ± 26.3)	(124± 29)
DOPAC	Patients	860 ±300	1621 ±236	1112±265
	Controls	912 ±220	1766 ±341	1858±225
		(126 ± 38)	(91.8± 13.4)	(60± 14)*

n = number of patients, d = number of assays. There were 7 controls for group D_1, 14 for group D_2 and 30 for group M.

The numbers under brackets refer to percent of patient values against corresponding controls.

Significative differences between patient and control values at * $0.01 < p < 0.05$.

3. *Pooled Metabolites.* In order to reveal alterations of a particular metabolic pathway, the urinary data were pooled as described in the paragraph "expression of results" (Fig. 2). As a whole, the deaminated pool was normal in either group of children (D or M) (Fig. 2 f), but aminated compound amounts (amines and methoxyamines) (Fig. 2 d and 2 e) were normal in M group and highly increased only in D_2 Duchenne patients.

Furthermore, the amount of adrenomedullary amines (E + MN) (Fig. 2 a) was about three-fold the normal value in D_2 boys, and normal in other groups. The amines reflecting peripheral sympathetic activity (NE + NMN; Fig. 2 b) were increased in the two Duchenne groups of patients. In contrast, the (DA + MT) pool was high only in young DDB I (D_1) (Fig. 2 c).

III. Catecholamine Metabolism at Different Stages of Disease Evolution

The urinary data obtained from Duchenne boys of D_2 group have been analyzed as to their relation with the degree of muscular dystrophy. In this aim, the patients were classified in two groups, in

terms of their ability to climb stairs and to walk without bracing (D_2^+) or not (D_2^{+++}). Precise clinical informations allowing such a classification have been obtained for 14 children. It can be seen from Fig. 3 that there was an increase of aminated compounds only in the severely affected children (D_2^{+++}); although the three pools of amines were high, the most important elevation above controls concerned (E + MN). Moreover, the D_2^{+++} group excreted significantly higher amounts of (E + MN) and (NE + NMN) than D_2^+ group.

