Br. J. Pharmac. (1970), **40**, 418–425.

Effects of chronic metaraminol treatment on the sympathetic activity of intact and adrenal demedullated rats kept in warm or cold environments

G. E. JOHNSON AND T. A. PUGSLEY

Department of Pharmacology, University of Toronto, Toronto 5, Canada

Summary

1. Rats were placed at 27° C or 4° C and given metaraminol ((10 mg/kg)/day) in their drinking water for 8 weeks. One experiment was run using adrenal demedullated rats. These animals were treated with metaraminol, as mentioned above, and kept at 4° C for 4 weeks.

2. Body temperature and metabolic rate were determined at selected intervals. Urine was collected on day 7 of each week and analysed for adrenaline, noradrenaline, metanephrine, normetanephrine and 3-methoxy-4-hydroxyphenylglycol (MHPG). At the end of each study the rats were killed and the tissues removed and analysed for metaraminol, adrenaline and noradrenaline.

3. All animals survived the metaraminol treatment and no change in metabolic rate or body temperature was seen. Metaraminol depressed the growth rate of the rats.

4. Metaraminol caused a fall in tissue noradrenaline concentrations, with only negligible quantities being found in brain, heart, lung, liver, kidney and spleen. Only the adrenals contained significant quantities of catecholamines. All tissues contained large amounts of metaraminol.

5. Despite the almost complete depletion of noradrenaline from sympathetic nerves, metaraminol did not depress the excretion of noradrenaline, normetanephrine and MHPG, in fact excretion of the latter two substances was higher in the treated animals. The failure of the drug to impair the normal cold-induced increase in noradrenaline secretion explains the survival of the rats at 4° C.

6. Adrenal demedullation did not prevent the metaraminol-treated rats from excreting large quantities of noradrenaline, normetanephrine and MHPG in the cold. It is apparent, therefore, that in the intact rats the noradrenaline emanated from the practically depleted nerves. The increase in MHPG excretion, seen during metaraminol treatment, suggests an increased rate of noradrenaline turnover.

7. In conclusion, although metaraminol uptake is accompanied by a fall in tissue noradrenaline concentrations, the presence of metaraminol does not depress noradrenaline release. These results do not support the concept that metaraminol can replace noradrenaline and function as a false transmitter.

Introduction

Metaraminol is taken up and bound within sympathetic nerves, causing a fall in tissue noradrenaline stores (Carlsson & Lindqvist, 1962; Shore, Busfield & Alpers, 1964; Andén, 1964; Udenfriend & Zaltzman-Nirenberg, 1964). Following its uptake into nervous tissue, metaraminol behaves, in many ways, like noradrenaline. It is subject to release by catecholamine releasing drugs or by *in vitro* or *in vivo* sympathetic stimulation. Its rate of synthesis, from α-methyl-*m*-tyrosine, is increased by *in vitro* or *in vivo* sympathetic stimulation (Crout, Alpers, Tatum & Shore, 1964; Johnson & Mickle, 1966; Johnson & Pugsley, 1968; Costa, Neff & Ngai, 1969). Because of these facts metaraminol has been referred to as a false sympathetic transmitter.

Most of the research on metaraminol has been restricted to studying the short term effects of the drug. The possibility that metaraminol can replace noradrenaline within sympathetic nerves and function as a sympathetic transmitter led us to investigate the significance of this replacement over a prolonged period. The experiments were run under conditions of rest and sympathetic stimulation. For the purpose of maintaining a constant sympathetic stress, rats were kept at 4° C for several weeks.

Methods

Male Wistar rats, obtained from the Canadian Breeding Laboratories, were used in the study. The animals were placed at 27° C or 4° C and given metaraminol in their drinking water. Control rats, not given metaraminol, were kept at both ambient temperatures.

In the present study both intact and adrenal demedullated rats were used. Adrenal demedullation, under pentobarbital anaesthesia, was performed 5 to 6 weeks before use. Experiments involving the use of intact rats were continued for 8 weeks, adrenal demedullated animals were studied for 4 weeks.

The daily dose of metaraminol was 10 mg/kg. A close check of the quantity of drinking water consumed assured the ingestion of at least that amount.

Body temperature was measured by inserting a thermocouple 3 to 4 cm inside the rectum. Metabolic rate was calculated from the oxygen consumption of the rats placed in a constant temperature water bath of 4° C $\pm$ 1° (Ferguson & Sellers, 1949).

