Brit. J. Pharmacol. (1962), **19**, 13–41.

COMPARISON OF BRETYLIUM AND GUANETHIDINE: TOLERANCE, AND EFFECTS ON ADRENERGIC NERVE FUNCTION AND RESPONSES TO SYMPATHOMIMETIC AMINES

BY

A. L. A. BOURA AND A. F. GREEN

From the Wellcome Research Laboratories, Beckenham, Kent

(*Received September* 6, 1961)

Bretylium depresses the slope of regression lines relating frequency of sympathetic nerve stimulation to magnitude of contractions of the cat nictitating membrane. In contrast, guanethidine and reserpine preferentially abolish responses to low rates of nerve stimulation and cause a roughly parallel shift of the regression lines. The hypersensitivity of the nictitating membranes of cats to intravenous adrenaline or noradrenaline is far greater after a series of small daily doses of bretylium or guanethidine than after single large doses. The maximal sensitivity produced was similar to that after postganglionic sympathetic nerve section and exceeded that produced by ganglion blockade. The development of hypersensitivity to catechol amines is accompanied by some return of responses of the nictitating membranes to sympathetic nerve stimulation despite continued daily administration of bretylium or guanethidine. In cats given bretylium daily, responses to low rates of nerve stimulation become greater than in controls unless the dose of bretylium given subcutaneously is 50 mg/kg or more. When marked hypersensitivity to catechol amines has been produced by giving bretylium or guanethidine daily for 7 or 14 days, the sympathomimetic effects of these compounds are greater. Responses to intravenous dimethylphenylpiperazinium are also increased and the results suggest that even large daily doses of adrenergic neurone blocking agents do not appreciably impair the functioning of the adrenal medulla. The pressor effects of intravenous adrenaline, noradrenaline and dimethylphenylpiperazinium iodide increase less than the corresponding nictitating membrane responses. These results are discussed in relation to tolerance to adrenergic neurone blockade, and differences between the effects of bretylium and guanethidine found in man. Bretylium and guanethidine depress the slopes of the dose-response curves for the pressor and nictitating membrane contracting effects of tyramine. When single doses or a short series of daily doses were given, guanethidine caused more depression of the slopes than did bretylium, but nevertheless large depressions of slope were found after giving bretylium daily for several weeks. The magnitude of the responses can be greater or less than in controls depending on the dose of the sympathomimetic amine, the dose of the adrenergic neurone blocking agent and the duration of its administration. The results suggest that injection of tyramine produces a progressively smaller release of adrenaline or noradrenaline during the daily administration of bretylium (or guanethidine) but that in some test situations this is more than compensated for by the development of hypersensitivity to the catechol amine released. Some corresponding changes in responses to amphetamine and ephedrine are also described.

Bretylium depresses the slope of curves relating frequency of sympathetic nerve stimulation and the magnitude of nictitating membrane contractions produced in cats (Boura & Green, 1959). In this paper we describe experiments showing that guanethidine has a relatively different effect on the responses to various rates of stimulation from that of bretylium, and compare the changes that occur during the daily administration of these compounds. These investigations together with estimates of changes in sensitivity of the nictitating membranes and blood pressure to adrenergic transmitters provide information on likely causes of tolerance to adrenergic neurone blocking agents.

Increased responses to adrenaline and noradrenaline have been found after administration of the adrenergic neurone blocking agents, bretylium (Boura & Green, 1959 ; Vernikos-Danellis & Zaimis, 1960) and guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960a ; Maxwell, Plummer, Povalski & Schneider, 1960b ; Vernikos-Danellis & Zaimis, 1960), and it has also been reported that the sensitivity to these catechol amines increases greatly during the daily administration of large doses of bretylium (Green, 1960). Comparable results for guanethidine or for lower doses of bretylium have not, however, been published hitherto. The effects observed have been compared, under similar conditions, with those produced by reserpine, ganglion blockade or nerve section.

We also describe some effects of daily subcutaneous administration of bretylium or guanethidine on the dose-response curves for tyramine, amphetamine and ephedrine, sympathomimetic amines that Burn & Rand (1958) suggest may act by releasing adrenaline or noradrenaline. Earlier findings (Green, 1960) showed that nictitating membrane contractions caused by some intravenous doses of tyramine were at first increased during the daily administration of large doses of bretylium, but later declined ; after 6 months' treatment the dose-response curves for tyramine effects on both blood pressure and the nictitating membranes were greatly depressed and responses to all doses were small. Vernikos-Danellis & Zaimis (1960) concluded that amphetamine and ephedrine continued to exert their full pressor effects in cats long after blockade of adrenergic neurones by bretylium or guanethidine, but Maxwell *et al.* (1960a and b) reported that the pressor responses to tyramine, amphetamine, ephedrine and certain other sympathomimetic amines in dogs were decreased after single doses of guanethidine.

METHODS

Sensitivities to intravenous injection of sympathomimetic amines were determined in cats under pentobarbitone sodium anaesthesia (*a*) from the rise of blood pressure in the left carotid artery, and (*b*) from the contractions of the nictitating membranes recorded with standard isotonic frontal writing levers. The amines were injected in 1 ml. saline over 30 sec through a fine needle inserted in the femoral vein. The usual procedure has been to inject in each animal a series of doses of each of the following in the order: adrenaline, noradrenaline, dimethylphenylpiperazinium iodide, tyramine and ephedrine (or amphetamine). In some experiments, however, only methylamphetamine, or tyramine and amphetamine, were injected. In others (Fig. 1) the sympathomimetic effects of increasing intravenous doses of bretylium or guanethidine were determined. The interval between doses, usually 5 to 20 min, was always sufficient to allow overt recovery from the effects of the previous dose, except when ephedrine, amphetamine or methylamphetamine were used or when the sympathomimetic effects of

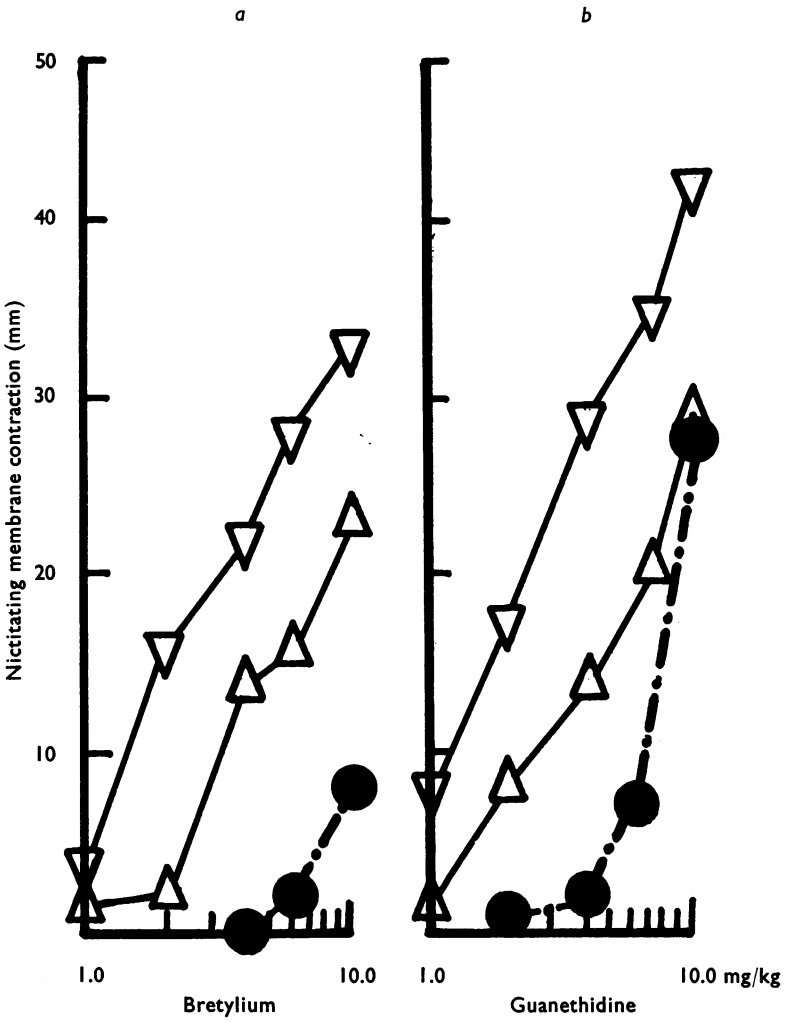

Fig. 1. Increasing sympathomimetic responses to intravenous bretylium and guanethidine during daily subcutaneous injection of these compounds. Nictitating membrane contractions in controls (● — . — ●) and after treatment with 10 mg/kg daily for 7 days (△ —— △) and for 14 days (▽ —— ▽). Means for groups of 5 cats.

bretylium or guanethidine were being tested. Then progressively increasing doses were given at intervals sufficient to allow each dose to produce its maximum effect (at least 2 min). Responses of the nictitating membranes to preganglionic stimulation were determined by applying to the nerve supramaximal square-wave shocks of 0.6 msec duration at each of a series of progressively increasing frequencies for 1-min periods, always prior to any administration of the sympathomimetic amines.

These sensitivity determinations were made in control cats and in animals given subcutaneous injections of bretylium, guanethidine, a ganglion blocking agent (BW 189C56) or reserpine daily for various periods. The interval between the last dose of these drugs and the determinations of sensitivity to sympathomimetic amines or intravenous bretylium or guanethidine was approximately 24 hr. The general condition of cats given bretylium or guanethidine remained

good throughout, but moderately severe necrosis often occurred at the site of the guanethidine injections. On the other hand, reserpine caused severe depression and diarrhoea and the ganglion blocking agent caused anorexia and the various severe incapacities that accompany full parasympathetic paralysis. For these reasons they were given for short periods only and to a small number of cats. A severe fall in temperature after reserpine treatment was avoided by keeping the cats in an environment of 30° C.

Some nictitating membranes were denervated under ether anaesthesia. Preganglionic denervation was effected by removing a 2-cm length of the cervical sympathetic trunk. For postganglionic denervation the superior cervical ganglion was excised.

Doses of all compounds are expressed in terms of the base or salt used. These were bretylium tosylate, guanethidine sulphate, reserpine (base), N^1-5:5-di-(4-chlorophenyl)-5-cyanopentyl-N^1:N^1:N^2-trimethylethylene-1-ammonium-2-morpholinium sulphate (BW 189C56), (−)-adrenaline acid tartrate, (−)-noradrenaline acid tartrate, tyramine hydrochloride, amphetamine sulphate, ephedrine hydrochloride, methylamphetamine hydrochloride and dimethylphenylpiperazinium iodide.

The dosage of sympathomimetic amines was extended to high levels in order fully to explore dose-response curves.

