Brit. J. Pharmacol. (1964), **22**, 238–245.

MODIFICATION OF THE EFFECTS OF GUANETHIDINE ON CARDIAC CATECHOL AMINES BY VARIOUS AGENTS

BY

B. BHAGAT*

From the Department of Pharmacology, University of Minnesota, Minneapolis 14, Minnesota, U.S.A.

(*Received June* 29, 1963)

A study has been made of the effect of injections of guanethidine in rats, in depleting catechol amines from the whole cardiac ventricles and from various subcellular fractions. Unlike reserpine, guanethidine first affected the concentration of the amines in the soluble fraction of the cell. Neither [2-(2,6-dimethylphenoxy)-propyl]trimethylammonium chloride monohydrate (β-methyl xylocholine) nor hemicholinium affected the endogenous catechol amines or the uptake of injected noradrenaline, but each significantly reduced the action of guanethidine in depleting catechol amines. Administration of choline chloride after hemicholinium reversed its influence on guanethidine depletion. In cats, cocaine potentiated the pressor response to noradrenaline, but antagonized the response to tyramine and guanethidine, while bretylium and *N*-*o*-chlorobenzyl-*N'N''*-dimethylguanidine sulphate (BW392C60) potentiated the responses to noradrenaline, tyramine and guanethidine.

Guanethidine is a potent antihypertensive agent with a selective inhibitory effect on the sympathetic nervous system (Maxwell, Plummer, Povalski & Schneider, 1960). It, like reserpine, depletes peripheral stores of catechol amines (Sheppard & Zimmerman, 1959; Cass & Spriggs, 1961; Bhagat & Shideman, 1963a). This paper reports observations on the action of guanethidine in depleting catechol amines from subcellular fractions of the ventricles of the rat heart.

The effects of β-methyl xylocholine and hemicholinium on the catechol amines of rat ventricles have been tested in three ways: firstly, to determine whether they depleted the catechol amines, secondly to determine whether they affected the depletion caused by guanethidine, and thirdly, to determine their influence on the increase of the catechol amines in the ventricles after injection of noradrenaline.

Finally, observations have been made on the effects of cocaine and of adrenergic neurone blocking agents in cats on the pressor responses to guanethidine, tyramine and noradrenaline.

METHODS

Determination of catechol amines in the myocardium

Male albino rats of the Holtzman strain, weighing between 200 and 250 g, were used. Animals were killed by a blow at the base of the neck and their hearts removed. The

* Present address: Department of Pharmacology, Howard University College of Medicine, Washington, D.C., U.S.A.

ventricles, dissected free of atrial tissue, were homogenized immediately in two volumes of cold 0.01 N-hydrochloric acid. In experiments in which the subcellular distribution of catechol amines was to be determined, the ventricles were homogenized in ice-cold potassium (0.075 M) phosphate buffer at *p*H 7.5. The homogenate was separated into coarse, particulate and soluble fractions by differential centrifugation and prepared for analysis by the method of Campos & Shideman (1962). The catechol amine contents of the acidified homogenates and fractions were determined by the trihydroxyindole fluorimetric procedure of Shore & Olin (1958) and are expressed as μg of noradrenaline per g of fresh tissue.

Cats, of 2 to 3.5 kg body weight and of either sex, were anaesthetized by the intraperitoneal injection of 30 mg/kg of sodium pentobarbitone; additional doses were given intravenously if required. The trachea was cannulated, the adrenal glands were excluded from the circulation, and the arterial pressure was recorded from a carotid artery with a mercury manometer. All drugs were injected into a cannula tied into a femoral vein unless otherwise stated, and flushed in with 0.5 ml. of 0.9% saline. Heparin (500 U/kg) was always injected. All animals were injected with atropine sulphate (1 mg/kg, intravenously) 30 min before the injection of test drugs.

The following drugs were used: (−)-noradrenaline bitartrate, expressed as base, and atropine sulphate, bretylium tosylate (Darenthin), choline chloride, cocaine hydrochloride, guanethidine sulphate, tyramine hydrochloride, [2-(2,6-dimethylphenoxy)propyl]trimethylammonium chloride monohydrate (β-methyl xylocholine), *N*-*o*-chlorobenzyl-*N'N''*-dimethylguanidine sulphate (BW392C60), α,α'-dimethylaminoethanol-4,4'-biacetophenone (hemicholinium; HC-3), all expressed as the salts. Details of dosage and times of administration are given in Results. Student's *t*-test (Fisher, 1946) was employed for all tests of significance.