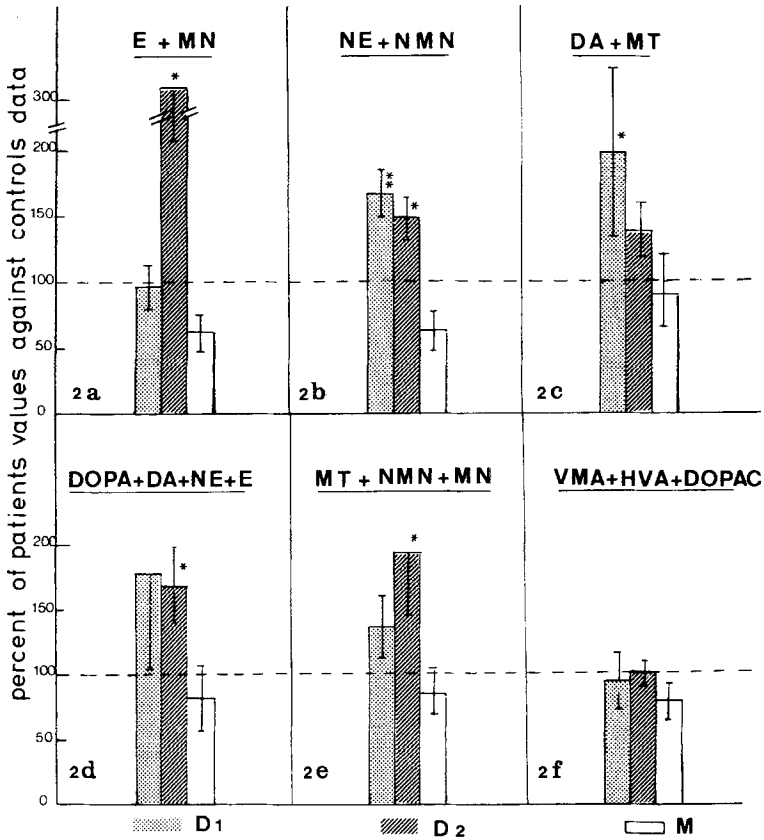

Fig. 2. Daily excretion (μg/24 hours, m ± S.E.) of pooled catecholamine metabolites expressed as percent of patients values against corresponding controls data in the three groups of dystrophic children D_1 (2 patients, 4 assays), D_2 (18 patients, 27 assays) and M (7 patients, 13 assays). *a-b-c* metabolites possibly related to functional adrenergic activity, *d-e-f* groups of chemical related metabolites. Significative difference between patients and corresponding controls values at * $0.01 < p < 0.05$, ** $0.005 < p < 0.01$, *** $p < 0.005$

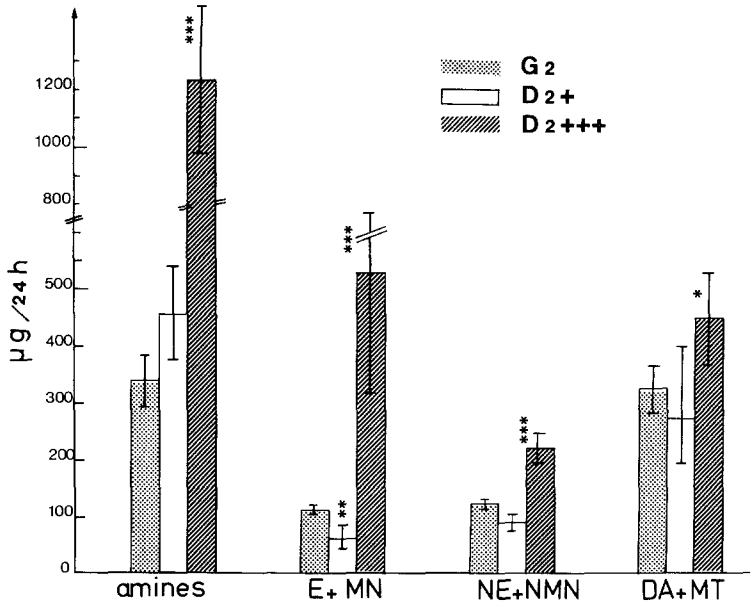

Fig. 3. Amine excretion (μg/24 hours, m $\pm$ S.E.) at different stages of disease evolution in Duchenne patients of D_2 group. D_2^+: 7 ambulant boys (7 assays) (mean age = 10 ± 0.8 years; mean body weight = 22.5 ± 2.1 kg. D_2^{+++}: 7 highly handicaped boys (12 assays) (mean age = 9.05 ± 0.4 years; mean body weight = 24.02 ± 3.8 kg). G_2: 14 corresponding control boys (21 assays) (mean age = 9.3 ± 0.3 years; mean body weight = 29 ± 0.97 kg). Amines: sum of DA + NE + E + MT + NMN + MN (μg/24 hours). Significative difference between patient and control values at * $0.01 < p < 0.05$, ** $0.005 < p < 0.01$, *** $p < 0.005$

Discussion

Previous reports on adrenergic function in human progressive myopathy were limited to the study of catecholamines in urines (E, NE) (*Mendell et al.*, 1972; *Stern et al.*, 1956).

Because several mechanism may influence catecholamine levels (changes in synthesis, release and/or degradation pathways), the interpretation of urinary data may be easier when the simultaneous determination of catecholamines and their metabolites have been performed. For these reasons, we have studied catecholamines (E, NE, DA, DOPA) their methoxylated amines (MN, NMN and MT) and deaminated acidic metabolites (VMA, HVA and DOPAC) in order to determine whether or not there is an alteration of catecholamine metabolic pattern in genetic human myopathy. Furthermore, additional informations may be obtained on the relative activity of

the peripheral adrenergic components by analyzing more precisely the amounts of adrenomedullary amines (E + MN) and those of sympathetic released amines (NE + NMN and probably DA + MT) (*Peyrin* and *Dalmaz*, 1975). With regard to urinary VMA, there is evidence that, in contrast to methoxylated amines, it can be derived from intraneuronal catecholamines (under the effect of mitochondrial monoaminoxydase [MAO]) and thus might reflect intraneuronal events (synthesis, reuptake) rather than active release of catecholamines (*Costa* and *Sandler*, 1972; *Kopin*, 1972; *Maas* and *Landis*, 1971).

