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THE INFLUENCE OF COLD EXPOSURE ON THE *IN VIVO* RELEASE OF METARAMINOL

BY

G. E. JOHNSON AND D. MICKLE

From the Department of Pharmacology, University of Toronto, Toronto, Canada

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The depression of noradrenaline levels in tissue by metaraminol has been well documented (Udenfriend & Zaltzman-Nirenberg, 1962; Gessa, Costa, Kuntzman & Brodie, 1962; and others). Metaraminol is taken up and stored within sympathetic nerves replacing endogenous noradrenaline (Carlsson & Lindquist, 1962; Shore, Busfield & Alpers, 1964; Andén, 1964), and may be released by catecholamine releasers, such as reserpine or tyramine, or by sympathetic nerve stimulation *in vitro* (Shore *et al.*, 1964; Crout, Alpers, Tatum & Shore, 1964). Thus it has been speculated that metaraminol can replace noradrenaline within sympathetic nerves and serve, at least temporarily, as an adrenergic transmitter. However, evidence from experiments *in vivo* is lacking to demonstrate an increased release of tissue-bound metaraminol under conditions of sympathetic stimulation.

The exposure of rats to a cold environment results in an increased release of noradrenaline from sympathetic nerves (Leduc, 1961; Johnson, 1963; and others). Experiments were undertaken to ascertain if cold-induced sympathetic stimulation increased the release of metaraminol from heart tissue, as would be expected if it were stored on binding sites normally containing noradrenaline. It has been suggested that the raised secretion of noradrenaline is essential for the maintenance of normothermia at a low ambient temperature and for the development of acclimatization to cold (Carlson, 1960; Leduc, 1961). The ability of metaraminol to depress tissue noradrenaline levels provided a tool to investigate further the role of catecholamines in cold survival, and for this purpose both tissue and urinary catecholamine concentrations were measured in the cold-stressed rats. Parallel determinations of adrenaline and noradrenaline in the animals kept in a warm environment allowed a comparison of the effect of metaraminol on animals kept at rest or exposed to a stressful situation.

METHODS

Male Wistar rats, 225 to 300 g, obtained from Canadian Breeding Laboratories, were used throughout the study. The animals were kept in our animal house for at least seven days before use. Food (Fox Cubes) and water were allowed *ad libitum*.

Metaraminol (Aramine) was injected intraperitoneally in a dose of 1 mg/kg (6 u-mole/kg). Control animals received an equivalent volume of saline.

All rats were placed in a room controlled at $27° \pm 1°$ C (warm room) one day before use. Following the injection of metaraminol or saline the animals were divided into two groups. One group

remained at 27° for the duration of the experiment, while the second group was transferred to 4° (cold room) 2 hr after treatment.

Rats were sacrificed by decapitation either 2, 6, 9 or 12 hr after injection and the hearts quickly removed. Urine was obtained from rats placed in individual wire collection cages. The cages were coated with a polyester resin to prevent an interaction between the amines and the metals in the wire. Care was taken to maintain the urine acid (approximately *p*H 3) by the addition of N HCl.

Hearts were extracted twice with ice-cold perchloric acid by high-speed homogenization (Virtis Tissue Homogenizer). Subsequent titration of the extract to *p*H 4.5 was effected with potassium carbonate and the precipitate centrifuged off. Samples of the supernatant were removed for metaraminol determination, adjusted to *p*H 9 and shaken with n-butanol:n-heptane (1:1). Following a re-extraction into 0.01N HCl, metaraminol was condensed with o-phthalaldehyde and measured fluorimetrically (Shore & Alpers, 1964). In agreement with Shore & Alpers (1964), who reported a 60% recovery with this method, our recoveries of metaraminol from heart tissue averaged 57% ±, a standard error of 3%. All results concerning extracts of hearts are corrected for incomplete recovery.

The fluorimetric method employing o-phthalaldehyde was found to give comparable results to the colorimetric reaction employing Gibbs Reagent (Udenfriend & Zaltzman-Nirenberg, 1964). Hearts removed from guinea-pigs 1½ hr after treatment with metaraminol, 3 mg/kg I.P., contained 12.1 n-mole/g as determined in our laboratory using the o-phthalaldehyde method. Previously published work, using the colorimetric assay, determined 9.7 n-mole metaraminol/g of guinea-pig heart 1½ hr after an injection of 3 mg/kg metaraminol (Udenfriend & Zaltzman-Nirenberg, 1964).