RESULTS

Bretylium

Adrenergic neurone blocking and sympathomimetic effects

The subcutaneous injection of a single dose of bretylium in the cat is followed by gradual relaxation of the nictitating membranes, due to blockade of their postganglionic sympathetic innervation. The time taken for relaxation of the membranes increases with the dose of the drug, as also does the duration and degree of relaxation. The delay in relaxation is probably due to the initial sympathomimetic effect of bretylium (Boura & Green, 1959; Green, 1960). Injection of bretylium in cats whose membranes were already relaxed by a previous dose of bretylium caused contraction of the membranes. When the drug was injected daily in doses of 10 mg/kg or more, the degree and duration of this sympathomimetic action greatly increased, whereas the degree of relaxation due to the adrenergic neurone blocking effect slightly increased during the first 2 or 3 days but later declined (Green, 1960). The increased sympathomimetic effect has now also been shown by injecting bretylium intravenously in anaesthetized cats given daily subcutaneous injections of the drug (Fig. 1). With lower subcutaneous doses of bretylium, e.g., 3 mg/kg, there was no relaxation of the membranes on the first day of injection, but moderate relaxation on the second and third days and full relaxation later on (Fig. 2). This suggests that even these low doses have a cumulative action. The relaxation caused by these doses was rapid in onset, lasted for several hours, and then declined; there was no sign of a sympathomimetic action during the first few days such as is seen with larger doses. The degree of membrane relaxation became progressively less when this amount was given for a week or more, but the apparent onset of "tolerance" was slower than with larger amounts.

Earlier experiments showed that single intravenous doses of bretylium depress the slope of the regression line relating the magnitude of nictitating membrane contractions to the frequency of stimulation applied to the postganglionic cervical sympathetic nerve, and also that the depression of slope increases with the dose

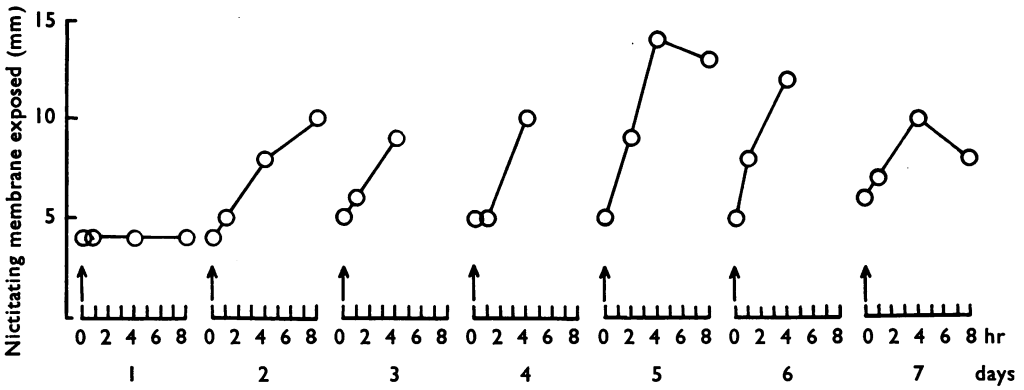

Fig. 2. Relaxation of nictitating membranes in unanaesthetized cats injected subcutaneously with 3 mg/kg bretylium daily (at arrows). Means for 2 cats.

(Boura & Green, 1959). Responses to preganglionic nerve stimulation were affected similarly, provided that the rate of impulses through the ganglion did not exceed 20/sec. Depressions of slope and diminished responses to all rates of nerve stimulation have also been found 24 hr after single doses of 10 mg/kg bretylium, but after daily injection of this amount for 19 weeks responses to all but the highest rates of stimulation were greatly increased (2 cats). In cats given 3 mg/kg bretylium daily for 14 days responses to low rates of stimulation tended to exceed those in control animals even though responses to high rates were greatly reduced, and after continuing treatment for a year the responses to the lower rates of stimulation again greatly exceeded those in controls (Fig. 3). Single doses of 50 mg/kg practically abolished responses to nerve stimulation 24 hr later in each of 3 cats, but this amount was not sufficient, when administered daily, to maintain blockade of responses in all cats. Considerable responses to nerve stimulation were found in 1 of 4 cats given 50 mg/kg daily for 14 days and in 3 of 5 cats in which treatment was continued for 6 months. The results obtained at 6 months were reported elsewhere (Green, 1960) and show a marked depression of the slopes of the nerve frequency-nictitating membrane response curves; the possibility that, in some cats, responses to very low rates of stimulation might have been at least as great as those in control animals was not excluded.

The possibility that permanent adrenergic nerve blockade might occur after repeated injection of large doses of bretylium was examined. When, after giving 50 mg/kg bretylium daily to 2 cats for 10 weeks, treatment was withdrawn for 2 weeks, the responses of the nictitating membranes to stimulation of the preganglionic cervical sympathetic nerves at 10 pulses/sec were similar to those of controls.

Although the sympathomimetic action of bretylium on the nictitating membranes of anaesthetized cats had greatly increased when 14 daily doses of 10 mg/kg bretylium had been given (Fig. 1), in 2 cats examined in the same way after continuing treatment for 19 weeks the sympathomimetic effect of intravenous bretylium was only slightly greater than in controls. Moreover, in these animals intravenous bretylium caused sharp transient falls of blood pressure instead of its

usual brief pressor effects. In a cat given 3 mg/kg bretylium daily for 1 year, intravenous injection of 5 mg/kg bretylium caused large contractions of the nictitating membranes (17 and 27 mm) but again a brief depressor effect (80 mm Hg).

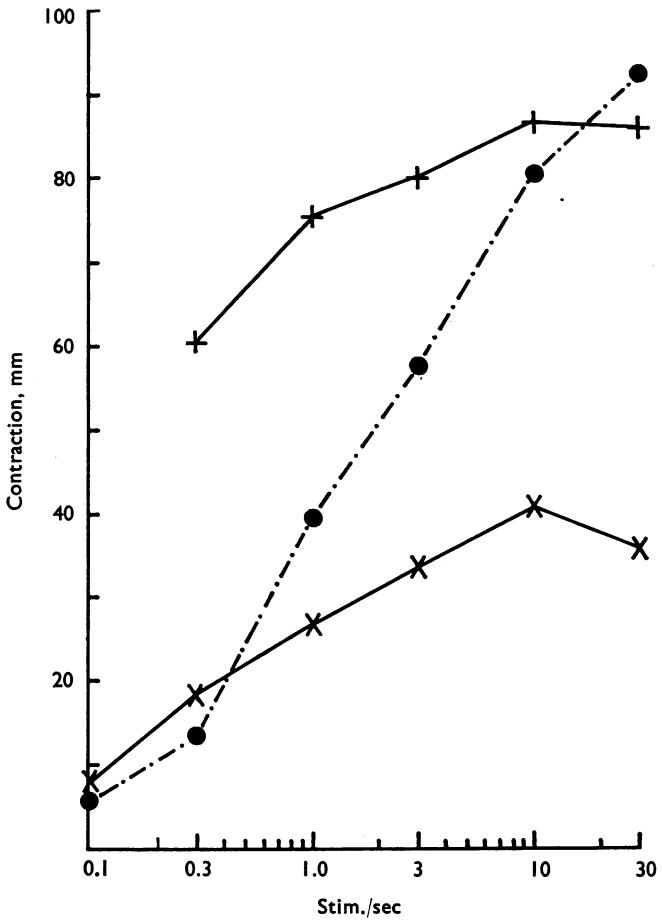

Fig. 3. Mean contractions of the nictitating membranes of cats caused by stimulation of the preganglionic cervical sympathetic nerve at various frequencies for 1 min periods: ●—·—● 11 control cats; x——x, 3 cats given 3 mg/kg bretylium daily for 2 weeks; +——+, 2 cats given 3 mg/kg bretylium daily for 13 months.

Adrenaline and noradrenaline

Nictitating membranes. In Fig. 4 are shown the dose-response curves for adrenaline and noradrenaline in cats given 1 dose or 14 daily doses of 10 mg/kg bretylium. The single dose increased the responses to all but threshold amounts of the catechol amines, and after 14 daily doses the minimal amounts of the amines required to contract the membranes had decreased at least 10-fold. The sensitivity to noradrenaline was increased more than that to adrenaline. Dose-response curves similar to those in Fig. 4 have been used to determine the doses of adrenaline and

noradrenaline that produce equal effects on the membranes of cats given various treatments with bretylium, and these are shown in Table 1. Because of some variation in the slopes of the curves obtained in different test situations, the estimates of changes in sensitivity must necessarily vary with the level at which comparison is made, but the Table nevertheless illustrates most of the main conclusions to be drawn: (1) With doses of bretylium varying between 3 and 50 mg/kg, noradrenaline sensitivity is increased more than adrenaline sensitivity ; (2) maximal hypersensitivity, about 20-fold for adrenaline and about 100-fold for noradrenaline, occurred when

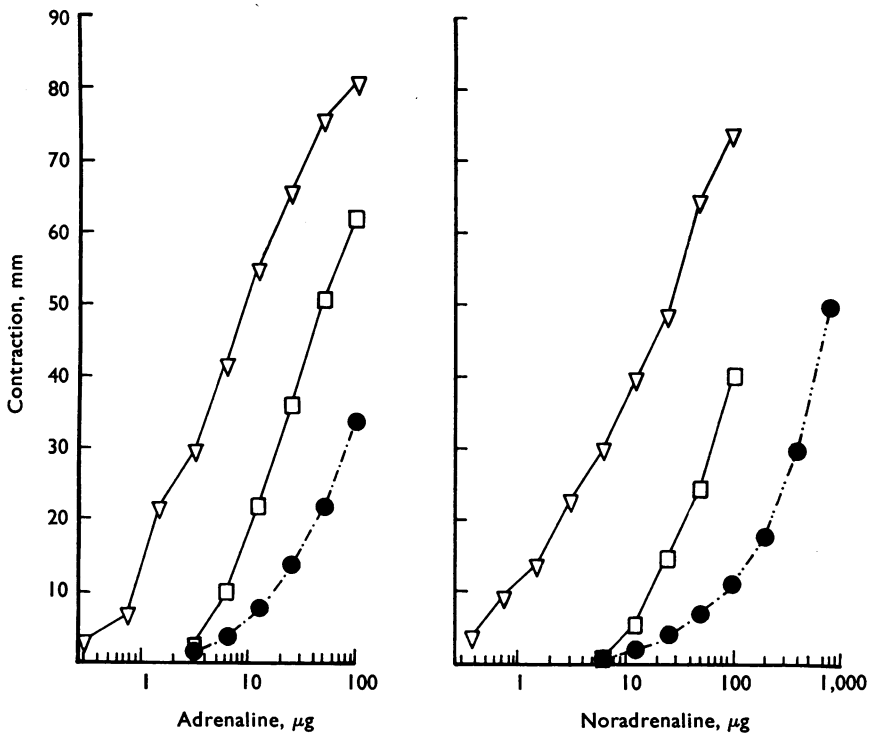

Fig. 4. Mean contractions of the nictitating membranes of cats caused by intravenous adrenaline or noradrenaline: ● — · — ●, controls (23 cats with doses up to 100 μg, 2 cats with higher doses of noradrenaline); □—— □, after 1 dose of 10 mg/kg of bretylium (4 cats); ▽—— ▽, after 14 daily doses of 10 mg/kg bretylium (4 cats).

10 or 50 mg/kg bretylium had been given daily for 7 to 14 days ; (3) daily doses of 3 mg/kg caused a marked, but probably submaximal, hypersensitivity within 14 days, and this level of sensitivity tended to increase when dosage was continued for a year ; (4) with 50 mg/kg dosage comparison of the effects of threshold doses of catechol amines indicated that near maximal hypersensitivity to threshold doses of catechol amines was maintained when treatment was continued for 6 months, but, on the other hand, responses to higher doses of the amines were less than at 14 days.