RESULTS

Effect of guanethidine on the intracellular distribution of catechol amines in rat ventricles

Table 1 shows the effect of 20 mg/kg of guanethidine, given by intramuscular injection, on the concentration of catechol amines in subcellular fractions of the ventricles. Within 45 min after administration of guanethidine there was a small but significant reduction in the concentration of catechol amines in the soluble fraction without a statistically significant decrease in the total concentration of the amines. Later, at 120 min, the concentration in the particulate fraction also declined.

TABLE 1

EFFECT OF GUANETHIDINE ON THE INTRACELLULAR DISTRIBUTION OF NORADRENALINE IN THE RAT HEART

Rats were killed at different times after the intramuscular injection of 20 mg/kg of guanethidine, and the concentration of cardiac catechol amines in various fractions, separated by centrifugation, were determined. Catechol amine concentrations (means and standard errors) are expressed in terms of noradrenaline. An asterisk indicates that the mean is significantly different ($P<0.05$) from that of the controls

Time after guanethidine (min)	No. of rats	Catechol amine concentration (μg/g of fresh tissue)			
		Coarse fraction	Particulate fraction	Soluble fraction	Whole homogenate
Control	6	0·23±0·026	0·29±0·02	0·29±0·015	0·76±0·05
45	7	0·27±0·024	0·31±0·024	0·20±0·02*	0·70±0·02
60	7	0·24±0·024	0·30±0·02	0·18±0·014*	0·71±0·04
75	9	0·19±0·02	0·26±0·013	0·15±0·014*	0·59±0·045*
90	4	0·16±0·02	0·23±0·03	0·16±0·017*	0·57±0·034*
120	7	0·14±0·014*	0·19±0·01*	0·12±0·012*	0·54±0·025*

Effect of β-methyl xylocholine on catechol amine content of rat ventricles

β-Methyl xylocholine is a congener of xylocholine and has been shown to possess a similar adrenergic neurone blocking action (McLean, Geus, Pasternack, Mattis & Ullyot, 1960). In one series of experiments rats were given 15 mg/kg of β-methyl xylocholine intramuscularly. The animals were killed 2.75 hr later and their hearts analysed for catechol amines. In some rats noradrenaline (1.5 mg/kg) was administered intraperitoneally, 1.75 hr after β-methyl xylocholine. The results, summarized in Table 2, show that β-methyl xylocholine effected neither the catechol amine levels in the heart nor the uptake of injected noradrenaline by the heart.

TABLE 2

CARDIAC CATECHOL AMINE CONCENTRATIONS AFTER THE INJECTION OF VARIOUS DRUGS

When noradrenaline or guanethidine was given in conjunction with another test drug, the former was injected 1 hr and guanethidine 2 hr before the rats were killed. Choline chloride was injected 15 min after hemicholinium. Other animals were killed 2.75 hr after drug administration. Catechol amine concentrations (means and standard errors) are expressed in terms of noradrenaline. An asterisk indicates the mean is significantly different ($P<0.01$) from that of the controls. I.m.= intramuscular; i.p.=intraperitoneal

Treatment	Dose (mg/kg)	Route	No. of rats	Catechol amine concentration (μg/g of fresh tissue)
None		—	34	1·0 ±0·03
β-Methyl xylocholine	15	I.m.	6	1·08±0·04
Choline chloride	50	I.m.	6	1·03±0·04
Guanethidine	20	I.m.	16	0·63±0·03*
Hemicholinium	0·1	I.m.	8	1·06±0·04
Noradrenaline	1·5	I.p.	18	1·81±0·05*
β-Methyl xylocholine and noradrenaline	15 and 1·5	I.m. I.p.	6	1·76±0·05*
Hemicholinium and noradrenaline	0·1 and 1·5	I.m. I.p.	12	1·70±0·08*
β-Methyl xylocholine and guanethidine	15 and 20	I.m. I.m.	12	0·85±0·03*
Hemicholinium and guanethidine	0·1 and 20	I.m. I.m.	12	0·78±0·02*
Hemicholinium, choline chloride and guanethidine	0·1, 50 and 20	I.m. I.m. I.m.	6	0·64±0·04*

In order to determine whether β-methyl xylocholine could reduce the ability of guanethidine to deplete noradrenaline from rat heart the following experiment was performed. Rats were given 15 mg/kg of β-methyl xylocholine intramuscularly 45 min before guanethidine (20 mg/kg, intramuscularly). The animals were killed 2 hr after the injection of guanethidine. β-Methyl xylocholine significantly reduced the depleting actions of guanethidine (Table 2).