The main conclusions of our work are that, as a whole, the sum of deaminated metabolites are not modified in either form of myopathy (D or M), whatever is the clinical state of patients (Fig. 2 f). In contrast, the most striking feature is that Duchenne patients, contrarily to other children (M) eliminate excessive amounts of most amines (Fig. 2), in relation to age and degree of disease evolution (Fig. 3). Thus, among genetically myopathic children, only highly handicaped Duchenne boys (D_2^{+++}) exhibit a marked disturbance of catecholamine metabolism. The finding that the metabolic alteration is highly dependent on the degree of disease evolution, explains perhaps the contradictory results reported in the literature, since catecholamine excretion in Duchenne boys was found increased by *Stern et al.* (1956) and normal by *Mendell et al.* (1972).

It must be noted however, that too much weight cannot be attached to the results in group D_1 as they are based on only two patients.

The unchanged VMA excretion is in agreement with previous results indicating that MAO activity was not altered in platelets (*Murphy et al.*, 1973) nor in skeletal muscle of Duchenne patients (*Kar* and *Pearson*, 1974). However, it is surprising that although the E + MN + NE + NMN sum is raised, the excretion of VMA is unaltered. A possible explanation might be an alteration in the renal excretion process affecting more especially organic acids. Indeed, there is evidence that glomerular filtration is the main mechanism involved in catecholamine excretion in man (*Juchmes*, 1977), whilst, in addition to glomerular filtration, a probenecid-sensitive mechanism, similar to that described in brain vessels, contributes to the renal excretion of acidic metabolites (*Meek* and *Neff*, 1972; *Spector* and *Lorenzo*, 1974). The alteration of this active transport process cannot be excluded in myopathic children. This statement has no direct experimental support; our results indicate only that daily urine output is low in Duchenne boys (Table 1). On the other hand, since a fraction of VMA can originate from catecholamine reuptake, an alternative explanation for the urinary unchanged VMA associated with high

amounts of catecholamines and methoxyamines might be the possible alteration of uptake 1, resulting in reduced NE neuronal uptake (see below).

Our own observations suggest that the catecholamine alteration does not seem to be the primary disorder in Duchenne disease, since it only becomes really apparent in children with advanced myopathy.

What are the factors causally involved in the catecholamine elevation and what role may play these amines on disease evolution? . . .

Two kinds of arguments may explain the considerably elevated excretion of catecholamines and methoxyamines in severely affected Duchenne boys (D_2^{+++}). Firstly, alterations in some enzymes related to catecholamines have been reported in these patients:

a) The adenylcyclase of erythrocyte membrane in Duchenne patients is not able to be stimulated by epinephrine (*Mawatari et al.*, 1976) suggesting a genetic alteration in the response of the amine. This would provide a possible explanation as to why Duchenne patients may need elevated levels of circulating epinephrine to fulfill the metabolic role of this catecholamine.

b) In erythrocyte ghosts from Duchenne boys, (Na^+-K^+-)ATPase with abnormal properties has been reported (*Mawatari et al.*, 1974; *Pearson*, 1978; *Sha'Afi et al.*, 1975; *Souweine et al.*, 1978). This enzyme is involved in the energy-dependent transport of catecholamines across neuronal membrane, during neuronal uptake process U_1. In contrast, catecholamines are allowed to penetrate freely by diffusion into extraneuronal cells, without energy requirements (*Iversen*, 1973). According to previous results obtained after pharmacological blockade of uptake 1 (*Hendley*, 1976; *Maas*, 1977), it could be expected that a defect in U_1 process induced by abnormal (Na^+-K^+-)ATPase in Duchenne patients would increase the overflow of catecholamines, their extraneuronal uptake U_2 and consequently the excretion of these amines and their corresponding methoxyamines.