TABLE 1

HYPERSENSITIVITY OF NICTITATING MEMBRANES TO INTRAVENOUS ADRENALINE AND NORADRENALINE IN CATS GIVEN BRETYLIUM DAILY FOR VARIOUS PERIODS

Bretylium treatment		No. of cats	Equivalent doses	
mg/kg daily	Duration		Adrenaline μg	Noradrenaline μg
Controls	—	23	100	100
3	14 days	3	10	3
	13 months	2	11	3
10	1 day	4	22	19
	3 days	5	9	3
	7 days	4	6	3
	14 days	3	4	1·0
	19 weeks	2	5	1·5
50	1 day	6	28	13
	7 days	5	13	3
	14 days	6	3	1·5
	6 months	5	40	10

Hypersensitivity to adrenaline and noradrenaline persisted long after withdrawal of treatment when 50 mg/kg bretylium had been given daily for 10 weeks. Thus sensitivity to noradrenaline was still about six times that of controls when treatment had been withdrawn for 4 weeks, but similar to that of controls at 20 weeks (2 cats —not in Table).

Blood pressure. Table 2 shows that the mean carotid blood pressure of anaesthetized supine cats given sufficient bretylium to block adrenergic nerves was usually less than or similar to that in controls. The highest mean value was for 2 cats that had received 3 mg/kg daily for a year. In these animals the effects of sympathetic nerve stimuli on the nictitating membrane were greatly enhanced (Fig. 3) and the membranes of these cats were very sensitive to catechol amines. Whereas in most of the cats blood pressure was fairly steady, in the animals given large doses of bretylium (50 mg/kg) cyclic variations of up to 40 mm Hg at 1 cycle every 1 to 2 min were often seen.

TABLE 2

MEAN RESTING BLOOD PRESSURES AND PRESSOR RESPONSES TO INTRAVENOUS INJECTION OF 10 μG ADRENALINE OR 5 μG NORADRENALINE IN CATS GIVEN BRETYLIUM DAILY

The equivalent doses of adrenaline and noradrenaline are derived from dose-response curves

Bretylium		No. of cats	Mean blood pressure mm Hg	Adrenaline		Noradrenaline	
				Response mm Hg	Equivalent dose μg	Response mm Hg	Equivalent dose μg
mg/kg daily	Duration						
Controls	—	8	121	44	10	42	5·0
3	14 days	3	—	42	11	38	6·5
	13 months	2	150	40	12	33	8·5
10	1 day	4	103	73	2·6	72	1·2
	3 days	5	113	64	4·3	72	2·1
	14 days	3	103	58	6·5	50	3·5
50	1 day	6	108	62	5·0	62	2·2
	14 days	4	143	76	1·7	72	0·9
	6 months	5	120	55	5·6	50	2·5

The pressor responses to adrenaline and noradrenaline in Table 2 were presumably influenced by the resting level of blood pressure and by vasomotor reflexes. Nevertheless, after most bretylium treatments, hypersensitivity to catechol amines was distinct. The cats given 3 mg/kg doses are exceptions, but their responses, though less than controls, were greater than might perhaps be expected in animals with raised blood pressures. The group showing the greatest hypersensitivity, about 5-fold, had been given the largest dose (50 mg/kg) for 14 days, but hypersensitivity was less pronounced in cats given this dose for 6 months.

Tyramine, amphetamine, ephedrine and methylamphetamine

Nictitating membranes. The slope of the dose-response curve for the action of intravenous tyramine on the nictitating membranes of cats was depressed 24 hr after administration of single adrenergic neurone blocking doses of bretylium and to an extent that varied with the dose (Fig. 5*a*). As hypersensitivity to catechol amines developed during daily administration of bretylium there was a tendency for the dose-response curve to shift to the left, but the slope remained depressed. This is illustrated for animals given 3 daily 10 mg/kg doses in Fig. 5*b*, but similar dose-response curves were found in cats examined after 7 or 14 days. When 10 mg/kg had been given daily for 19 weeks (2 cats) responses to tyramine were smaller than at 14 days. Similarly, responses to tyramine were less in cats given 3 mg/kg bretylium for 1 year or 50 mg/kg daily for 10 weeks than after giving these amounts for 14 days (Fig. 5*c*). Responses to tyramine were small in 5 cats given 50 mg/kg daily for 6 months (Green, 1960).

The mean slope of the dose-response curve for intravenous amphetamine (0.1 to 10 mg) in cats given single doses of 10 or 50 mg/kg bretylium (groups of 4 cats) or 14 daily doses of 10 mg/kg (2 cats) was slightly less than in controls (10 cats). Responses to 0.1 to 3 mg amphetamine in the treated animals were greater than in controls (representing a 2 to 3-fold increase in sensitivity), but responses to 10 mg amphetamine were less than in controls (approximately 3-fold decrease in sensitivity). When 50 mg/kg bretylium had been given daily for 14 days (4 cats) the slope of the dose-response curve for amphetamine was greatly depressed—changes in response to threshold doses of amphetamine were minimal, but the mean response to 10 mg amphetamine was greatly reduced (12 mm in treated group, 48 mm in controls). The slopes of the log dose-response curves for ephedrine (1 to 9 mg) and methyl-amphetamine (0.3 to 10 mg) were depressed, and responses to all but perhaps threshold doses of these amines were reduced in 2 cats given 50 mg/kg bretylium daily for 5 or 10 weeks.

In the experiments so far described, adrenaline and noradrenaline sensitivities had been determined before recording the responses to tyramine and amphetamine in bretylium-treated and control animals alike. Nevertheless it seemed possible that the administration of the catechol amines might have had a different influence in the treated animals. That this is not an important factor is suggested by the finding that, in one cat given 10 mg/kg and another 50 mg/kg bretylium daily for 14 days, the responses to tyramine and amphetamine, tested without first giving catechol amines, were similar to those in animals tested after giving catechol amines.

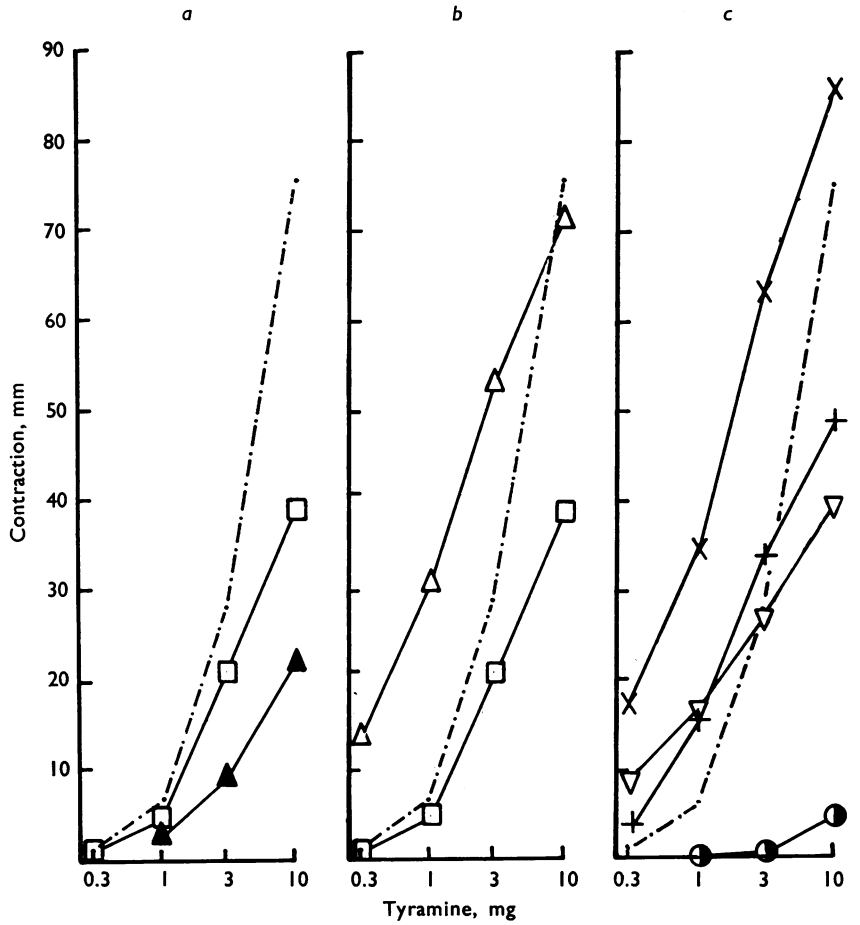

Fig. 5. Mean contractions of the nictitating membranes of cats caused by intravenous tyramine: — · —, controls (23 cats). (*a*) The slope of the dose-response curve is depressed by bretylium within 24 hr: □ —— □, 10 mg/kg (4 cats); ▲ —— ▲, 150 to 200 mg/kg (2 cats). (*b*) The dose-response curve shifts to the left during daily administration of 10 mg/kg bretylium: □ —— □, after 1 dose (4 cats); △ —— △, after 3 daily doses (5 cats). (*c*) Responses diminish when daily injection of bretylium is continued for a longer period: × —— ×, 3 mg/kg for 14 days (3 cats); + —— +, 3 mg/kg for 13 months (2 cats); ▽ —— ▽, 50 mg/kg for 7 or 14 days (9 cats); ◑ —— ◑, 50 mg/kg for 10 weeks (2 cats).

Tyramine administration may conceivably have influenced the amphetamine responses, but the changes in responses to amphetamine observed were similar to those for methylamphetamine in experiments where no other sympathomimetic amine was given.

Blood pressure. The slopes of the dose-response curves for the pressor effects of tyramine were reduced by bretylium (Fig. 6). After single doses of 10 or 50 mg/kg bretylium, responses to the higher doses of tyramine were reduced, but responses to low doses exceeded those in controls. Pressor responses to tyramine,

in contrast to nictitating membrane responses, were less in animals given 10 or 50 mg/kg daily for 14 days than in animals given the single doses. Greater reduction of responses was found in cats given bretylium daily for 6 months. In 3 cats given 3 mg/kg bretylium daily for 14 days the mean pressor responses to 0.1 to 0.3 mg tyramine were greater than in controls, but responses to higher doses were reduced. When, however, this dosage had been given daily for 13 months the mean responses to all doses of tyramine (0.3 to 10 mg) were reduced and the mean dose-response curve was similar to that after giving 10 mg/kg daily for 14 days (Fig. 6).