Effect of hemicholinium on the catechol amine content of rat ventricles

Rats were given 100 μg/kg of hemicholinium intramuscularly. They were killed 2.75 hr later and the concentrations of catechol amines in their hearts determined.

In some rats noradrenaline (1.5 mg/kg) was administered intraperitoneally 1.75 hr after hemicholinium. The results (Table 2) show that hemicholinium had no effect on the catechol amine content of the ventricles or on the uptake of injected noradrenaline by the ventricles.

In a second series of experiments rats were given 100 μg/kg of hemicholinium 45 min before guanethidine (20 mg/kg, intramuscularly). The animals were killed 2 hr after the injection of guanethidine. Hemicholinium significantly reduced the depleting action of guanethidine (Table 2).

Choline chloride antagonizes some of the actions of hemicholinium (MacIntosh, Birks & Sastry, 1956). It was, therefore, of interest to study its effect on the action of hemicholinium in preventing the guanethidine-induced depletion. Experiments were performed similar to those described above, except that choline chloride (50 mg/kg) was administered intramuscularly 15 min after the injection of hemicholinium. Choline chloride reversed the effect of hemicholinium in preventing the guanethidine-induced depletion (Table 2).

Effect of cocaine and of adrenergic-blocking agents on the pressor response to guanethidine in cats

In preliminary experiments it was found that the pressor response to guanethidine in the cat depended on the dose (two experiments) and was not influenced by adrenalectomy (one experiment). No tachyphylaxis was observed when 1 mg/kg of guanethidine was injected at intervals of 20 to 40 min.

Cocaine (10 mg/kg, subcutaneously) potentiated the pressor response (Fig. 1) to noradrenaline but antagonized the pressor responses to tyramine and guanethidine (six experiments). Bretylium (5 mg/kg, intravenously) potentiated the pressor responses (Fig. 2) to noradrenaline, tyramine and guanethidine both in height and duration (five experiments). In one experiment bretylium increased only the dura-

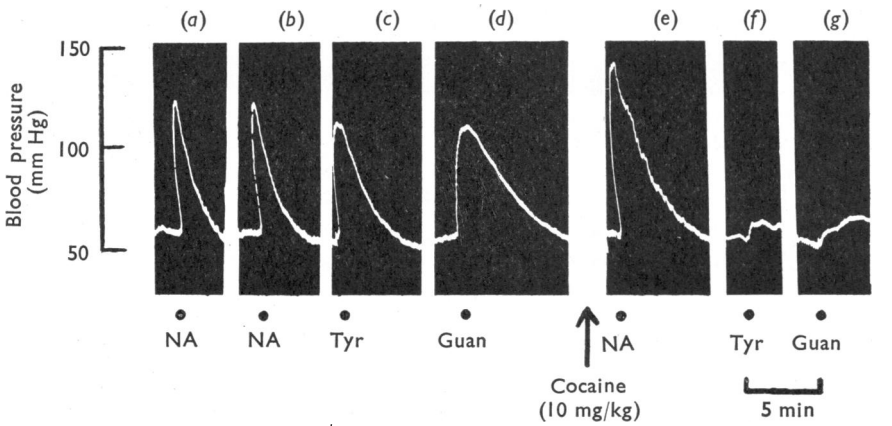

Fig. 1. Cat, 3 kg. Effect of cocaine on pressor responses to noradrenaline (NA, 1 μg/kg), tyramine (Tyr, 100 μg/kg) and guanethidine (Guan, 1 mg/kg). (*a*) to (*d*) before and (*e*) to (*g*) after cocaine (10 mg/kg), given subcutaneously 20 min before (*e*). Time intervals between panels, 15 to 25 min.

