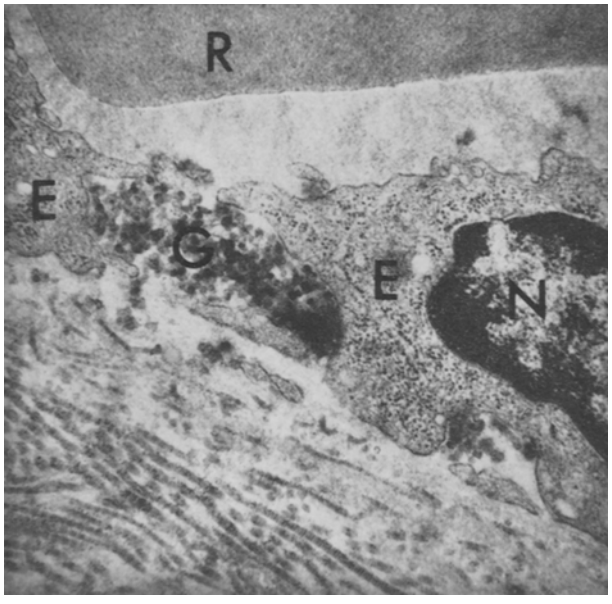

Fig. 2. Electron micrograph of synovial venule following intra-articular injection of serotonin. Carbon particles have accumulated in a gap (G) between 2 endothelial cells (E, E); R, erythrocyte in lumen; N, nucleus of endothelial cell. × 20.000.

approximately 70 times greater potency than histamine, maximal labelling of vessels being obtained by a dose of 0.25 µg. The synovial membranes of joints injected with 2.5 µg serotonin exhibited a similar amount of vascular labelling as those of joints injected with 0.25 µg, but labelling of vessels also occurred in muscle tissue adjacent to the joints. Bradykinin had approximately twice the potency of histamine in a dose range of 0.005 to 0.5 µg per joint. Maximal labelling of synovial vessels was not obtained with the highest test dose of bradykinin.

The relative potency of histamine, serotonin and bradykinin on the vascular permeability of synovialis therefore approximates the relative potency reported for these substances in rat's skin[4].

Electron microscopic examination of synovial vessels obtained from joints 8 min after intra-articular injection of histamine, serotonin or bradykinin revealed changes for each substance similar to those described for cremaster muscle of the rat[8]. Carbon particles were found beneath, and in gaps between, the endothelial cells of venules (Figure 2). Carbon deposits were not found in the walls of capillaries with either continuous or fenestrated endothelium[9]. In labelled venules, the nuclei of endothelial cells frequently showed prominent indentations of the nuclear membrane, a change which has been interpreted as resulting from contraction of the cytoplasm of the endothelial cell[10].

The above results indicate that in the rat, histamine, serotonin and bradykinin induce increased vascular permeability in the synovialis, that the mechanism of action of these substances is the same in synovialis as in skin and muscle, and that the relative potency of these substances is similar in synovialis and skin.

Résumé. La perméabilité vasculaire de la membrane synoviale du rat est augmentée par l'histamine, la sérotonine et la bradykinine. Dans cette réaction la sérotonine est plus active que l'histamine et la bradykinine.

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5 February 1975.

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Increased Catecholamine Excretion in the Rat After Administration of α-Methyl-Tyrosine

Alpha-methyl-*para*-tyrosine (α-MpT), an analogue of tyrosine, has been shown to inhibit tyrosine-hydroxylase competitively, in vitro[1] as in vivo[2]. Thus this drug has been widely used as an inhibitor of norepinephrine (NE) synthesis in various experimental conditions, mainly for the estimation of both the rate of synthesis and the tissular turnover of NE in the rat[3–5]. As a matter of fact, injection of α-MpT to guinea-pigs or rats has been found to decrease the tissue levels of NE[2,3]. Furthermore, because of its inhibitory effect on catecholamine production, α-MpT has also been used clinically, especially for the treatment of amine producing tumors[6].

As far as we know, no study has been devoted, however, to the possible effect of α-MpT on both the excretion and the oxidation of catecholamines. The purpose of the present work was to test this hypothesis by determining the effects of α-MpT on the urinary excretion of both epinephrine (E) and NE as well as of their metabolite 3-methoxy-4-hydroxy-mandelic acid (VMA) in normal rats. The effects of this drug on NE excretion were also studied in adrenalectomized rats.

Materials and methods. Male Wistar rats, weighing 180–200 g and fed on stock laboratory diet, were injected i.p. with a single dose (100 or 200 mg/kg) of either α-MpT (Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.), as a 0.5 *M* phosphate buffer solution (20 mg/ml), or α-MpT methyl ester (compound H 44/68,

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Effects of α-MpT (200 mg/kg) on the 4 h urinary excretion of epinephrine (E) and norepinephrine (NE) in the rat

	Controls	Injected α-MpT
E (μg)	0.026 ± 0.009	0.168 ± 0.090 [a]
NE (μg)	0.108 ± 0.011	0.993 ± 0.640 [a]

[a] $p < 0.01$ as compared to control values.