Urine was collected on day 7 of each week and analysed for noradrenaline and adrenaline by the method of Euler & Lishajko (1961). Free metanephrine and normetanephrine were purified on Amberlite CG-120 Type 2 and assayed fluorimetrically (Häggendal, 1962). Total 3-methoxy-4-hydroxyphenylglycol (MHPG) in the urine was estimated by a modification (Johnson & Pugsley, 1968) of the Ruthven & Sandler (1965) method. At the end of each experiment the rats were killed and the tissues removed and analysed for metaraminol (Pugsley & Johnson, 1968) and adrenaline and noradrenaline (Euler & Lishajko, 1961).

Results

Treatment of the cold-stressed rats with metaraminol for 8 weeks did not affect survival. No significant change in core temperature or metabolic rate was noted as a result of drug treatment (Table 1). Metaraminol diminished the growth rate

of the rats. Treated animals kept at 27° C gained only 49 g in 8 weeks whereas the control rats gained 129 g during this time. At 4° C the metaraminol treatment for 4 weeks caused a loss of 4 g compared with a gain of 20 g in the controls.

The influence of metaraminol on catecholamine storage and excretion from intact rats is shown in Figs. 1, 2 and 3 and Table 2. High concentrations of metaraminol were found in all tissues. With the exception of the adrenal glands, the quantities of metaraminol present in the tissues at the time of killing greatly exceeded the amount of noradrenaline normally present. Evidence of the ability of metaraminol to lower tissue catecholamine concentrations may be seen in the low estimates of noradrenaline in organs removed from the animals. One exception was noted. Metaraminol increased the catecholamine concentration in the adrenal glands of the cold-stressed rats. The excretion of the catecholamines was not decreased by metaraminol treatment at 27° C. Urinary normetanephrine values were at times higher in the treated rats kept in the warm. The levels of MHPG were significantly higher at 27° C ($P<0{\cdot}01$) in the drug-treated rats. Cold exposure increased noradrenaline excretion and this effect was not altered by metaraminol. After the third week at 4° C, metaraminol caused a further increase in the already elevated excretion of normetanephrine and MHPG.

Metaraminol treatment did not impair the survival of the cold-stressed adrenal demedullated rats during 4 weeks' exposure to 4° C. Core temperature was not lowered by metaraminol. Tissue analysis after the fourth week of treatment showed an uptake of metaraminol combined with a lowering of noradrenaline in the heart and spleen (Table 3). Adrenals, containing presumably only cortical tissue, taken from the control rats contained only trace amounts of adrenaline and noradrenaline. After 4 weeks of treatment with metaraminol the surgically demedullated glands contained significant quantities of adrenaline. Metaraminol initially depressed noradrenaline, normetanephrine and MHPG excretion (Fig. 4). This effect was reversed later in the experiment as the excretion of all substances rose to exceed that of the control adrenal demedullated animals ($P<0{\cdot}05$).

Discussion

The ability of metaraminol to replace noradrenaline within sympathetic nerves is recognized. In spite of the depression of endogenous noradrenaline levels, animals treated acutely with metaraminol show little sympathetic impairment (Andén & Magnusson, 1965; Johnson & Mickle, 1966; Johnson & Pugsley, 1968). The present set of experiments was begun to investigate the effects of "flooding" the tissues with a false transmitter, over many weeks, on the ability of the rats to meet a known situation demanding increased sympathetic activity.

Metaraminol was given orally as it was felt to be the best way of supplying the drug continually over the 8 week period. Proof of the absorption of metaraminol can be

TABLE 1. *Core temperature and oxygen consumption ((ml/m²)/min at NTP) of metaraminol treated and control rats at week 7 of study, of rats exposed to 4° C*

	Control	Treated
Oxygen consumption	275·3±22·84	307·0±13·61
Core temperature (°C)	38·3±0·1	37·6±0·3

Data are presented as mean±S.E.M. of four animals.