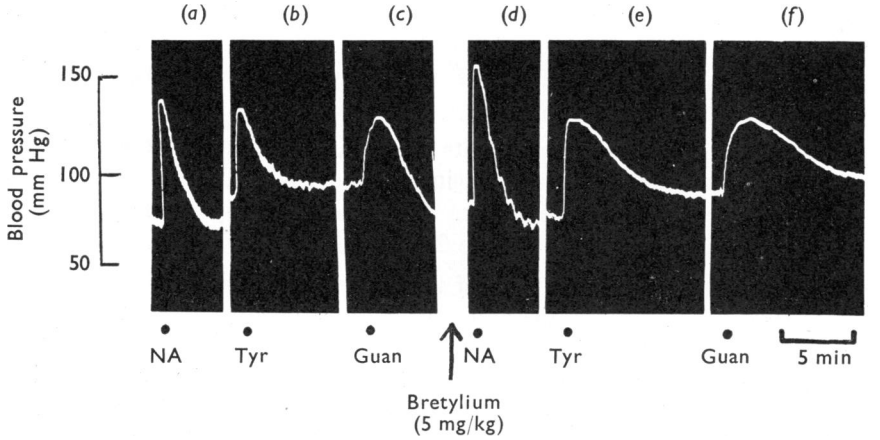

Fig. 2. Cat, 2.7 kg. Effect of bretylium on pressor responses to noradrenaline (NA, 1 μg/kg), tyramine (Tyr, 100 μg/kg) and guanethidine (Guan, 1 mg/kg). (*a*) to (*c*) before and (*d*) to (*f*) after bretylium (5 mg/kg), given intravenously 20 min before (*d*). Time intervals between panels, 15 to 25 min.

tion of the pressor responses to tyramine and guanethidine but the heights of the responses were reduced. BW392C60, a potent adrenergic-blocking agent which antagonizes the catechol amine-depleting effect of guanethidine (Kuntzman, Gessa & Brodie, 1962a, b), potentiated the pressor responses (Fig. 3) to noradrenaline, tyramine and guanethidine (four experiments).

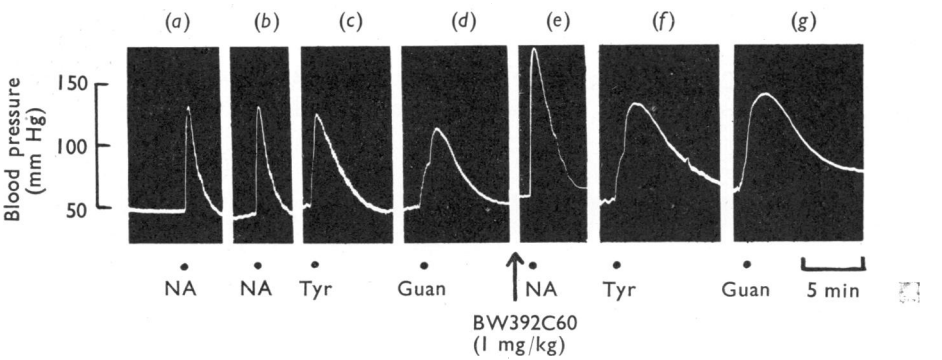

Fig. 3. Cat, 3.1 kg. Effect of BW392C60 on pressor responses to noradrenaline (NA, 1 μg/kg), tyramine (Tyr, 100 μg/kg) and guanethidine (Guan, 1 mg/kg). (*a*) to (*d*) before and (*e*) to (*g*) after BW392C60 (1 mg/kg), given intravenously 20 min before (*e*). Time intervals between panels, 15 to 25 min.

DISCUSSION

The results described show that the first effect of guanethidine was to deplete noradrenaline from the soluble fraction of heart homogenates. This contrasts to the effect of reserpine, which first releases noradrenaline from the bound fraction (Campos & Shideman, 1962).

Responses to stimulation of sympathetic nerves can be mimicked by acetylcholine (in the presence of atropine) or by nicotine (Kottegoda, 1953 ; Burn & Rand, 1958a, b). These effects of acetylcholine and nicotine are no longer seen in animals treated with reserpine or in organs in which the sympathetic fibres have degenerated after sympathectomy. Burn & Rand (1959a, b) therefore advanced the hypothesis that the release of noradrenaline by adrenergic fibres involves the action of acetylcholine.