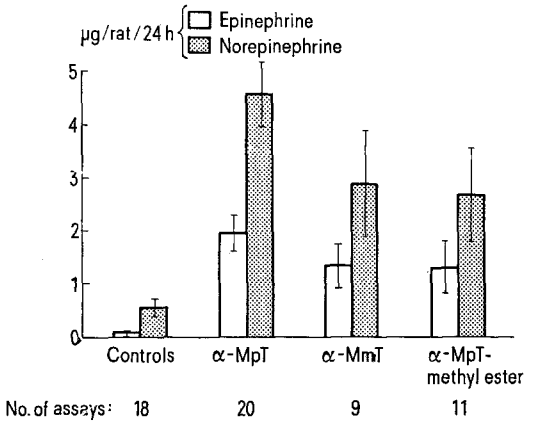

Fig. 1. Effects of α-MpT, α-MmT and α-MpT-methyl ester (200 mg/kg) on the daily urinary excretion of epinephrine and norepinephrine in the rat. The vertical bars represent 2 SEM.

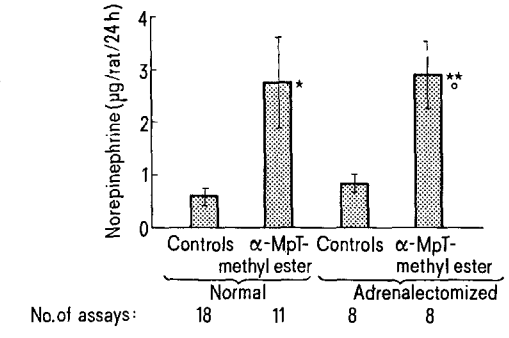

Fig. 2. Influence of adrenalectomy on the effect of α-MpT-methyl ester (200 mg/kg) on the daily urinary excretion of norepinephrine in the rat. * $p < 0.001$ as compared to control normal rats. ** $p < 0.001$ as compared to control adrenalectomized rats. ○, non significant as compared to normal methyl ester treated rats.

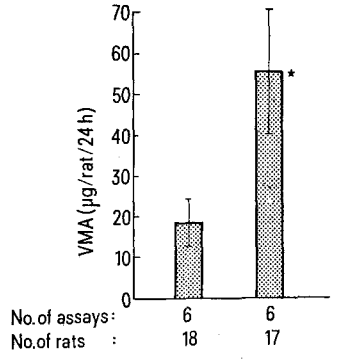

Fig. 3. Influence of α-MpT-methyl ester (200 mg/kg) on the daily urinary excretion of 3-methoxy-4-hydroxy-mandelic acid (VMA) in the rat. * $p < 0.01$ as compared to control values.

Labkemi AB, Göteborg, Sweden), as a saline solution (20 mg/ml). In some experiments, the meta isomer of α-MpT, α-methyl-*meta*-tyrosine (α-MmT, Nutritional Biochemical Corporation, Cleveland, Ohio) was also administered i.p. in a dose of 200 mg/kg as a saline solution (20 mg/ml). Control animals received i.p. the same volume of saline solution. In another set of experiments, α-MpT methyl ester (200 mg/kg) was administered intraperitoneally to adrenalectomized rats. Bilateral adrenalectomy was performed under ether anesthesia 6 to 8 weeks prior to the treatment. Adrenalectomized rats received the same food as the controls, except that saline was given in place of water.

Animals, placed in metabolic cages immediately after the injection, had free access to food and drink durirg the course of the experiments. Four or 24 h following the injection, urines were collected on 6 *N* HCl (0.5 ml) and filtrated. Contamination of urines by faeces was avoided by means of glass funnels covered with filters. Catecholamines (free and conjugated) were separated on acid-activated aluminium oxide (3 g/100 ml) added with EDTA (10 g/100 ml), according to the method of Euler and Orwen[7] as modified by de Schaepdryver[8]. E and NE were eluted in 0.25 *N* sulfuric acid and assayed fluorometrically, according to the method of Euler and Lishajko[9], using an Aminco-Bowman spectrofluorometer. In these conditions, neither α-MpT nor α-MmT interfered with the estimation of E and NE. Determination of VMA was performed according to the method of Sunderman et al.[10], as modified by Pisano et al.[11]. Results are expressed as means ± SEM. The statistical significance has been computed with the Student's *t*-test.

Results. As shown in Figure 1, α-MpT, α-MmT or α-MpT- methyl ester, administered i.p. in a single dose of 200 mg/kg, enhances the urinary excretion of both E and NE. Compared to controls, E and NE levels are increased from 13 to 20 times and 4.5 to 8 times respectively, maximal effects being observed with α-MpT.

When a lower dose (100 mg/kg) of α-MpT or α-MpT-methyl ester was administered, similar effects were obtained. In these conditions, compared to the control values, urinary excretion of E was significantly ($p < 0.01$) increased by both compounds (×17 by α-MpT and ×7 by α-MpT-methyl ester), whereas urinary excretion of NE increased also significantly ($p < 0.01$), but to a lesser extent (×5 by α-MpT and ×3.5 by α-MpT-methly ester).