Samples of urine, 20 ml., were titrated to *p*H 9 and extracted for metaraminol with the organic solvent mixture. In view of the large number of substances appearing in the urine some doubt could exist as to the ability of the method specifically to determine metaraminol. However, the fluorophore produced by the addition of o-phthalaldehyde to the extract obtained from the urine of rats injected with metaraminol possessed the same activation and fluorescent spectra as pure metaraminol. Also extracts of urine obtained from rats not treated with metaraminol failed to fluoresce when mixed with o-phthalaldehyde.

Adrenaline and noradrenaline were obtained by passing the tissue extracts or urine through alumina columns at a *p*H of 8.3 to 8.5 and eluting with 0.25N acetic acid. Catecholamines were estimated fluorimetrically according to the method of Euler & Lishajko (1961). A 95% recovery of noradrenaline from tissue extracts was obtained using this method. In one experiment the adrenals were removed 2 hr after metaraminol and their catecholamine content estimated.

Metabolic rate was calculated by measuring the oxygen consumption of the rats placed in sealed containers in a water bath of a constant temperature (Ferguson & Sellers, 1949).

RESULTS

Rats treated with metaraminol appeared in good physical condition throughout the experiment. The administration of metaraminol failed to produce signs of impaired sympathetic function, in that both treated and control rats placed at 4° were normothermic at the time of sacrifice.

Hearts taken from rats killed 2 hr after injection contained 3.15 n-mole metaraminol/g of tissue. As shown in Fig. 1 the concentration of metaraminol in the hearts of rats kept at 27° fell throughout the experiment. Exposure to 4° 2 hr after injection produced an abrupt fall in cardiac metaraminol levels as indicated by the low concentration (0.83 n-moles/g) found at hour six. This value is significantly less ($P<0.001$) than the concentration of 2.80 seen in the warm-room rats. Twelve hours after treatment no metaraminol was found in the hearts of the cold-stressed rats, while the animals kept at 27° still possessed 1.62 n-mole/g.

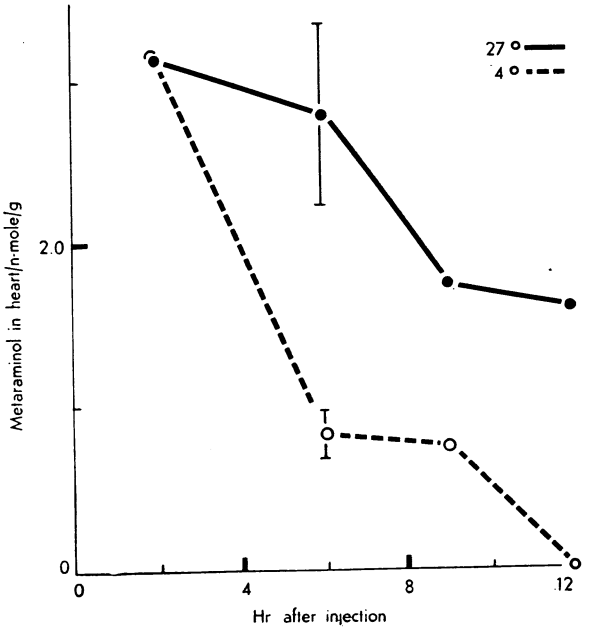

Fig. 1. Concentrations of metaraminol in the hearts of rats kept at 27° or transferred to 4° 2 hr after injection with metaraminol. Points represent means of 6 to 16 values. Standard errors are plotted at hour 6.