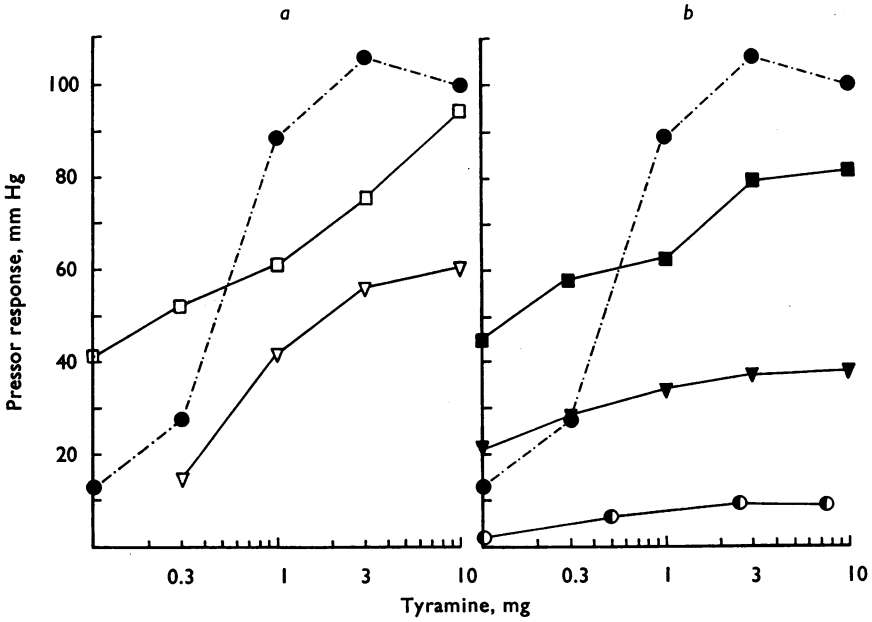

Fig. 6. Mean pressor responses to intravenous tyramine in cats given bretylium daily. ●—·—● Controls (4 cats). (*a*) 10 mg/kg dosage: □——□ after 1 day (4 cats); ▽——▽ after 14 days (3 cats); (*b*) 50 mg/kg dosage: ■——■ after 1 day (3 cats); ▼——▼ after 14 days (4 cats); ◐——◐ after 6 months (5 cats).

Dimethylphenylpiperazinium iodide

Changes in the responses to this substance were followed to give some indication of possible subacute effects on the adrenal medulla. Dimethylphenylpiperazinium iodide produces contractions of the nictitating membrane and pressor responses which, at low dose levels, are mainly due to activation of the adrenal medulla. With higher doses stimulation of sympathetic ganglia is of increasing importance. In acute experiments intravenous bretylium inhibits pressor responses to dimethylphenylpiperazinium iodide in adrenalectomized cats, but does not depress effects mediated by the adrenal medulla (Boura & Green, 1959).

Nictitating membranes. Bretylium caused marked hypersensitivity to intravenous dimethylphenylpiperazinium iodide (Table 3). Responses to threshold doses of the latter were affected less than responses to higher doses. The increased sensitivity was, however, less than that to the catechol amines, and this is compatible with

TABLE 3

EQUIVALENT DOSES OF INTRAVENOUS DIMETHYLPHENYLPIPERAZINIUM IODIDE FOR PRODUCING SMALL CONTRACTIONS OF THE NICTITATING MEMBRANE (14 MM ON TRACE) IN ANAESTHETIZED CATS GIVEN BRETYLIUM DAILY

(Derived from dose-response curves)

Bretylium		No. of cats	Equivalent dose μg	Dose as % of control
mg/kg daily	Duration			
Controls	—	15	300	100
3	14 days	3	25	8
	13 months	2	54	18
10	3 days	5	43	14
	7 days	4	56	19
	14 days	3	63	21
	19 weeks	2	50	17
50	1 day	6	100	33
	7 days	5	40	13
	14 days	6	50	17
	6 months	5	50	17

bretylium blocking the effect of dimethylphenylpiperazinium iodide that is produced via sympathetic ganglia but not the release of amines from the adrenal medulla. However, the indication that the sensitivity to dimethylphenylpiperazinium was greater after 3 days than after 14 days' treatment with 10 mg/kg bretylium, whereas catechol amine sensitivity was greater at 14 days, suggests that bretylium may possibly have depressed the adrenal medulla response to a small degree.

Blood pressure. Pressor responses to dimethylphenylpiperazinium iodide (30 to 100 μg) in cats given 10 mg/kg bretylium for 1, 3 or 14 days, or 50 mg/kg for 1 or 14 days or for 6 months, were usually greater than in control animals. The greatest increase in mean sensitivity, about 3-fold, was found in the groups given 10 mg/kg for 14 days (3 cats), 50 mg/kg once (6 cats) or 50 mg/kg daily for 6 months (5 cats).

Adrenal medulla

The adrenal glands were removed aseptically under ether anaesthesia in 2 cats at a time when daily subcutaneous injections of 10 mg/kg bretylium no longer caused relaxation of the nictitating membranes. On the following day administration of this dose of bretylium still did not relax the membranes.

Guanethidine

Adrenergic neurone blockade and sympathomimetic effects

Guanethidine, like bretylium, causes adrenergic neurone blocking and sympathomimetic effects on the nictitating membranes of cats. A single subcutaneous dose of 5 mg/kg partially relaxes and 10 mg/kg fully relaxes the nictitating membranes. The relaxation is slow in onset and with the higher dose lasts for over 24 hr. Part of the delay is probably due to the sympathomimetic action of the compound. This is seen as a contraction when a second dose is given at a time when guanethidine has relaxed the membranes. The effects of daily subcutaneous injections of guanethidine are highly cumulative, and relaxation is seen with amounts (2.5 mg/kg)

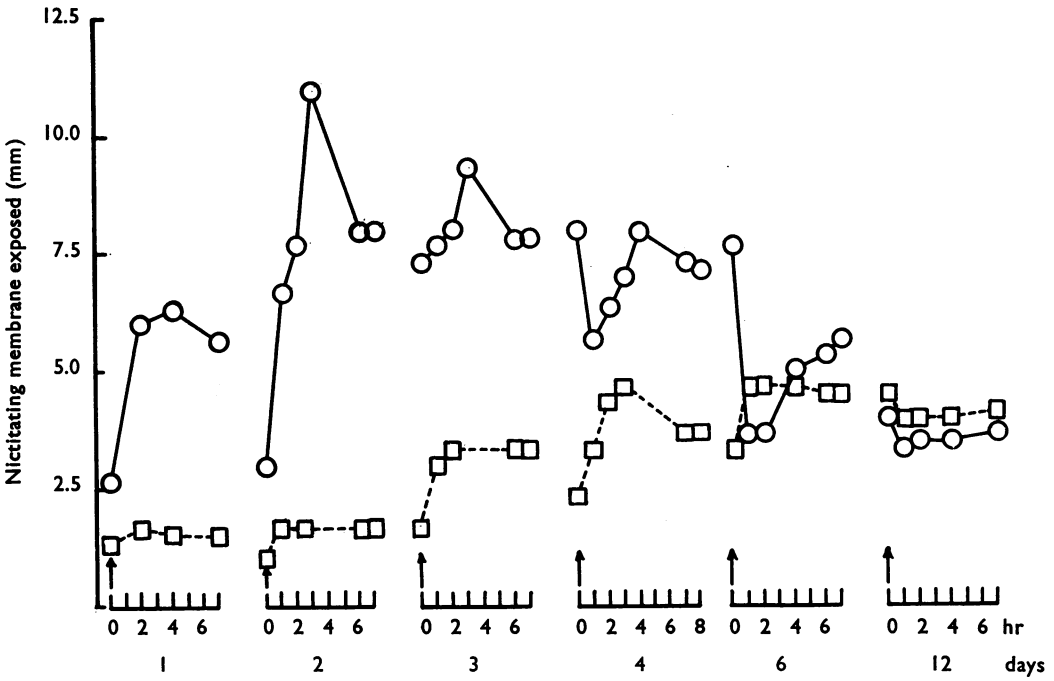

Fig. 7. Relaxation of the nictitating membranes of cats injected daily (at arrows) with guanethidine, 2.5 mg/kg (□---□) or 5 mg/kg (○——○). Means for groups of 3.

that are ineffective as single doses (Fig. 7). Fig. 7 also shows that the sympathomimetic action of subcutaneous doses of 5 mg/kg guanethidine increases when this amount is injected daily. Sensitivity to the sympathomimetic action of intravenous guanethidine increased about 5 to 10-fold when 10 mg/kg doses had been injected subcutaneously daily for 2 weeks (Fig. 1), and a similar increase was seen in 2 cats which had received a single dose of 50 mg/kg guanethidine on the day before the test.

The effect of guanethidine on the curve relating the frequency of sympathetic nerve stimulation to the magnitude of nictitating membrane contractions contrasts with that of bretylium. Whether the stimulation was applied to the pre- or the post-ganglionic cervical sympathetic nerve, the change produced by intravenous guanethidine was similar to that in Fig. 8*a*. The effects of the lower rates of stimulation were abolished and the frequency response curve shifted to the right but remained roughly parallel to that obtained before treatment. The extent of the shift increased with the dose, the minimal effective intravenous dose being about 1 mg/kg. A depression of slope was seen only after doses of 5 mg/kg or more and after 10 mg/kg responses to nerve stimulation were practically abolished.

Similar parallel shifts of the nerve frequency-nictitating membrane response curves were found after daily administration of guanethidine. There was a tendency for the curve to shift to the right during treatment, in keeping with the cumulative blocking action of the drug observed in non-anaesthetized animals. Thus responses to nerve stimulation in cats given 1.5 mg/kg guanethidine daily for 2 weeks were

much less than in a cat examined after a single dose of 1.5 mg/kg. Similarly blockade of response to nerve stimulation was complete in each of 4 cats given 10 mg/kg daily for 14 days, but 2 of 5 cats given 1 dose showed contractions of at least 20% of maximal with the highest rates of stimulation. On the other hand, there was an opposing tendency for the nerve frequency-nictitating membrane

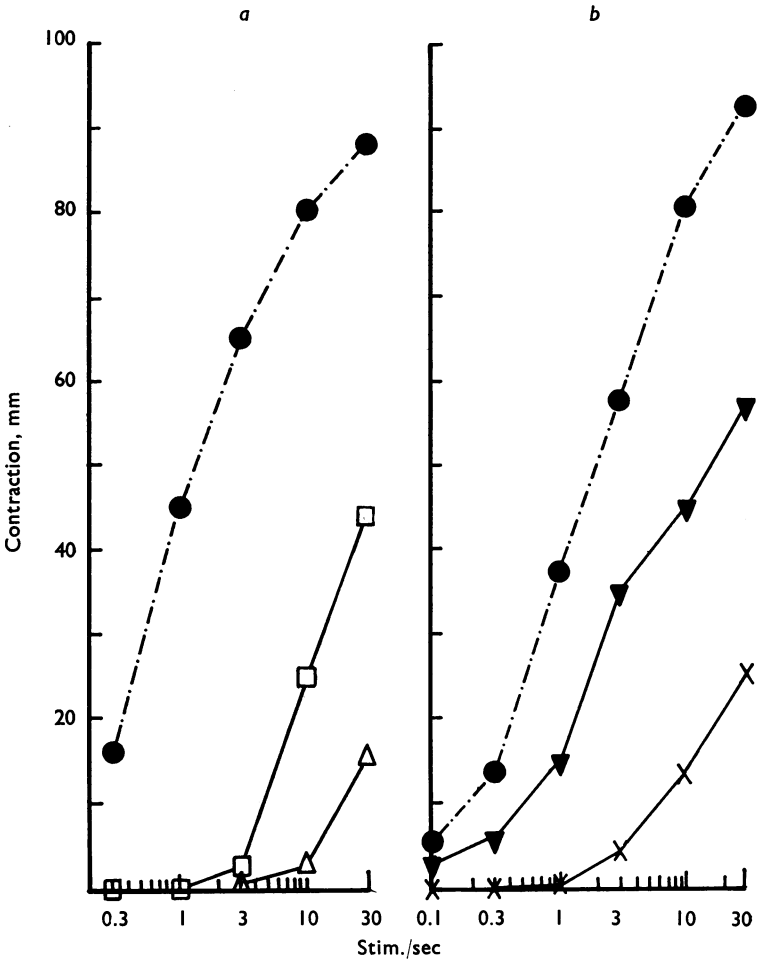

Fig. 8. Mean contractions of the nictitating membranes of cats caused by stimulation of the preganglionic cervical sympathetic nerve at various frequencies for 1 min periods. (*a*) Effect of intravenous guanethidine in an acute experiment: ●—·—● initial; □——□ after 2.5 mg/kg; △——△ after 5 mg/kg guanethidine. (*b*) Effect of daily subcutaneous injection of 5 mg/kg guanethidine: ●—·—● controls (11 cats); x——x after 3 days (3 cats); ▼——▼ after 5 weeks (3 cats).

response curve to shift to the left as hypersensitivity to adrenergic transmitter increased. Thus the mean curve for cats given 5 mg/kg guanethidine daily for 5 weeks was to the left of that found when this dose had been given for only 3 days (Fig. 8*b*).