As shown in the Table, this action of α-MpT appears early since, as soon as 4 h after the administration of α-MpT (200 mg/kg), the urinary excretion of E and NE were significantly increased (×6.5 and 9.0 respectively).

In order to determine the contribution of adrenal medulla in these effects, we have studied the influence of α-MpT-methyl ester (200 mg/kg) on the urinary excretion of NE in adrenalectomized rats. In these conditions, the increase in the urinary excretion of NE was of the same order of magnitude in both normal and adrenalectomized rats (Figure 2).

Enhancement of the urinary excretion of catecholamines induced by α-MpT could be related to a reduction of catecholamines oxidation. In order to test this hypothesis,

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we have measured the daily excretion of one of the urinary metabolites of catecholamines, namely VMA. As can be seen in Figure 3, α-MpT-methyl ester did not reduce the excretion of VMA but, on the contrary, markedly increased the latter.

Discussion. Administration of α-MpT to guinea-pigs[2] or rats[3] has been shown to result in a sharp decrease of the heart, spleen and brain NE levels. From these experiments, it was postulated that this decrease was a consequence of NE synthesis inhibition. On the other hand, the *meta* isomer of α-MpT, α-MmT, a potent inhibitor of dopa-decarboxylase[12], has been reported to increase the release of catecholamines[13]. In the same conditions, our experiments show that administration of either α-MpT or α-MmT is followed by a drastic rise in the urinary excretion of E and NE. It seems, therefore, that the effects of these drugs described above are not only related to their inhibitory action on catecholamine biosynthesis, but may also be the consequence of a modification of their release, their oxidation, as well as on their uptake.

SPECTOR et al.[2] have tested the possibility that α-MpT may increase the tissular release of NE. As α-MpT is methylated in vivo and transformed into α-methylated amines which are known to induce the release of NE, these authors have assayed methyl tyramine and α-methyl norsynephrine in both heart and brain of guinea-pig after the administration of α-MpT. Since these assays were unsuccessful, SPECTOR et al. concluded that the NE tissular decrease induced by α-MpT was unrelated to an effect on catecholamine release, and was only the consequence of tyrosine-hydroxylase inhibition.

Another mechanism which could explain the increase of urinary excretion of catecholamines is an inhibitory effect of α-MpT on the catabolism of catecholamines. As shown by the increase of the urinary excretion of VMA, it can be assumed that α-MpT does not inhibit the oxidation of catecholamines.

The effects of α-MpT or α-MmT on the excretion of E and NE could also be attributed to an interference of these compounds with the mechanism involved in the catecholamine uptake. As a matter of fact, HESS et al.[12] have shown that α-MmT decreases the ability of tissues to take up and bind exogenous NE. The same effects were obtained with α-MpT by BRALET et al.[5], who concluded that a decrease in the re-uptake of NE enhances the release of NE for a few hours. Our present data showing that α-MpT and α-MmT also increase the excretion of catecholamines therefore support this finding.

From our results, it is not possible to determine precisely the mechanism by which α-MpT enhances the urinary excretion of catecholamines. However, we can postulate, like ENNA et al.[14], that the inhibition of tyrosine-hydroxylase is not the only action of α-MpT. We can furthermore conclude that this drug stimulates the tissular release of E and NE into the blood, the part taken by adrenal medulla in this release being negligible[15].

Résumé. Une injection à des rats d'α-méthyl-*para*-tyrosine (α-MpT), d'α-MpT-méthyl ester ou d'α-méthyl-*méta*-tyrosine (α-MmT), aux doses de 200 mg/kg, produit une augmentation rapide et importante de l'excrétion urinaire de l'adrénaline et de la noradrénaline, ainsi que de l'acide vanyl-mandélique. La surrénalectomie ne modifie pas l'augmentation de l'excrétion urinaire de noradrénaline produite après injection d'α-MpT-méthyl ester. L'élévation des taux des catécholamines urinaires résulte vraisemblablement de leur libération tissulaire, sous l'action de l'α-MpT ou de l'α-MmT.

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Amphetamine-Induced Changes in Body Temperature and Glycogen Content of the Encephalon in the Chicken[1]

Thirty min after an i.p. injection of D-amphetamine, 5 mg/kg, the concentration of glycogen in the mouse brain decreased by 30%; this depletion was closely associated with an increase in phosphorylase a, and the subsequent marked resynthesis of the polysaccharide seemed achieved by the conversion of glycogen synthetase D to I[2,3]. In these conditions, the depletion of glycogen may occur in glial cells in response to the release of catecholamines[4,5]. On the other hand, injection of amphetamine in the rat, the NMRI-strain mouse and the rabbit produced an increase in body temperature, though in the C3H-strain mouse no change appeared[6–8]. The possible role of dopamine in the amphetamine-induced hyperthermia was suggested[7,9,10]. We may expect that some connection exists between cerebral glycogenolysis and body hyperthermia, which is mediated by the level of biogenic amines

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