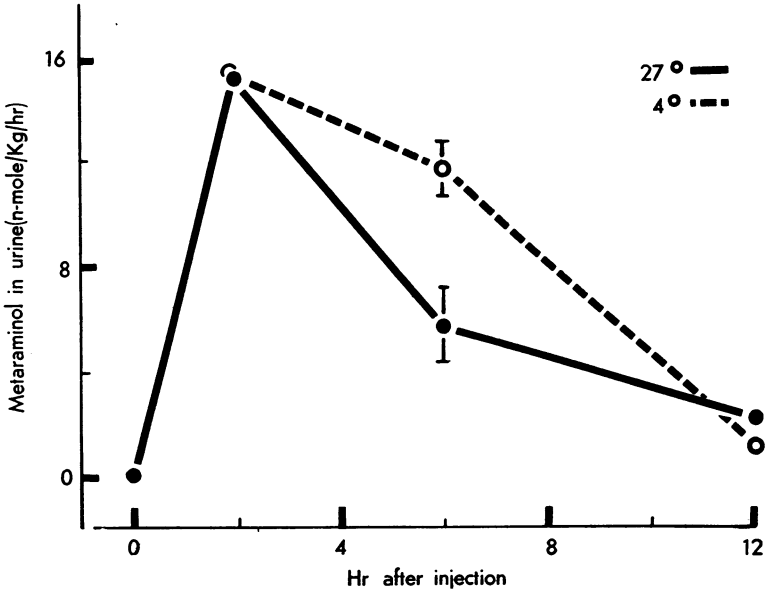

Fig. 2. Urinary excretion of metaraminol from rats kept at 27° or transferred to 4° 2 hr after injection with metaraminol. Points are means of eight rats per group. Standard errors are plotted at hour 6.

The results of the urine analyses for metaraminol are given in Fig. 2. The cold-stressed animals excreted significantly more drug between 2 and 6 hr than did the rats left in the warm room ($P<0.005$). The time of the increase in metaraminol excretion coincided with the cold-induced fall in heart levels.

Metaraminol depressed heart noradrenaline levels in both the warm and cold-exposed rats (Fig. 3). However, in contrast to the continued fall seen in the warm room, with

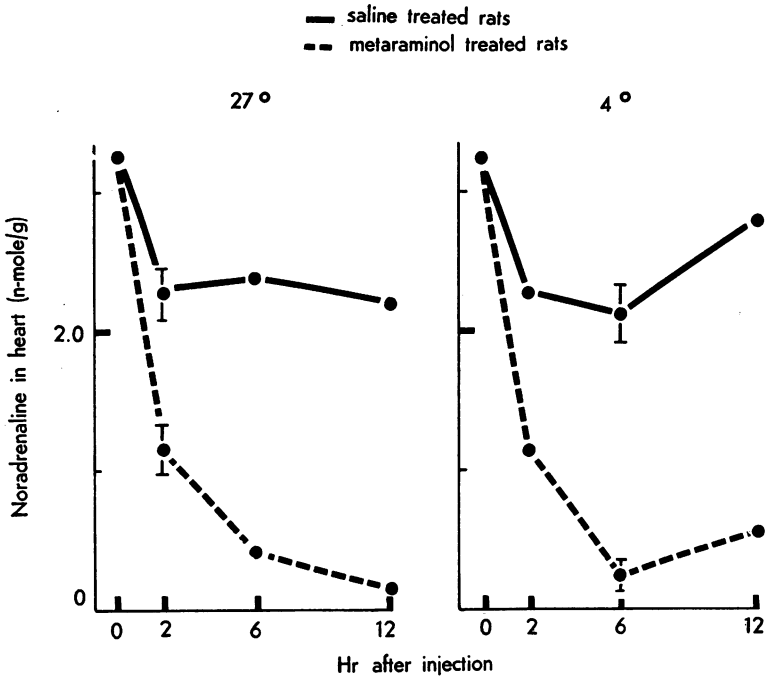

Fig. 3. Concentrations of noradrenaline in the hearts of rats injected with saline or metaraminol and left at 27° or transferred to 4° 2 hr after injection. Standard errors are plotted at hour 2 at 27° and at hour 6 at 4°. Points are means of 4 to 8 values.

the lowest values occurring 12 hr after treatment the cold-stressed rats showed an increase in noradrenaline levels between 6 and 12 hr following metaraminol. The cardiac noradrenaline content 12 hr after metaraminol was significantly higher in the cold-stressed animals than in their warm-room counterparts ($P<0.05$).

Two hours after metaraminol the adrenal glands contained normal amounts of catecholamines. The respective quantities of adrenaline and noradrenaline estimated were 108 and 17 μg/kg body weight. Although adrenals taken from untreated rats were not assayed for their catecholamine content previous work in our laboratory has shown these to contain between 70–120 μg adrenaline and 10–20 μg noradrenaline per kg body weight.

Metaraminol produced a transient increase in noradrenaline excretion within the first 2 hr after treatment. As indicated in Fig. 4 rats kept at 27° subsequently showed a fall in noradrenaline excretion. These results can be contrasted with the observations in the cold. Animals transferred to 4° 2 hr after metaraminol maintained a continued rise