The present studies indicate that hemicholinium, which acts as a specific and potent inhibitor of acetylcholine synthesis (MacIntosh *et al.*, 1956), was effective, like bretylium and xylocholine and its congener β-methyl xylocholine, in reducing the depletion of catechol amine in rat heart by guanethidine. This action of hemicholinium was reversed by administration of choline chloride ; choline also reverses the inhibition of acetylcholine synthesis by hemicholinium (MacIntosh *et al.*, 1956).

The present studies, also, confirm the rapid onset of supersensitivity to noradrenaline which develops after the acute administration of cocaine (Fröhlich & Loewi, 1910 ; Fleckenstein & Bass, 1953 ; Fleckenstein & Stöckle, 1955) or bretylium (Boura & Green, 1959). But the mechanisms of the two sensitizations are not exactly the same. The action of bretylium is roughly that of chemical sympathectomy while the action of cocaine is to block the nonspecific binding sites for noradrenaline around the nerve endings ; this results in an effective increase in the concentration of the amines available for receptor combination (Whitby, Hertting & Axelrod, 1960 ; Hertting, Axelrod, Kopin & Whitby, 1961 ; Bhagat, 1964).

The results suggest that pressor responses to guanethidine and tyramine are similiar in mechanism, namely that they act on the same store of noradrenaline at or near postganglionic sympathetic nerve endings. This explains the inhibitory action of guanethidine to pressor responses to tyramine (Maxwell *et al.*, 1960 ; Bhagat & Shideman 1963b). Both guanethidine and tyramine compete for the same store, but when guanethidine is given in large doses it perhaps attaches itself permanently to the store, thus preventing release by tyramine.

Bhagat & Shideman (1963a, b) showed that cocaine did not influence the depletion of myocardial catechol amines by guanethidine, while previous treatment with bretylium or xylocholine prevented it. This seems to conflict with the present findings of the inhibitory effects of cocaine and the potentiation of the vasopressor effect of guanethidine by bretylium, shown to be mediated through the release of catechol amines (Bhagat & Shideman, 1963a). These apparent discrepancies may possibly be explained on the basis of separate stores of catechol amines. One of the stores may be located within the nerve endings and its noradrenaline may be released directly by nerve stimulation. The other store may perhaps be located near the nerve endings and may consist of " easily mobilized " noradrenaline. It may be this store that is influenced by indirectly-acting sympathomimetic amines, such as tyramine ; and the released noradrenaline is then exposed to catechol-*O*-methyl transferase (Kopin & Gordon, 1962). On the basis of this hypothesis, cocaine would be acting on the store near the nerve endings, thus preventing the release due to tyramine and guanethidine but apparently not the release due to nerve stimulation

(Trendelenburg, 1959). Responses to nerve stimulation might thus persist after the development of tachyphylaxis to sympathomimetic amines (Cown, Cannon, Koppanyi & Maengwyn-Davies, 1961). If bretylium and related compounds acted on the neuronal store, they would block the release of noradrenaline by nerve impulses (Boura & Green, 1959) and its depletion by guanethidine (Bhagat & Shideman, 1963a) but not the release by tyramine and guanethidine. Thus, guanethidine would act on both stores, but preferentially on the neuronal store. Although this hypothesis of separate stores leads to a coherent picture of reported facts, it cannot, of course, be regarded as established, and it continues to be necessary to consider an alternative explanation.

I am indebted to Dr R. A. McLean of Smith, Kline & French Laboratories for generous supplies of β-methyl xylocholine; to Dr J. J. Burns of Burroughs Wellcome for bretylium and BW392C60; to Dr A. J. Plummer of Ciba Pharmaceutical Products for guanethidine; to Dr J. P. Long of the State University of Iowa for hemicholinium; and to Mr Richard Landry for technical assistance.

REFERENCES

BHAGAT, B. (1964). Tyramine-induced depletion of cardiac catecholamines and the effects of cocaine and bretylium. *Arch. int. Pharmacodyn.*, **147**, 26–35.

BHAGAT, B. & SHIDEMAN, F. E. (1963a). Mechanism of the positive inotropic responses to bretylium and guanethidine. *Brit. J. Pharmacol.*, **20**, 56–62.