TABLE 2. *Concentrations of noradrenaline (NA), adrenaline (A) and metaraminol (MA) in tissues of rats kept at 27° C and 4° C for 8 weeks*

	Heart	Brain	Adrenals	Lungs	Liver	Kidneys	Spleen
27° C							
NA (Control)	0·99±0·11	0·66±0·007	48·98±6·95	0·16±0·03	0·04±0·003	0·15±0·004	1·41±0·03
NA (MA treated)	0·07±0·03**	0·28±0·02**	21·58±2·73*	None detected	None detected	0·02±0·002**	None detected
A (Control)			359·57±3·78				
A (MA treated)			178·18±5·25**				
MA	4·22±0·33	1·63±0·16	10·43±1·35	4·86±0·19	1·42±0·08	1·79±0·07	8·35±0·87
4° C							
NA (Control)	0·99±0·06	0·92±0·08	149·21±9·29	0·22±0·03	0·02±0·008	0·03±0·006	2·13±0·18
NA (MA treated)	0·03±0·002**	0·63±0·003*	174·92±4·29*	0·05±0·006**	0·0004±0·0002	None detected	None detected
A (Control)			485·16±3·44				
A (MA treated)			561·61±5·04**				
MA	6·21±0·42	1·51±0·04	4·93±0·29	2·65±0·16	8·21±0·07	2·75±0·38	11·26±1·50

Rats received at least 10 mg of metaraminol daily in their drinking water. Values in table are expressed as nmol/g of tissue except for adrenals, which are presented as nmol/kg of body weight. Each treated value is a mean±S.E.M. of five determinations. Control values of tissues of rats placed at 4° C are means of four determinations. Tissue values for metaraminol are corrected for incomplete recovery and tissue blanks.

* $P < 0.05$ when compared with control rats.

** $P < 0.01$ when compared with control rats.

seen in the high tissue concentrations at the time of killing. However, in spite of the uptake of metaraminol, and the concomitant fall in tissue noradrenaline, no impairment in the excretion of noradrenaline and normetanephrine was seen at 27° C. At 4° C metaraminol increased normetanephrine excretion. In the past we have measured excretion rates of noradrenaline and normetanephrine and equated changes in these with alterations in noradrenaline release (Shum, Johnson & Flattery, 1969). Normetanephrine is the major metabolite of secreted noradrenaline (Wurtman, 1966) and it has been reasoned that measurement of its excretion, together with noradrenaline, provides a better index of noradrenaline release than measurements of catecholamine excretion alone. Based on this assumption it would appear that at 4° C the metaraminol-treated rats released even more noradrenaline than the

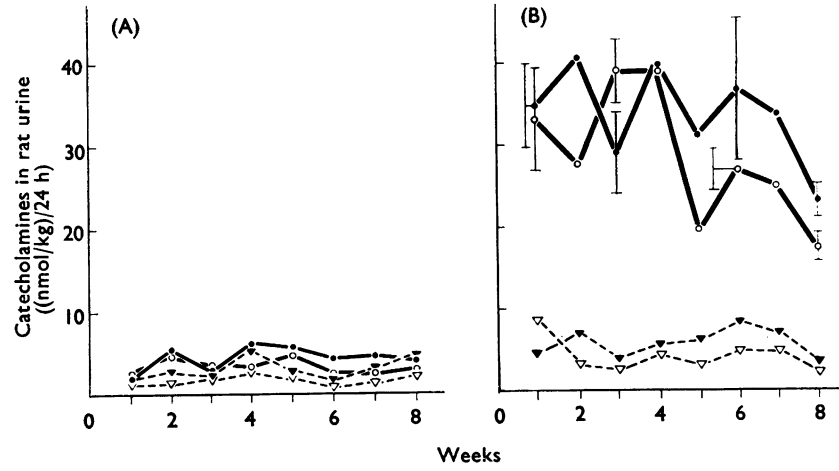

FIG. 1. Urinary excretion of adrenaline (▼---▼, treated rats; ▽---▽, control rats) and noradrenaline (●——●, treated rats; ○——○, control rats) from rats kept at 27° C (A) or 4° C (B). Points are means of five rats for metaraminol treated and four rats for controls. Standard errors are plotted for noradrenaline at 4° C.