Adrenaline and noradrenaline

Nictitating membrane. The equivalent doses of adrenaline and noradrenaline in cats given guanethidine daily, in Table 4, show that the degree of hypersensitivity that developed was similar to that with bretylium (Table 1). The rate of onset of hypersensitivity was different, however. Thus one day after a single dose of 10 mg/kg, when adrenergic nerves seemed fully blocked in some animals, there was no change in the threshold doses of adrenaline and noradrenaline required to contract the membranes and only a relatively small increase in the responses to higher doses of the amines, in comparison to that seen with equivalent adrenergic neurone blocking doses of bretylium (10 to 50 mg/kg). Hypersensitivity was prominent, however, 1 day after a single dose of 50 mg/kg or 3 daily doses of 5 or 10 mg/kg guanethidine. Maximal hypersensitivity occurred after 7 to 14 daily injections of 10 mg/kg, and the sensitivity of cats given 5 mg/kg guanethidine daily for 5 weeks was of similar magnitude. In cats given 1.5 mg/kg guanethidine daily for 14 days the sensitivity to catechol amines was high but submaximal.

TABLE 4

HYPERSENSITIVITY OF NICTITATING MEMBRANES TO INTRAVENOUS ADRENALINE AND NORADRENALINE IN CATS GIVEN GUANETHIDINE DAILY FOR VARIOUS PERIODS

Guanethidine treatment		No. of cats	Equivalent doses	
mg/kg daily	Duration		Adrenaline μg	Noradrenaline μg
Controls	—	23	100	100
1·5	14 days	3	15	5
2·5	4 weeks	3	8	6
5·0	3 days	3	18	6
	5 weeks	3	7	3
10	1 day	3	54	51
	3 days	5	13	2
	7 days	5	5	1
	14 days	5	7	1
50	1 day	3	9	4

Blood pressure. The mean blood pressures of cats given 1 dose of 10 or 50 mg/kg guanethidine (Table 5) were lower than those in controls, but the numbers of animals used was small and the differences not significant at the 5% level. However, the mean blood pressure of the group given 2.5 mg/kg for 4 weeks was significantly greater than that in controls ($P<0.01$) and the apparent elevation with 5 mg/kg for 5 weeks bordered on significance levels ($P=0.054$). The pressor effects of both adrenaline and noradrenaline were increased by daily injection of 1.5 to 10 mg/kg guanethidine, and the change was greater with 3 daily doses of 10 mg/kg than with the single dose. The extent of the increase was much less than seen on the nictitating membranes, as was the case after bretylium.

Tyramine and amphetamine

Nictitating membranes. Single doses of 10 mg/kg guanethidine depressed the slope of the dose-response curve for the action of tyramine on the nictitating

membranes. In Fig. 9, tyramine responses were determined before catechol amine responses in 4 of the 10 cats and after the determination of catechol amine sensitivity in the remaining 6 cats—no significant difference was found between these 2 groups. Nevertheless, in 2 of these cats in which sensitivity to tyramine was determined both before and after giving catechol amines, the latter seemed to restore slightly the responses to tyramine. All other responses to tyramine were determined after testing sensitivity to catechol amines. When 10 mg/kg guanethidine had been given daily for 3 days, responses to low doses of tyramine tended to be greater than after 1 dose of guanethidine. The mean dose-response curve for tyramine in 4 cats given a single dose of 50 mg/kg was similar to (or below) that in cats given 10 mg/kg guanethidine in Fig. 9, and when 50 mg/kg had been given daily for 5 weeks (5 cats) the slope of the dose-response curve was still similarly depressed but the sensitivity to tyramine was about 5 times that in the cats given the single dose. In these animals, as in those given 5 mg/kg guanethidine daily for 5 weeks (Fig. 9), the mean responses to low doses of tyramine exceeded those in controls, but the responses to high doses

TABLE 5

MEAN RESTING BLOOD PRESSURE AND THE PRESSOR RESPONSES TO INTRAVENOUS INJECTION OF 10 μG ADRENALINE OR 5 μG NORADRENALINE IN CATS GIVEN GUANETHIDINE DAILY

The equivalent doses are derived from dose-response curves

Guanethidine		No. of cats	Mean blood pressure mm Hg	Adrenaline		Noradrenaline	
mg/kg daily	Duration			Response mm Hg	Equivalent dose μg	Response mm Hg	Equivalent dose μg
Controls	—	8	121	44	10	42	5
1·5	14 days	3	—	70	5	55	2·1
2·5	4 weeks	3	180	70	4·5	75	1·2
5	3 days	3	113	60	5	56	1·8
	5 weeks	3	162	70	4·5	65	1·3
10	1 day	2	93	72	5·7	64	2·1
	3 days	4	116	75	1·8	73	0·9
50	1 day	3	81	92	2·1	70	1·3

of tyramine were less than in controls. A similar situation was found when 1.5 mg/kg guanethidine had been given daily for 2 weeks (3 cats) and when 2.5 mg/kg had been given for 4 weeks (2 cats), but then the depression of slope was less than with the 5 mg/kg doses and mean responses to all but the highest dose of tyramine (10 mg) exceeded those in controls. These animals showed an approximately 2-fold increase in sensitivity to doses of tyramine in the region of 0.3 to 1 mg.

Responses of the nictitating membranes to intravenous amphetamine were examined shortly after determination of responses to tyramine in several cats that had not received other sympathomimetic agents. One of 5 animals tested after a single dose of 10 mg/kg guanethidine showed responses within the control range, but 4 others showed only small responses to amphetamine. Mean responses to amphetamine in 5 cats given this amount of guanethidine daily for 14 days were greater than in those given only one dose, but the slope of the dose-response curve was again greatly depressed; the mean response to 0.03 mg amphetamine

tended to exceed those in controls, but responses to 0.3 to 10 mg were less than in controls. Responses to amphetamine in a small number of cats (groups of 2 or 3) examined after giving 1.5, 2.5 or 5 mg/kg guanethidine daily for 2, 4 and 5 weeks respectively were similar to those in controls, but there was again a tendency for

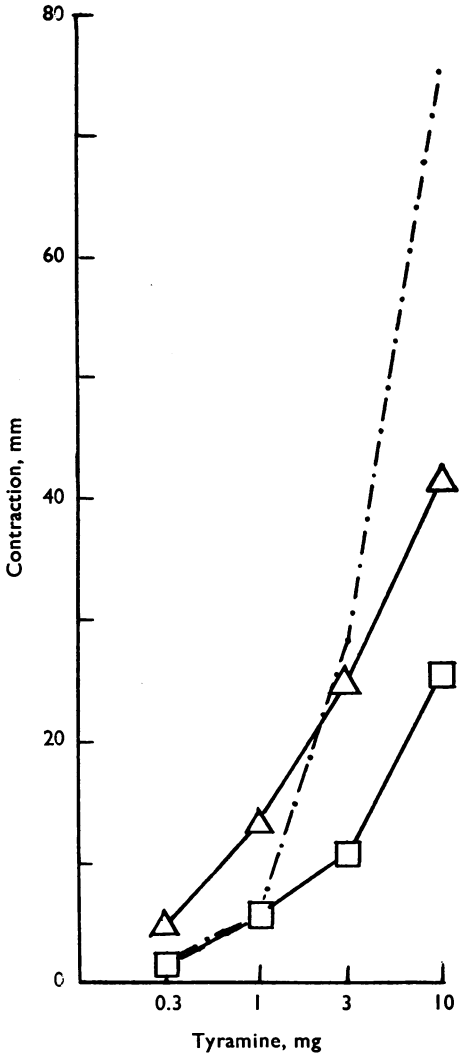

Fig. 9. Effect of guanethidine on mean nictitating membrane responses to intravenous tyramine: —·— 23 control cats; □——□ after a single dose of 10 mg/kg guanethidine (10 cats); △——△ after 5 mg/kg daily for 5 weeks (3 cats).

the effects of low doses of amphetamine to be greater than in controls and for the effects of high doses to be less than in controls (responses to catechol amines and dimethylpiperazinium iodide and tyramine had already been determined in these animals).

Responses to amphetamine were examined in a small number of cats soon after inducing anaesthesia and before giving any other substance. The mean slope of the dose response for 4 controls was steeper than that for 2 cats given 10 mg/kg guanethidine and another given 50 mg/kg, 24 hr earlier. (The mean responses to amphetamine in these controls were greater than in those animals in which amphetamine was given after testing the effects of other sympathomimetic amines.)

Blood pressure. Guanethidine depressed the slope of the regression line relating the log dose of tyramine to the pressor response. This was found in groups of 3 animals given 1.5 mg/kg daily for 2 weeks, 5 mg/kg for 5 weeks, 10 mg/kg on 3 successive days or one dose of 50 mg/kg. The mean response to 0.3 mg/kg tyramine in controls (4 cats) was about 30 mm Hg and similar or slightly higher means were found in all the treated groups except that given 5 mg/kg daily for 5 weeks (mean = 16 mm). On the other hand, whereas the mean response to 3 mg tyramine in controls was 106 mm Hg, the mean responses to this dose after the guanethidine treatments were all substantially less, varying between 42 and 58 mm Hg. In these

TABLE 6

EQUIVALENT DOSES OF INTRAVENOUS DIMETHYLPHENYLPIPERAZINIUM IODIDE FOR PRODUCING SMALL CONTRACTIONS OF THE NICTITATING MEMBRANE (14 MM ON TRACE) IN ANAESTHETIZED CATS GIVEN GUANETHIDINE DAILY

(Derived from dose-response curves)

Guanethidine		No. of cats	Equivalent dose μg	Dose as % of control
mg/kg daily	Duration			
Control	—	15	300	100
1·5	14 days	3	52	17
2·5	4 weeks	3	38	13
5	5 weeks	3	42	14
10	1 day	3	approx. 200	approx. 60
	3 days	5	130	43
	7 days	5	66	22
	14 days	5	56	19
50	1 day	2	48	16

experiments tyramine was given after testing sensitivity to catechol amines. In 2 cats given a single dose of 10 mg/kg guanethidine, the mean pressor responses to 0.3 mg and 3 mg tyramine were 23 and 63 mm Hg respectively before giving catechol amines and 38 and 84 mm Hg afterwards. The slope of the dose-response curve for the pressor effect of amphetamine in 2 cats given 5 mg/kg guanethidine daily for 5 weeks was also shallow—pressor responses of 24 mm and 35 mm Hg were produced by 0.3 mg amphetamine, but the responses were no greater with doses up to 10 mg amphetamine. Similar responses to these were found in 2 of 3 cats given 2.5 mg/kg guanethidine daily for 4 weeks.