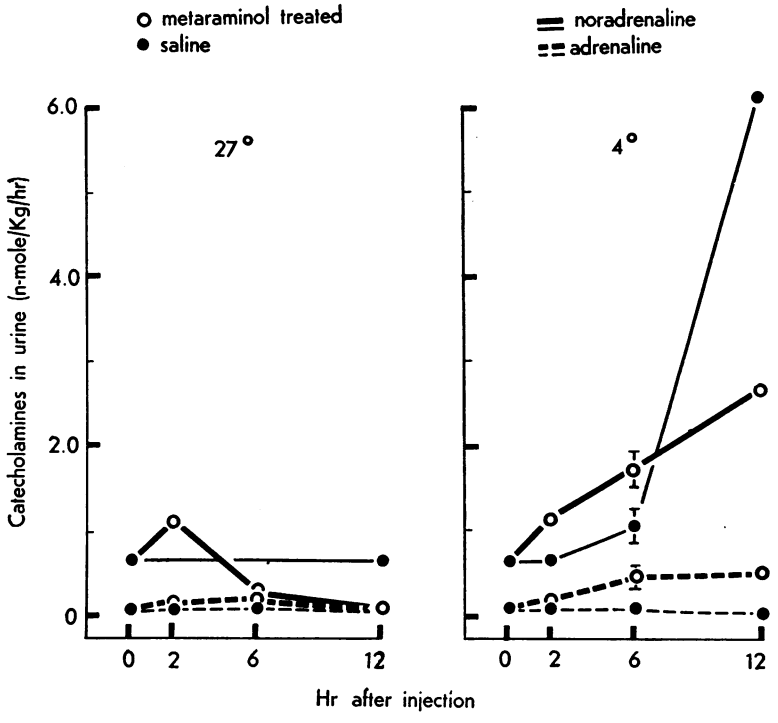

Fig. 4. Urinary excretion of noradrenaline and adrenaline from rats administered saline or metaraminol and left at 27° or transferred to 4° 2 hr after injection. Standard errors are plotted at hour 6 at 4°. Points are means of eight rats.

in noradrenaline excretion with an accompanying increase in adrenaline levels. This increased noradrenaline excretion following metaraminol, although significant, was far less than the normal cold-induced rise seen in the saline-treated controls.

In metabolic rate studies conducted at 27° metaraminol failed to increase the oxygen consumption of rats during a 3-hr observation period after injection. The pretreatment value (cc/M^2/min at N.T.P.) was 117 ± 4.4 whereas the maximum value noted after metaraminol was 121 ± 4.5.

DISCUSSION

The depression of tissue noradrenaline levels by metaraminol has been attributed to the uptake of metaraminol within sympathetic nerves replacing the endogenous catecholamine (Carlsson & Lindquist, 1962 ; Shore *et al.*, 1964 ; Andén, 1964). Furthermore, as a result of studies with catecholamine releasing drugs (Shore *et al.*, 1964) and sympathetic nerve stimulation *in vitro* (Crout *et al.*, 1964) it has been postulated that metaraminol, bound within sympathetic nerves, may be released to serve as a "false transmitter." Evidence was sought in this study, on the basis of experiments *in vivo*, to support or refute the possibility that a foreign amine, metaraminol, could replace endogenous noradrenaline within sympathetic nerves and function, at least temporarily,

as a "false transmitter." Because of our previous interests in the role of the sympathetic nervous system in survival at a low ambient temperature it was decided to employ cold exposure as the sympathetic stress in the current study.

Rats placed at 4° show an immediate increase in noradrenaline excretion (Fig. 4). Consistent with earlier work showing the increased noradrenaline release to be derived from sympathetic nerves (Leduc, 1961), Oliverio & Stjärne (1965) reported an increased turnover of ^{3}H-noradrenaline in the hearts of mice placed in a cold environment. Mice injected with ^{3}H-Dopa showed an increased disappearance of heart ^{3}H-noradrenaline following exposure to 5° (Drazkóczy, Pulley & Burack, 1966). We observed that rats kept at 27° possessed 2.80 n-mole metaraminol/g of heart tissue 6 hr after injection as compared with the value of 0.83 n-mole seen in the group transferred to 4°. This indicates that exposure of rats to the cold increases the disappearance of cardiac stores of metaraminol. At the same time cold increases the loss of metaraminol in the urine. These changes are consistent with the concepts of Carlsson and Lindquist (1962), Shore *et al.* (1964), Andén (1964), and Crout *et al.* (1964), which were outlined before. Therefore, one may postulate that the increased loss of metaraminol seen at 4° resulted from its increased secretion from sympathetic nerves. However, other factors not directly related to sympathetic function, such as a cold-induced increase in renal clearance of metaraminol or an elevated detoxification of the drug at 4°, could produce identical tissue changes. These possibilities remain to be investigated.