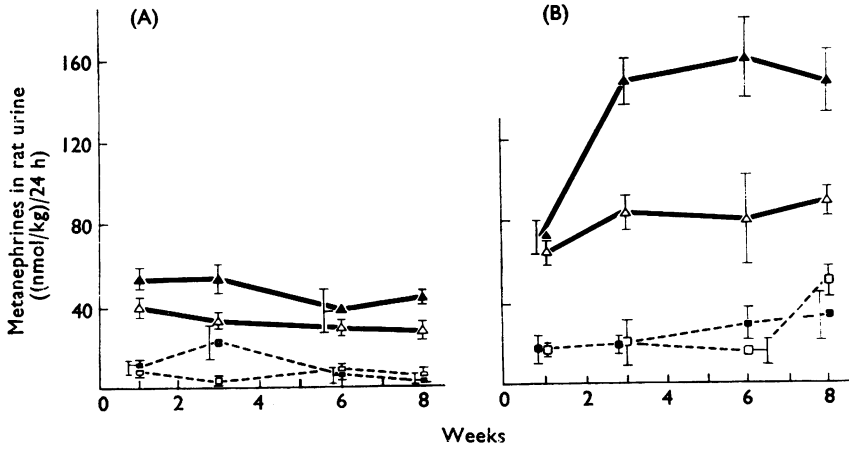

FIG. 2. Urinary excretion of metanephrine (■---■, treated rats; □---□, control rats) and normetanephrine (▲——▲, treated rats; △——△, control rats) from rats kept at 27° C (A) or 4° C (B). Points are means ± standard errors of five rats for metaraminol treated and four rats for controls.

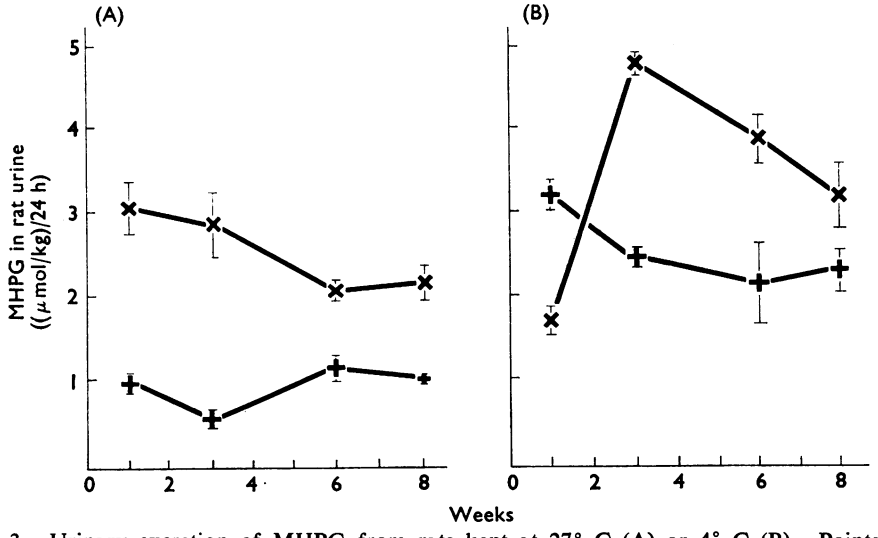

FIG. 3. Urinary excretion of MHPG from rats kept at 27° C (A) or 4° C (B). Points are means ± standard errors of five rats for metaraminol treated (×——×) and four rats for controls (+——+).

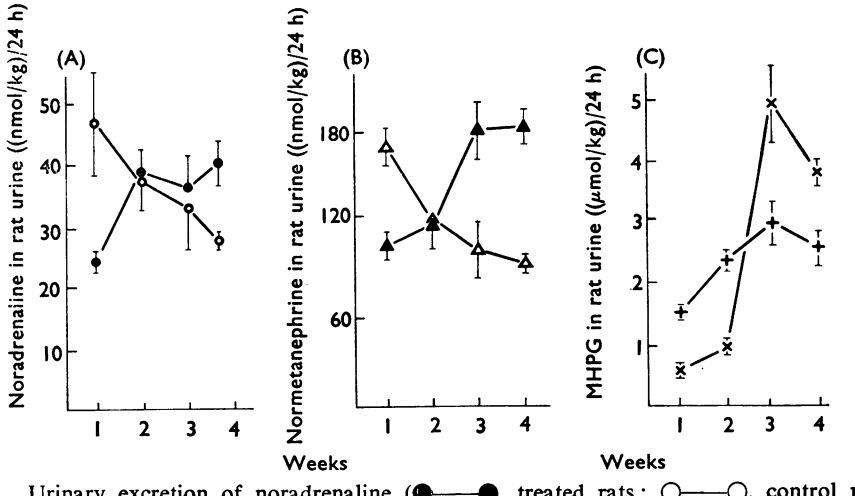

FIG. 4. Urinary excretion of noradrenaline (●——●, treated rats; ○——○, control rats), normetanephrine (▲——▲, treated rats; △——△, control rats) and MHPG (×——×, treated rats; +——+, control rats) from adrenal demedullated rats kept at 4° C. Points are means ± standard errors of five rats for metaraminol treated and four rats for controls.