In the above experiments amphetamine was given after determining responses to catechol amines and tyramine. When only amphetamine was tested, in 2 cats given a single dose of 10 mg/kg guanethidine, the responses were not appreciably different from those in controls.

Dimethylphenylpiperazinium iodide

Nictitating membrane. Guanethidine increased the sensitivity to dimethylphenylpiperazinum iodide (Table 6). The increase was less than that to adrenaline or noradrenaline (Table 4), indicating that guanethidine may block the effects of dimethylphenylpiperazinium iodide that are mediated by adrenergic nerves but not those mediated by the adrenal medulla as seemed also to be the case for bretylium.

Blood pressure. The mean sensitivities to the pressor action of dimethylphenylpiperazinium iodide (30 to 100 μg) in the cats given guanethidine varied between half and twice those of the control group. A low sensitivity was found in cats where there was effective adrenergic neurone blockade (e.g., after 10 mg/kg for 3 days), and a high sensitivity was often associated with treatments that had maintained only slight blockade but greatly increased sensitivity to catechol amines (e.g., after 2.5 mg/kg for 28 days).

Reserpine

Reserpine is known to block the effects of adrenergic nerve stimulation, but its action on various rates of stimulation has received scant attention. In Fig. 10 it is shown that 24 hr after 0.25 mg/kg reserpine, given intraperitoneally, the effects on the nictitating membrane of low rates of stimulation applied to the preganglionic cervical sympathetic nerve were abolished, and that the frequency-response curve shifted to the right but remained roughly parallel to the control curve. After doses of 2 mg/kg only small contractions of the membranes could be produced and then only with the higher rates of nerve stimulation. In these same cats, the responses of the nictitating membranes to intravenous adrenaline or noradrenaline were not appreciably changed, either when 0.25 or 2 mg/kg reserpine had been given. However, the slope of the dose-response curve for intravenous tyramine (1 to 10 mg) and the magnitude of the responses were reduced ; the mean responses to 10 mg tyramine were about 30% and 6% of the mean for controls in cats given 0.25 and 2 mg/kg reserpine respectively.

Ganglion blockade

Emmelin (1959) found that the sensitivity of the nictitating membrane to adrenaline increased in cats injected daily with chlorisondamine. This compound has a long duration of action, but except at very high doses does not maintain full ganglion blockade when injected once a day. In our experiments we used BW 189C56, which causes ganglion blockade lasting over 24 hr. Daily injection of 2 mg/kg for 15 days increased the sensitivity to adrenaline and noradrenaline approximately 8-fold in each of 2 cats. When 5 mg/kg was given daily the increase in sensitivity was about 20-fold (1 cat). Sensitivities to tyramine and ephedrine tended to increase in the cats given 2 mg/kg BW 189C56 daily, but the hypersensitivity, in contrast to that to the adrenalines, never exceeded 2-fold. No major change in sensitivity to dimethylphenylpiperazinium iodide (100 to 300 μg) was observed in 2 cats given 5 mg/kg BW 189C56 daily for 8 days, despite the development of marked hypersensitivity to catechol amines.

Nerve section

Denervation experiments were done to confirm that the main conclusions reached by others in more detailed studies (Bülbring & Burn, 1938 ; Lockett, 1950 ; Fleckenstein & Burn, 1953) were applicable under the conditions we have used in studying the changes in sensitivity to sympathomimetic amines produced by various drug treatments. We found that under these conditions the sensitivity of the nictitating membranes to adrenaline and noradrenaline was increased about 5-fold when the

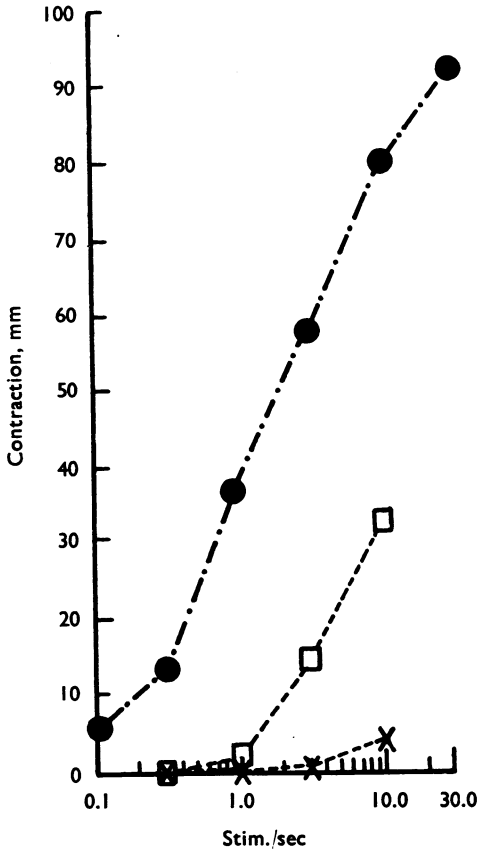

Fig. 10. Contractions of the nictitating membranes after stimulating the preganglionic cervical sympathetic nerve for 1 min at various frequencies. The frequency-response line (● — · — ● = mean for 11 control cats) was shifted to the right 24 hr after the intraperitoneal injection of 0.25 mg/kg reserpine (□ - - - □, 4 cats). Responses were almost abolished after 2 mg/kg (x - - - x, 4 cats).

preganglionic cervical sympathetic nerves had been cut 3 weeks previously (2 cats). Seven days after removing the superior cervical ganglion, the sensitivity to adrenaline was increased about 40 times and that to noradrenaline was increased about 100 times (2 cats). In contrast in a cat examined 4 months after removing the ganglion, sensitivities to adrenaline and noradrenaline were both increased by only 10 to 20 times.

DISCUSSION

Development of hypersensitivity to catechol amines. Increased responses to catechol amines after single doses of bretylium or guanethidine that block adrenergic nerves have been described by Boura & Green (1959); Maxwell *et al.* (1960a, b); Vernikos-Danellis & Zaimis (1960); and Zaimis (1960). In the present paper it is shown that the increase in the sensitivity of the nictitating membranes of cats, caused by single doses of either drug, is much smaller than that caused by repeated daily administration. The greatest increases in sensitivity, about 100-fold for noradrenaline and about 20-fold for adrenaline, were observed after daily injection of 10 mg/kg or 50 mg/kg of bretylium or 10 mg/kg guanethidine for 7 to 14 days. These increases are similar to those following postganglionic nerve section which reach their maximum after a similar time (Bülbring & Burn, 1938; Lockett, 1950; Fleckenstein & Burn, 1953). Marked hypersensitivity was also produced by daily injection of lower doses of bretylium (3 mg/kg) or guanethidine (1.5 mg/kg) in amounts that caused little adrenergic nerve blockade when given once only, but which, because of their cumulative effect, produced blockade when given daily for 3 days or more.

It has happened, for example, during the daily administration of low doses of bretylium or guanethidine, or after discontinuing a series of large doses of bretylium, that the effects of injected catechol amines were greatly increased at a time when the membranes gave a good response to stimulation of the preganglionic nerve. This does not mean that the sensitization was independent of an action on the adrenergic mechanism. The sensitization may outlive the interference with the nerve mechanism. There is another explanation of the apparent separation of these effects. Hypersensitivity to the injected amines may be expected to be accompanied by similar sensitization to transmitter amines released at the nerve endings. Thus, for example, in a cat given bretylium or guanethidine, the response of the nictitating membrane to nerve stimulation may be equal to that in controls, even when only a tenth of the amount of transmitter is released, if the sensitivity to this transmitter is increased 10-fold.

Emmelin (1959) showed that the sensitivity of the nictitating membranes to intravenous adrenaline increased at least 10-fold when ganglion blockade was maintained by giving chlorisondamine twice daily for 2 to 3 weeks. In our experiments a similar increase in sensitivity to both adrenaline and noradrenaline was found after daily administration of BW 189C56, a ganglion blocking agent with a particularly long duration of action. The hypersensitivity caused by ganglion blockade is similar to that caused by preganglionic nerve section but less than that caused by postganglionic nerve section or by bretylium or guanethidine. Increased responses to adrenaline, noradrenaline and dopamine have been reported in cats given reserpine (2.5 to 5.0 mg/kg) on two successive days (Burn & Rand, 1958). Since in our experiments there was no increase in sensitivity 1 day after single doses of reserpine, it seems that sensitization occurs later, as after moderate doses of guanethidine or bretylium.

Tolerance. Tolerance, manifested by a progressive lessening of the nictitating membrane relaxation, occurs during daily administration of either guanethidine or

bretylium in cats, but tends to be more prominent with the latter drug. There are several ways whereby hypersensitivity of the smooth muscle to catechol amines may be instrumental in causing this tolerance.

It is to be expected that the hypersensitivity to intravenous catechol amines will be accompanied by a comparable increase in responses to the transmitters released from adrenergic nerve endings. Since bretylium and guanethidine impair adrenergic nerve function by inhibiting transmitter release (Boura & Green, 1959; Hertting, Axelrod & Patrick, 1962), the degree of impairment of the smooth muscle responses to stimulation will depend on the balance between the amount of transmitter released and the sensitivity of the smooth muscle to this amount. "Tolerance" may be expected during the daily administration of an adrenergic neurone blocking agent if the inhibitory action on transmitter release increases less than the sensitivity of the smooth muscle to the transmitter. Despite the large increase in sensitivity to the injected transmitter amines following daily administration of guanethidine, impairment of the responses of the nictitating membranes to nerve stimulation is fairly well maintained, suggesting that the drug has a highly cumulative effect on transmitter release. A cumulative effect is also manifested by a progressive relaxation of the nictitating membranes during the first few days of administering doses of guanethidine that produce no apparent relaxation when given once only (Fig. 7). Nevertheless with 5 mg/kg dosage the impairment of responses to nerve stimulation was less after 3 days' than after 5 weeks' treatment. After prolonged administration of bretylium but not after guanethidine, responses of the nictitating membranes to low rates of sympathetic nerve stimulation may greatly exceed those in controls. This difference between the effects of the two compounds can be related to the finding that, whereas bretylium depresses the slope of the curve relating frequency of nerve stimulation and the magnitude of the nictitating membrane contraction produced (Boura & Green, 1959), guanethidine preferentially abolishes the effects of low stimulation frequencies causing a roughly parallel shift of the curve (Fig. 8). This relationship is illustrated in Fig. 11. The results agree with the presumption made here that the curves relating frequency of nerve stimulation and the response produced by the released transmitter tend to shift to the left during daily administration of the blocking agents, in keeping with the roughly parallel shift of the dose-response curves for intravenous adrenaline and noradrenaline. When marked hypersensitivity to injected adrenergic transmitters has developed during bretylium administration, exaggerated responses to low rates of stimulation may be avoided only by giving sufficient drug to largely abolish responses to all rates of stimulation. This may require that the transmitter release is reduced to substantially less than 1% of normal when hypersensitivity to transmitter amines is maximal (50- to 100-fold for noradrenaline, 20-fold for adrenaline). To do this, daily subcutaneous doses of 50 mg/kg bretylium are scarcely adequate in some cats, since fair responses to nerve stimulation were sometimes obtained after giving this amount for 2 weeks or 6 months. This dose is equivalent to about 200 mg/kg orally in cats or 12 g/man.