BHAGAT, B. & SHIDEMAN, F. E. (1963b). Mechanism of the inhibitory action of guanethidine on cardiovascular responses to tyramine and amphetamine. *J. Pharmacol. exp. Ther.*, **140**, 317–326.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BURN, J. H. & RAND, M. J. (1958a). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

BURN, J. H. & RAND, M. J. (1958b). Noradrenaline in artery walls and its dispersal by reserpine *Brit. med. J.*, **i**, 903–908.

BURN, J. H. & RAND, M. J. (1959a). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol. (Lond.)*, **147**, 135–143.

BURN, J. H. & RAND, M. J. (1959b). Sympathetic adrenergic mechanism. *Nature (Lond.)*, **184**, 162–166.

CAMPOS, H. A. & SHIDEMAN, F. E. (1962). Subcellular distribution of catecholamines in the dog heart. Effect of reserpine and norepinephrine administration. *Int. J. Neuropharmacol.*, **1**, 13–22.

CASS, R. & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Brit. J. Pharmacol.*, **17**, 442–450.

COWN, F. F., CANNON, C., KOPPANYI, T. & MAENGWYN-DAVIES, G. D. (1961). Reversal of phenylalkylamine tachyphylaxis by norepinephrine. *Science*, **134**, 1069–1070.

FISHER, R. A. (1946). *Statistical Methods for Research Workers.* Edinburgh: Oliver & Boyd.

FLECKENSTEIN, A. & BASS, H. (1953). Die Sensibilisierung der katzen-Nickhaut fur Sympathomimetica der Brenzkatech-in-reihe. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **220**, 143–156.

FLECKENSTEIN, A. & STÖCKLE, D. (1955). Die Hemmung der Neuro-sympathomimetica durch Cocain. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **224**, 401–415.

FRÖHLICH, A. & LOEWI, O. (1910). Uber eine Steigerung der Adrenalinempfindlichkeit durch Cocain. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **62**, 159–169.

HERTTING, G., AXELROD, J., KOPIN, I. J. & WHITBY, L. G. (1961). Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature (Lond.)*, **189**, 66.

KOPIN, I. J. & GORDON, E. K. (1962). Metabolism of norepinephrine-H^3 released by tyramine and reserpine. *J. Pharmacol. exp. Ther.*, **138**, 351–359.

KOTTEGODA, S. R. (1953). The action of nicotine and acetylcholine on the vessels of the rabbit's ear. *Brit. J. Pharmacol.*, **8**, 156–161.

KUNTZMAN, C. E., GESSA, G. L. & BRODIE, B. B. (1962a). Reserpine and guanethidine action on peripheral stores of catecholamines. *Life Sciences*, **3**, 75–80.

KUNTZMAN, C. E., GESSA, G. L. & BRODIE, B. B. (1962b). Structural requirements for bretylium and guanethidine-like activity in a series of guanidine derivatives. *Life Sciences*, **3**, 75–80.

MACINTOSH, F. C., BIRKS, R. I. & SASTRY, P. B. (1956). Pharmacological inhibition of acetylcholine synthesis. *Nature (Lond.)*, **178**, 1181.

MCLEAN, R. A., GEUS, R. J., PASTERNACK, J., MATTIS, P. A. & ULLYOT, G. E. (1960). Pharmacology of trimethyl[2-(2,6-dimethylphenoxy) propyl] trimethylammonium chloride monohydrate: compound 6890 or β-TM10. *J. Pharmacol. exp. Ther.*, **129**, 17–23.

MAXWELL, R. A., PLUMMER, A. J., POVALSKI, H. & SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (SU-5864) in smooth-muscle. *J. Pharmacol. exp. Ther.*, **129**, 24–30.

SHEPPARD, H. & ZIMMERMAN, J. (1959). Effect of guanethidine (SU-5864) on tissue catecholamines *Pharmacologist*, **7**, 69.

SHORE, P. A. & OLIN, J. S. (1958). Identification and chemical assay of norepinephrine in brain and other tissues. *J. Pharmacol. exp. Ther.*, **122**, 295–300.

TRENDELENBURG, U. (1959). The supersensitivity caused by cocaine. *J. Pharmacol. exp. Ther.* **125**, 55–65.

WHITBY, L. G., HERTTING, G. & AXELROD, J. (1960). Effect of cocaine on the disposition of noradrenaline labelled with tritium. *Nature (Lond.)*, **187**, 604–605.