TABLE 3. *Concentration of noradrenaline (NA), adrenaline (A) and metaraminol (MA) in tissues of adrenal demedullated rats kept at 4° C for 4 weeks*

	Heart	Spleen	Adrenals
NA (control)	1·71±0·021	2·46±0·04	23·79±0·870
NA (MA treated)	0·12±0·012**	0·83±0·05**	2·40±2·19
MA	2·78±0·12	5·10±0·23	
A (control)			7·10±0·25
A (MA treated)			100·26±24·57**

Rats received at least 10 mg/kg of metaraminol daily in their drinking water. Values in table are expressed as nmol/g of tissue except for adrenals, which are stated in nmol/kg body weight. Treated values are means of five determinations. Control values are means of four determinations. Tissue values for metaraminol are corrected for incomplete recovery and tissue blanks. Each value is mean±S.E.M.

** $P<0·01$ as compared with control concentrations.

control animals. However, this may not necessarily be true. Metaraminol is known to show a greater affinity for the neuronal amine reuptake mechanism than does noradrenaline (Burgen & Iversen, 1965). It is possible that the presence of metaraminol around the sympathetic nerves blocked the reuptake of the released noradrenaline. This would explain the elevated urinary levels of normetanephrine, seen during metaraminol treatment, regardless of the quantities of noradrenaline secreted. At the present time it is impossible to determine which of the above postulates truly explains the increases in normetanephrine excretion caused by metaraminol. However, regardless of the mechanism(s) involved, the failure of metaraminol to depress the release of noradrenaline in the cold explains the survival of the animals.

The low tissue concentrations of noradrenaline seen in the intact rats, coupled with the high rate of excretion, raise the question as to the source of the noradrenaline. Adrenal demedullated rats were treated with metaraminol to determine if the noradrenaline emanated from the almost depleted nerves, or from the adrenaline medullae, where large stores still remain. As adrenal demedullation did not prevent the treated rats from excreting high levels of noradrenaline and normetanephrine, it is apparent that the noradrenaline originated in the practically depleted nerves.

In addition to the measurement of the catecholamines and metanephrines in urine, the excretion of MHPG was also determined. MHPG is the O-methylated, oxidatively-deaminated product of adrenaline and/or noradrenaline. In the conditions of our experiment, where the release of noradrenaline greatly exceeded that of adrenaline, MHPG is believed to result mainly from the degradation of noradrenaline. The urinary excretion of MHPG greatly exceeds the combined amounts of noradrenaline and normetanephrine and, under normal circumstances, is felt to result mainly from the intraneuronal oxidative deamination of noradrenaline followed by extraneuronal O-methylation (Wurtman, 1966). Changes in MHPG excretion are often felt to reflect alterations in noradrenaline synthesis (Kopin, 1964; Wurtman, 1966). If such is the case then it might be concluded that metaraminol increased the rate of noradrenaline synthesis at 27° C. At 4° C an initial depression in noradrenaline synthesis at week 1 was followed by an increased rate of formation. Such an increase in noradrenaline synthesis is consistent with the ability of the rats to maintain release, particularly at 4° C, in the face of low tissue concentrations. It is possible, however, that the increase in MHPG seen during metaraminol treatment reflects an increase in the extraneuronal degradation of noradrenaline. If, as suggested earlier, metaraminol can block the neuronal reuptake of noradrenaline, a higher percentage of that secreted would be available for extraneuronal O-methylation and oxidative deamination.

The possibility that metaraminol, replacing noradrenaline within sympathetic nerves, may function as a false transmitter has been well investigated (Carlsson *et al.*, 1962; Crout *et al.*, 1964; Crout, 1966; Kopin, 1968a, b). In our laboratory it has been shown that sympathetic stimulation, produced by cold exposure, increased both the formation and release of metaraminol (Johnson & Pugsley, 1968). In the present study, chronic daily treatment with metaraminol resulted in high tissue levels of the drug and virtual depletion of noradrenaline. However, no evidence of a depression of noradrenaline release was seen and all animals survived in the cold. This is in agreement with Andén & Magnusson (1965), who showed

that metaraminol can replace up to 95% of the cardiac noradrenaline stores without impairing sympathetic function. The evidence that the release of metaraminol does not serve as a replacement for noradrenaline release does not support the false transmitter concept. In spite of the fact that metaraminol is bound within sympathetic nerves and the rate of its release is dependent on nerve stimulation, it did not during chronic treatment influence the rate of noradrenaline release.