When sufficient bretylium or guanethidine is given daily to maintain full blockade of the responses of the nictitating membranes to sympathetic nerve stimulation,

the membranes still do not remain relaxed. Then some other factor must operate. In previous publications (Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim, 1959 ; Green, 1960) it was suggested that the hypersensitivity to catechol amines was such that circulating catechol amines might keep the smooth muscle

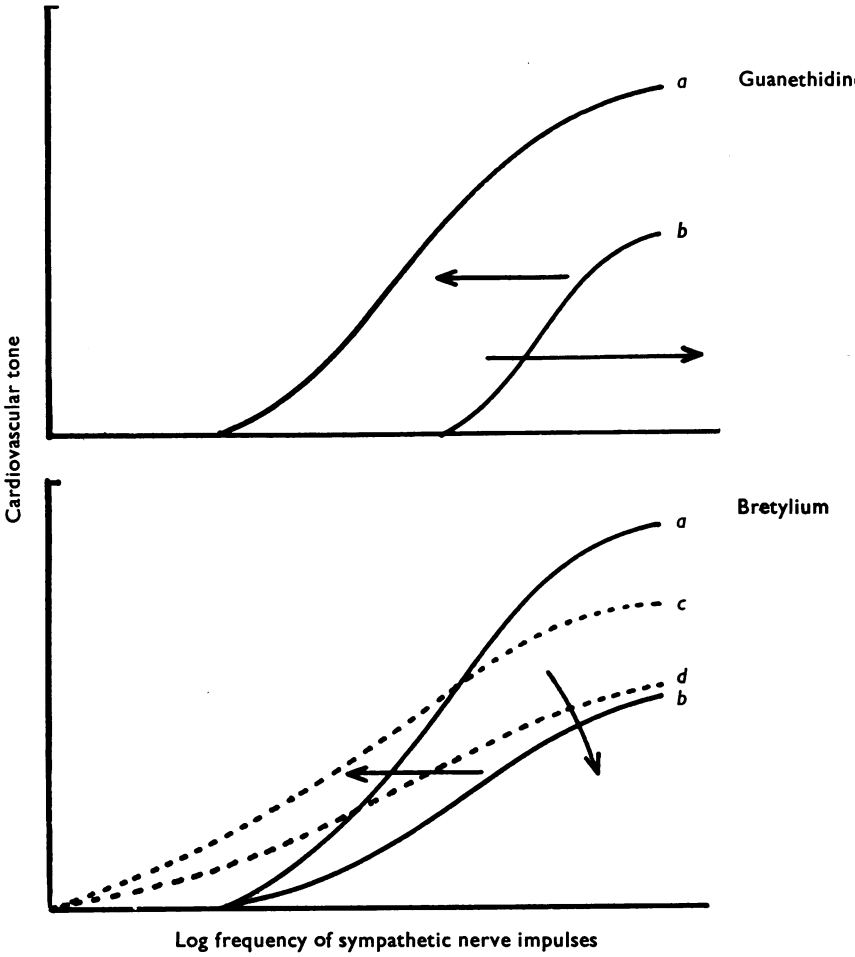

Fig. 11. Theoretical curves illustrating a possible explanation for the finding that the incidence of tolerance to adrenergic neurone blockade may be higher with bretylium than with guanethidine. *Guanethidine:* The curve relating frequency of stimulation to effect produced in untreated animals (*a*) shifts to the right after giving the drug (*b*). The tendency for the curve (*b*) to shift to the left during the development of hypersensitivity to the adrenergic nerve transmitter that accompanies daily administration of the drug is apparently offset by the cumulative effect of the drug or can be overcome by increasing the dosage. *Bretylium:* The slope of the curve (*a*) is depressed after giving bretylium (*b*) to an extent dependent on dosage. When bretylium has been given daily the developed hypersensitivity to adrenergic transmitter may be expected to cause a parallel shift of the curve (*b*) to a position (*c*), so that responses to low rates of stimulation tend to exceed those before treatment (curve *a*). Increased dosage of bretylium is expected to depress the slope of the curve (*c*) to position (*d*), but, except when the dosage is large, responses to the lowest rates of stimulation may continue to exceed those before treatment.

contracted. Neither bretylium nor guanethidine impairs the pressor responses to adrenal medulla activation, either by splanchnic nerve stimulation or intravenous injection of dimethylphenylpiperazinium iodide, in acute experiments, and the present experiments indicate that even when large doses of these adrenergic neurone blocking drugs are given daily there are no major changes in the nictitating membrane or pressor responses to dimethylphenylpiperazinium that are effected via the medulla. That nictitating membrane relaxation was better maintained during daily administration of a ganglion blocking agent in amounts sufficient to depress sympathetic ganglia and the adrenal medulla, than in cats given the adrenergic neurone blocking agents, may suggest that the lack of impairment of the functioning of the adrenal medulla is an important factor in the development of tolerance to the latter drugs, but the difference could be partly attributed to the lesser hypersensitivity of the smooth muscle produced by ganglion blockade. Removal of the adrenal gland in cats whose nictitating membranes had ceased to relax during the daily injection of 10 mg/kg bretylium was not followed by relaxation of the nictitating membranes, but the latter may still have been partly under the control of their sympathetic nerves.

Increased sympathomimetic responses to bretylium or guanethidine may also contribute to tolerance. The sympathomimetic effects of these drugs seem to be due to catechol amine release perhaps mainly from nerve endings (Boura & Green, 1959 ; Maxwell *et al.*, 1960a, b ; Gillis, 1960 ; Bein, 1960). As hypersensitivity to catechol amines developed, the sympathomimetic effects of bretylium and guanethidine also increased, but to a lesser extent. When, however, treatment with bretylium was continued for long periods of time, its increased sympathomimetic effect may have declined. Later doses of the adrenergic neurone blocking agents may produce less release of catechol amines. The changes may be analogous to the changes in responses to tyramine discussed below.

Though hypersensitivity to catechol amines may in these various ways lead to " tolerance " to adrenergic neurone blockade, when drug administration is continued for long periods other factors may also operate. This is suggested by the finding that the responses of the nictitating membranes to nerve stimulation after giving 50 mg/kg bretylium daily for 6 months or 3 mg/kg daily for a year were greater than those after giving the same doses for 2 weeks, whereas sensitivity of the membranes to catechol amines had not shown a correspondingly large increase. Similarly, although catechol amine sensitivity was greater in cats given 5 mg/kg guanethidine daily for 5 weeks than in cats given this amount for 3 days, the difference was insufficient to account readily for the much greater depression of responses to nerve stimulation seen at 3 days. One possibility is that transmitter release may partly return in the animals given the drug for the longer periods.

Results in man are compatible with tolerance to adrenergic neurone blocking agents, being due to sensitization of the peripheral smooth muscle to catechol amines (Laurence & Nagle, 1961), and the distinction between bretylium and guanethidine in their liability to cause tolerance seems to apply (Dollery, Emslie-Smith & Milne, 1960). Because of the results obtained using different rates of nerve stimulation of the nictitating membrane in cats, it is tempting to suppose that bretylium may be

more able to maintain a lowered blood pressure in those subjects in whom sympathetic nerve tone is high. It might also be expected that control by either drug will not be maintained when hypersensitivity to catechol amines is associated with a high output of these amines by the adrenal medulla. There have been reports of sympathomimetic effects being produced in man by bretylium (Laurence & Rosenheim, 1960 ; Blair, Glover, Kidd & Roddie, 1960) and guanethidine (Imhof, 1960), and these effects could be of greater importance after the development of hypersensitivity to catechol amines.

Tyramine and amphetamine

The slopes of the dose-response curves for the pressor and nictitating membrane contracting effects of tyramine were depressed by both bretylium and guanethidine in amounts that impaired the membrane responses to sympathetic nerve stimulation, the depression of slope increasing with the dose of the blocking agent. During the development of hypersensitivity to catechol amines the dose-response curve for the action of tyramine on the nictitating membrane shifted to the left, the change being more prominent after the bretylium treatments than after guanethidine, but, when bretylium had been given for long periods of time, responses to tyramine greatly declined. A tendency for some shift to the left of the dose-response curve for the pressor action of tyramine is suggested by some increase in the pressor responses to the lower doses after giving bretylium or guanethidine. However, pressor responses, in contrast to nictitating membrane responses, declined rapidly during the daily administration of the adrenergic neurone blocking agent.

These results can be interpreted using Burn & Rand's hypothesis (1958), now supported by more direct evidence (Lockett & Eakins, 1960 ; Schümann, 1961), that tyramine acts by releasing catechol amines. The application of this hypothesis suggests that the amount of catechol amines released by injection of tyramine declines rapidly during the daily administration of guanethidine and more slowly during the administration of bretylium. At first the development of marked hypersensitivity to the catechol amines released by tyramine may partly offset diminution of nictitating membrane responses that might otherwise result from a smaller release of catechol amines. When treatment with bretylium is continued for longer periods than those causing maximal sensitization to catechol amines (that is, longer than 14 days with 10 or 50 mg/kg dosage), the decline of nictitating membrane responses to tyramine found suggests that the amount of catechol amines released by tyramine is still declining. The more rapid decline of pressor responses to tyramine during administration of bretylium or guanethidine is to be expected, since the pressor responses to injected catechol amines increase much less than the nictitating membrane responses, so that there is a smaller tendency for diminished release of catechol amines being offset by the onset of increased sensitivity to these amines.

If the actions of tyramine were in fact exclusively due to release of catechol amines and if the sensitivity to the latter increased as greatly as that to injected adrenaline or noradrenaline, it would follow that the amount released was reduced whenever the sensitivity to the injected adrenaline or noradrenaline increased more than the sensitivity to tyramine ; this was the case for nearly all the treatments we have

used. For example, after giving 1.5 mg/kg guanethidine daily for 14 days, nictitating membrane responses to the lower dose ranges of tyramine indicated a 2-fold increase in sensitivity, but adrenaline and noradrenaline sensitivities had increased about 6- and 20-fold respectively. Likewise the depression of tyramine responses caused by single doses of 10 mg/kg bretylium contrasts with the increase in responses to catechol amines.