It has been reported that the administration of noradrenaline increases the loss of bound metaraminol (Shore *et al.*, 1964) and thus it could be suggested that the abrupt fall in heart metaraminol levels in the cold resulted from a competitive removal of metaraminol from noradrenaline binding sites due to the increased synthesis of noradrenaline. Such was not the mechanism in our experiments. The competitive removal of metaraminol from noradrenaline binding sites as a result of an increased synthesis of noradrenaline in the cold necessitates an increased retention of noradrenaline itself due to the availability of the freed binding sites. This did not occur and 6 hr after treatment the hearts of the cold-exposed rats contained less noradrenaline than the animals kept in the warm room.

It has been variously reported that metaraminol does (Andén, 1964 ; Shore *et al.*, 1964) or does not (Udenfriend and Zaltzman-Nirenberg, 1964) replace stoichiometrically the noradrenaline lost. As indicated in the section, Results, the concentrations of both metaraminol and noradrenaline fell steadily during the 12-hr period at 27°. Similar results were reported by Udenfriend & Zaltzman-Nirenberg (1964). The pattern of metaraminol and noradrenaline loss following treatment is given in Fig. 5. Two hours after treatment the metaraminol in the hearts exceeded the quantity of noradrenaline lost. The subsequent fall at 27° in the levels of both amines reduced their cumulative concentration to values slightly above (6 hr) or below (12 hr) control noradrenaline values. No statistical difference existed at hours 6 and 12 between the amounts of metaraminol plus noradrenaline found in the hearts of the drug-treated rats and the stores of noradrenaline in the animals given saline. However, if there is a fall in the levels of both metaraminol and noradrenaline an exact equivalence with noradrenaline control values must occur at some time after treatment. Therefore, whether or not one finds a mole for mole replacement under resting conditions depends upon the time of investigation.

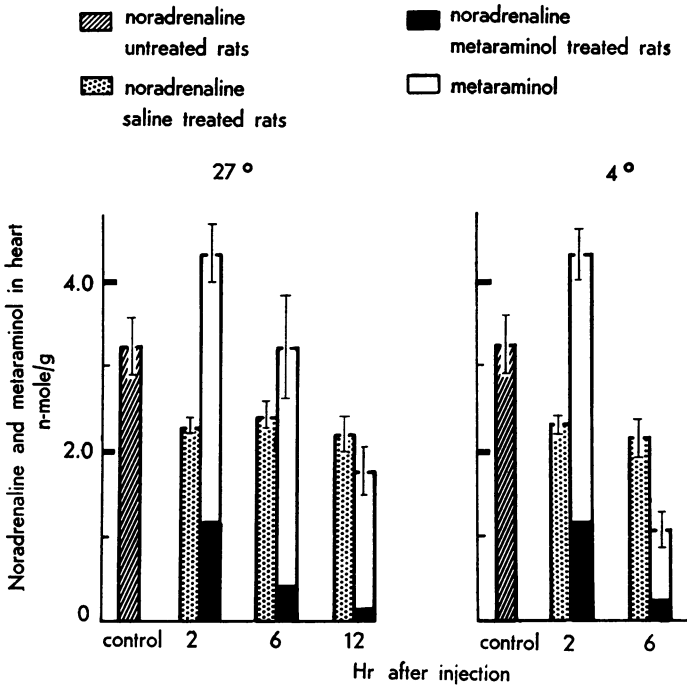

Fig. 5. Concentrations of noradrenaline and metaraminol in the hearts of rats injected with saline or metaraminol and left at 27° or transferred to 4° 2 hr after injection.

It is apparent from the results that a simple competition between metaraminol and noradrenaline for a limited number of binding sites does not exist in the cold environment. The total concentration of metaraminol plus noradrenaline after 6 hr was significantly below the noradrenaline levels of the saline-treated animals. Twelve hours after injection the hearts of the cold-stressed rats contained no metaraminol and only 20–25% of the control levels of noradrenaline. These results show that there is no necessity for a mole for mole replacement of noradrenaline with metaraminol. Therefore it must be stated that at least under conditions of sympathetic stress where there is an increased release of both catecholamines and metaraminol the prolonged depression of tissue noradrenaline by metaraminol is not dependent upon the presence of metaraminol on noradrenaline binding sites.