One additional point deserves comment. Daily metaraminol treatment for several weeks increased the catecholamine content of the intact adrenals and the adrenaline levels of the previously demedullated adrenal glands. No explanation can be offered at this time for this phenomenon. However, further studies are in progress.

This work was supported by grant MA 1595 from the Medical Research Council of Canada. T. A. P. was a recipient of a Medical Research Council of Canada Studentship.

REFERENCES

ANDÉN, N. E. (1964). On the mechanism of noradrenaline depletion by alpha-methyl-tyrosine and metaraminol. *Acta pharmac. tox.*, **21**, 260–271.

ANDÉN, N. E. & MAGNUSSON, T. (1965). *Pharmacology of Cholinergic and Adrenergic Transmission*, ed. Koelle, G. B., Douglas, W. W. and Carlsson, A., p. 319. Oxford: Pergamon Press.

BURGEN, A. S. V. & IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmac. Chemother.*, **25**, 34–49.

CARLSSON, A. & LINDQVIST, M. (1962). In vivo decarboxylation of alpha-methyl-meta-tyrosine. *Acta physiol. scand.*, **54**, 87–94.

COSTA, E., NEFF, N. H. & NGAI, S. H. (1969). Regulation of metaraminol efflux from rat heart and salivary gland. *Br. J. Pharmac. Chemother.*, **28**, 153–160.

CROUT, J. R., ALPERS, H. S., TATUM, E. L. & SHORE, P. A. (1964). Release of metaraminol (aramine) from the heart by sympathetic nerve stimulation. *Science, N.Y.*, **145**, 828–829.

CROUT, J. R. (1966). Substitute adrenergic transmitters. *Circulation Res.*, **18** and suppl. 1, 120–127.

EULER, U. S. VON & LISHAJKO, F. (1961). Improved technique for the fluorometric estimation of catecholamine. *Acta physiol. scand.*, **51**, 348–355.

FERGUSON, J. K. W. & SELLERS, E. A. (1949). The effect of iodide and other halides given with thiouracil. *J. Pharmac. exp. Ther.*, **97**, 177–181.

HÄGGENDAL, J. (1962). Fluorometric determination of 3-O-methylated derivatives of adrenaline and noradrenaline in tissues and body fluids. *Acta physiol. scand.*, **56**, 258–266.

JOHNSON, G. E. & MICKLE, D. (1966). The influence of cold exposure on the *in vivo* release of metaraminol. *Br. J. Pharmac. Chemother.*, **28**, 246–254.

JOHNSON, G. E. & PUGSLEY, T. A. (1968). The formation and release of metaraminol during exposure to warm and cold environments. *Br. J. Pharmac.*, **34**, 267–276.

KOPIN, I. J. (1964). Storage and metabolism of catecholamines: the role of monoamine oxidase. *Pharmac. Rev.*, **16**, 179–191.

KOPIN, I. J. (1968a). False adrenergic transmitters. *Ann. Rev. Pharmac.*, **8**, 377–394.

KOPIN, I. J. (1968b). The influence of false adrenergic transmitters on adrenergic neurotransmission. *Adrenergic Neurotransmission*, ed. Wolstenholme, G. E. W. and O'Connor, M., pp. 95–104. London: J. & A. Churchill Ltd.

PUGSLEY, T. A. & JOHNSON, G. E. (1968). Modified method for estimation of metaraminol and alpha-methyl-m-tyrosine. *J. Pharm. Pharmac.*, **20**, 490–491.

RUTHVEN, C. R. & SANDLER, M. (1965). The estimation of 4-hydroxy-3-methoxy-phenylglycol and total metadrenalines in human urine. *Clin. chim. Acta*, **12**, 318–324.

SHORE, P. A., BUSFIELD, D. & ALPERS, H. S. (1964). Binding and release of metaraminol: mechanism of norepinephrine depletion by alpha-methyl-m-tyrosine and related agents. *J. Pharmac. exp. Ther.*, **146**, 194–199.

SHUM, A., JOHNSON, G. E. & FLATTERY, K. V. (1969). Influence of ambient temperature on excretion of catecholamines and metabolites. *Am. J. Physiol.*, **216**, 1164–1169.

UDENFRIEND, S. & ZALTZMAN-NIRENBERG, P. (1964). On the mechanism of norepinephrine depletion by aramine. *Life Sci., Oxford*, **3**, 695–702.

WURTMAN, R. J. (1966). *Catecholamines*, p. 45. Boston: Little, Brown.

(*Received January* 21, 1970)