c) In Duchenne DDB I boys, a lowered activity of platelet diphenoloxidase has been demonstrated (*Demos*, 1968, 1973; *Demos* and *Berthelon*, 1972). This enzyme, present in several tissues (*Axelrod*, 1964; *Demos*, 1968; *Tuil et al.*, 1975), is able, in vitro, to oxidize catecholamines and DOPA to the corresponding aminochromes and further to lutines and rheomelanins (*Hegedus* and *Altschule*, 1970). In vivo, the physiological role of this enzyme is questioned (*Bacq*, 1949; *Fisher* and *Lecomte*, 1951). Nevertheless, even if such an oxidative pathway could normally contribute to remove some active catecholamines from blood, it would concern only 2 to 5 per cent of blood amines (*Goodall* and *Alton*, 1965; *Koch*, 1966; *Kopin*, 1960;

Labrosse et al., 1961). Thus, it is unlikely that this factor alone might explain the high increase of urinary amines observed in our patients.

Secondly, another possible explanation for the catecholamine disturbance in severely affected Duchenne boys might be related to increased reliance on the known metabolic effects of catecholamines to produce heat (*Himms-Hagen*, 1972). Indeed, as a consequence of a very low degree of physical activity and appreciable loss of muscle tissue able to produce heat with shivering thermogenesis, these children must have an increased reliance on the ability of epinephrine and norepinephrine to induce non-shivering thermogenesis, and to mobilize energetic substrates. This metabolic hypothesis is supported by the observation that the clinical state of Duchenne patients is aggravated in winter; on the other hand, this argument agrees with the alteration of adenylcyclase activity.

Besides a possible efficience of catecholamines in the regulation of body temperature in D_2^{+++} boys, some physiopathological or experimental arguments can suggest that these amines might enhance muscular impairment:

a) Catecholamines are able to induce ischaemia in muscle and it has been shown that, in some conditions, necrotic muscular lesions similar to those observed in human PMD have been obtained experimentally in animals after injection of small dosis of vasoactive agents (as norepinephrine or serotonin) associated to aortic ligation, or after treatment with monoaminoxidase inhibitors or neuronal uptake blockers (*Burch et al.*, 1969; *Engel* and *Derrer*, 1975; *Hathaway et al.*, 1970; *Parker* and *Mendell*, 1974; *Yu et al.*, 1974).

b) Some clinical features, often associated to Duchenne disease (*Herschberg et al.*, 1965; *Heymsfield et al.*, 1978; *Robert* and *Guibaud*, 1966) are observed also in human patients with catecholamine secreting tumors, pheochromocytoma (*Hermann* and *Mornex*, 1964; *Werning* and *Siegenthaler*, 1971): intense sudation, vasomotor disturbances of the extremities, long-lasting response to hyperglycemic test, cardiac dysfunction including electrocardiogram modifications (inversion of T wave) and heart necrotic lesions. In contrast, elevation of arterial blood pressure has not been reported in Duchenne patients.

As a conclusion, our results clearly demonstrate that the catecholamine metabolism is altered only in Duchenne boys but not in other groups of myopathic children; among Duchenne children, severely affected patients only (D_2^{+++}) have a considerably elevated excretion of catecholamines and methoxylated amines. These results strongly suggest that although the catecholamine alteration is specific for Duchenne patients, it is not primary but rather secondary to some

effects of the disease process. The possible factors contributing to such a catecholamine increase might be some enzymatic alterations observed in these patients [adenylcyclase, (Na^+-K^+-)ATPase, diphenoloxidase] or/and the strong requirements of metabolic generation of heat in these children because of their low degree of physical activity.

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Authors' address: Dr. *L. Peyrin*, Laboratoire de Physiologie, Faculté de Médecine, 8, avenue Rockefeller, 69373 Lyon Cédex 2, France.