The observation that all cold-stressed rats remained normothermic suggests an adequate heat production in spite of a relative deficiency of noradrenaline during the last 6 hr of the experiment. The inability of metaraminol to increase oxygen consumption discounts any direct thermogenic action from the drug. It was, however, of interest to note the increase in adrenaline excretion seen in the cold-exposed metaraminol treated rats. It has been a consistent observation in our laboratory as well as those of other workers (Leduc, 1961 ; Johnson & Pritzker, 1966) that cold exposure results in an immediate increase in noradrenaline release with little change in adrenaline secretion. However, any situation that produces a relative deficiency of noradrenaline—in this case metara-

minol treatment—elicits a compensatory increase in adrenaline secretion. Thus adrenaline has been referred to as a " second line of defence " (Leduc, 1961) in exposure to cold. The release of adrenaline from the adrenals, which still contained normal amounts of the amine, may well have contributed to the maintenance of normothermia.

It will be noted that the tissue metaraminol values reported in this study are lower than earlier published results following treatment of Sprague-Dawley rats with 1 mg/kg metaraminol (Shore *et al.*, 1964). With reference to this it was interesting to observe that the Wistar rats used in our experiments contained considerably less endogenous noradrenaline than the animals employed by the previous investigators. From these results it would appear that the quantity of metaraminol taken up by the heart is related to normal endogenous levels of noradrenaline.

SUMMARY

1. Rats, placed at 27°, were injected intraperitoneally with metaraminol in a dose of 1 mg/kg. Two hours after treatment one group of rats was transferred to 4°, while the remaining animals stayed at 27°. Rats were sacrificed 2, 6, 9, or 12 hr after injection. Exposure to cold increased (a) the loss of metaraminol from heart tissue and (b) the concentration of metaraminol in urine. This data supports the concept that metaraminol, bound within sympathetic nerves, may be released as a " false transmitter."

2. Following the administration of metaraminol the cardiac levels of noradrenaline in both groups of rats fell. At 27° the total concentration of metaraminol plus noradrenaline in heart tissue approximated to the quantity of noradrenaline found in saline-treated animals. In the cold environment, however, the metaraminol plus noradrenaline levels in the treated rats fell significantly below the noradrenaline determined in the hearts of animals injected only with saline. Twelve hours after injection with metaraminol, at a time when no drug could be found in the hearts of the rats placed at 4°, the cardiac levels of noradrenaline were only 25% of control values. It is thus concluded that there need be no mole for mole replacement of noradrenaline with metaraminol. Under conditions of sympathetic stress, where there is an increased release of both catecholamines and metaraminol, the prolonged depression of tissue noradrenaline by metaraminol is not dependent upon the presence of metaraminol on noradrenaline binding sites.

3. Metaraminol depressed the cold-induced increase in noradrenaline excretion. This was accompanied by a compensatory increase in adrenaline release which presumably aided in the maintenance of normothermia.

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REFERENCES

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

CARLSON, L. D. (1960). Nonshivering thermogenesis and its endocrine control. *Fedn Proc.*, **19**, No. 4, Part II, 25–30.

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

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.

DRAZKÓCZY, P. R., PULLEY, K. & BURACK, W. R. (1966). The effects of cold environment on the endogenously labeled catecholamine stores. *Pharmacologist*, **8**, 29.

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

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

GESSA, G. L., COSTA, E., KUNTZMAN, R. & BRODIE, B. B. (1962). On the mechanism of norepinephrine release by alpha-methyl-metatyrosine. *Life Sci. Oxford*, **1**, 353–360.

JOHNSON, G. E. (1963). The effect of cold exposure on the catecholamine excretion of adrenalectomized rats treated with reserpine. *Acta physiol. scand.*, **59**, 438–444.

JOHNSON, G. E. & PRITZKER, K. (1966). The influence of the dopa decarboxylase inhibitor Ro4-4602 on the urinary excretion of catecholamines in cold-stressed rats. *J. Pharmac. exp. Ther.*, **152**, 432–438.

LEDUC, J. (1961). Catecholamine production and release in exposure and acclimation to cold. *Acta physiol. scand.*, **53**, Suppl. 183.

OLIVERIO, A. & STJÄRNE, L. (1965). Acceleration of noradrenaline turnover in the mouse heart by cold exposure. *Life Sci. Oxford*, **4**, 2339–2343.

SHORE, P. A. & ALPERS, H. S. (1954). Fluorometric estimation of metaraminol and related compounds. *Life Sci. Oxford*, **3**, 551–554.

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.

UDENFRIEND, S. & ZALTZMAN-NIRENBERG, P. (1962). On the mechanism of the norepinephrine release produced by alpha-methyl-meta-tyrosine. *J. Pharmac. exp. Ther.*, **138**, 194–199.

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