The diminished responses to tyramine caused by guanethidine can be related to reduction of peripheral stores of catechol amines in the cat (Cass, Kuntzman & Brodie, 1960). In contrast, single doses of bretylium do not appreciably reduce the catechol amine content of the heart or spleen of rabbits, guinea-pigs or rats under conditions where guanethidine caused a gradual reduction within 24 hr (Brodie & Kuntzman, 1960 ; Cass & Spriggs, 1961 ; von Euler, 1960). However, the noradrenaline concentration of sympathetic ganglia, heart and spleen fell after daily administration of 30 mg/kg bretylium in cats (A. C. McCoubrey, personal communication). A slow decline of tissue catechol amines is in keeping with the inhibitory action of bretylium and guanethidine on the uptake of circulating [^{3}H]-noradrenaline by the heart in rats (Hertting, Axelrod & Patrick, 1962). Bretylium and guanethidine may, however, prevent the releasing action of tyramine on catechol amine stores without first causing an appreciable depletion of these stores, as, for example, is suggested by some results with guanethidine recently described (Bartlet, 1962). Again, only a small proportion of catechol amines may be available for release by tyramine as indicated by Nasmyth's finding (1960) that isolated guinea-pig hearts failed to respond to tyramine following its repeated administration at a time when their catechol amine contents were not appreciably different from those in controls. This available catechol amine might be reduced, perhaps during the sympathomimetic action of the adrenergic neurone blocking agents, but its loss might nevertheless escape detection by the usual assay procedures.

Amphetamine and ephedrine have been classed together with tyramine as being sympathomimetic amines that appear to act by releasing adrenaline or noradrenaline (Burn & Rand, 1958). In keeping with this Maxwell *et al.* (1960b) found that in dogs single doses of guanethidine (15 mg/kg intravenously) reduced the response to each of these amines, when these were given 48 hr later. In contrast, Vernikos-Danellis & Zaimis (1960) reported that amphetamine and ephedrine produced their full nictitating membrane and pressor effects in cats given large doses of guanethidine or bretylium. The present experiments suggest, however, that nictitating membrane responses to amphetamine like those to tyramine, in cats given bretylium or guanethidine, can be greater or less than in controls, depending on (*a*) the dose of the sympathomimetic amine, since dose-response curves showed depressed slopes and tended to move to the left, and (*b*) the dose of the adrenergic neurone blocking agent and the period over which it had been given. They also indicate that the slopes of the dose-response curves for ephedrine and methylamphetamine, like those for tyramine, are reduced when large doses of bretylium have been given daily for many weeks. These are only indications, however. Responses to amphetamine and ephedrine were nearly always determined soon after responses to tyramine and the cats had often also received large doses of catechol amines. The possibility

that this may have influenced the responses to amphetamine and ephedrine in cats given the adrenergic neurone blocking agents has not been entirely excluded even though controls received similar treatment.

Effects on various frequencies of sympathetic nerve stimulation

The preferential abolition by guanethidine of nictitating membrane responses to low frequencies of nerve stimulation, and the roughly parallel shift of curves relating frequency of stimulation to the response obtained, suggest that this compound could be a competitive antagonist of some factor necessary for adrenergic nerve function, whereas the depression of the slope of the frequency-response curve caused by bretylium suggests that it may be a non-competitive antagonist. It has been suggested that bretylium may prevent a facilitatory action of acetylcholine on adrenergic transmitter release (Burn, 1961). However, this suggestion is not supported by Hukovič's finding (1960) that blockade by bretylium of the sympathomimetic action of acetylcholine

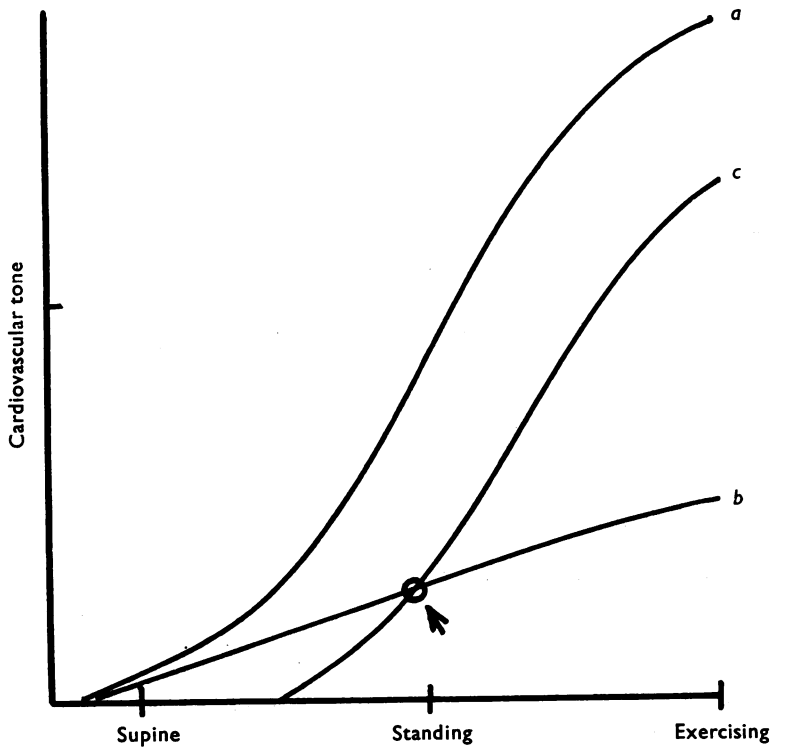

Fig. 12. Expected relative effects of bretylium and guanethidine on cardiovascular tone in man depending on whether a subject is supine, standing or exercising. It is assumed that the frequency of sympathetic nerve impulses is least when the subject is supine and greatest during exercise and that the relation of this frequency to cardiovascular tone (control=*a*) is affected by bretylium (*b*) and guanethidine (*c*) in like manner to the nerve frequency-nictitating membrane response curves. The dose of bretylium or guanethidine usually given is that which causes the desired lowering of cardiovascular tone with the subject standing—this is represented by the circle at the intersection of the curves.

on isolated atropinized rabbit atria was rapidly lost after washing the preparation whereas adrenergic neurone blockade persisted. Details of the mechanism whereby bretylium and guanethidine prevent transmitter release at the adrenergic nerve endings has still to be worked out. It is nevertheless interesting that small doses of reserpine alter the nerve frequency-nictitating membrane response curve in a manner similar to guanethidine, suggesting that this action is associated with a depleting effect on catechol amine stores. However, the depletion caused by guanethidine appears later than the adrenergic neurone blockade in rats (Cass & Spriggs, 1961) and the rapid " parallel shift " of the nerve frequency-response curves seen after intravenous guanethidine occurs at a time when it is unlikely that there is appreciable loss of catechol amines.

That the contrasting effects of bretylium and guanethidine on different rates of sympathetic nerve traffic may lead to differences in degrees of tolerance has already been suggested. There are also other possible consequences of clinical importance. In Fig. 12 it is assumed that the frequency of sympathetic stimuli is least when a subject is supine, greater when he stands and highest during exercise ; it is further assumed that the curve relating this frequency to the sympathetic cardiovascular tone in man is affected by bretylium or guanethidine in an analogous way to that found for the nictitating membranes of cats. When the dose of either drug is chosen as that required for a given lowering of cardiovascular tone with the subject in the erect position, then the following effects may be expected. (*a*) Guanethidine will cause a relatively greater fall in blood pressure when the patient is supine, i.e., when the level of sympathetic traffic is low. (*b*) Both drugs will cause exertional hypotension, since a high level of cardiovascular tone is no longer possible, but this will be more prominent with bretylium because of its relatively greater effect on the higher rates of traffic. (*c*) Bradycardia may be more common with guanethidine, since this agent causes the more complete blockade of low rates of sympathetic traffic. Some at least of these distinctions have been suspected during the use of these drugs in man (Taylor & Donald, 1960 ; Dollery *et al.*, 1960).

We have been particularly fortunate in having the able and enthusiastic assistance of Mrs D. R. Ferguson, Mr M. J. Follenfant and Mr E. J. R. Harry during these investigations. Guanethidine was kindly supplied by the Ciba Laboratories.

REFERENCES

BARTLET, A. L. (1962). The pressor action of guanethidine in the spinal cat. *J. Pharm. Pharmacol.*, **14**, 91–95.

BEIN, H. J. (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 162–170. London: Churchill.

BLAIR, D. A., GLOVER, W. E., KIDD, B. S. L. & RODDIE, I. C. (1960). Peripheral vascular effects of bretylium tosylate in man. *Brit. J. Pharmacol.*, **15**, 466–475.

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

BOURA, A. L. A., GREEN, A. F., MCCOUBREY, A., LAURENCE, D. R., MOULTON, R., & ROSENHEIM, M. L. (1959). Darenthin: hypotensive agent of new type. *Lancet*, ii, 17–21.

BRODIE, B. B. & KUNTZMAN, R. (1960). Pharmacological consequences of selective depletion of catechol amines by antihypertensive agents. *Ann. N.Y. Acad. Sci.*, **88**, 939–943.

BÜLBRING, E. & BURN, J. H. (1938). The action of tyramine and adrenaline on the denervated nictitating membrane. *J. Physiol. (Lond.)*, **91**, 459–473.

BURN, J. H. (1961). A new view of adrenergic nerve fibres, explaining the action of reserpine, bretylium and guanethidine. *Brit. med. J.*, i, 1623–1627.

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

CASS, R., KUNTZMAN, R. & BRODIE, B. B. (1960). Norepinephrine depletion as a possible mechanism of action of guanethidine (SU 5864), a new hypotensive agent. *Proc. Soc. exp. Biol. Med. N.Y.*, **103**, 871–872.

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

DOLLERY, C. T., EMSLIE-SMITH, D. & MILNE, M. D. (1960). Clinical and pharmacological studies with guanethidine in the treatment of hypertension. *Lancet*, **ii**, 381–387.

EMMELIN, N. (1959). Supersensitivity due to prolonged administration of ganglion blocking compounds. *Brit. J. Pharmacol.*, **14**, 229–233.

EULER, U. S. VON (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., p. 217. London: Churchill.

FLECKENSTEIN, A. & BURN, J. H. (1953). The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. *Brit. J. Pharmacol.*, **8**, 69–78.

GILLIS, C. N. (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 216–217. London: Churchill.

GREEN, A. F. (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 148–157. London: Churchill.

HERTTING, G., AXELROD, J. & PATRICK, R. W. (1962). Actions of bretylium and guanethidine on the uptake and release of [^{3}H]-noradrenaline. *Brit. J. Pharmacol.*, **18**, 161–166.

HUKOVIĆ, S. (1960). The action of sympathetic blocking agents on isolated and innervated atria and vessels. *Brit. J. Pharmacol.*, **15**, 117–121.

IMHOF, P. (1960), cit. RICHARDSON, D. W. & WYSO, E. M. Human pharmacology of guanethidine. *Ann. N.Y. Acad. Sci.*, **88**, 944–955.

LAURENCE, D. R. & NAGLE, R. E. (1961). The interaction of bretylium with pressor agents. *Lancet*, **i**, 593–594.

LAURENCE, D. R. & ROSENHEIM, M. L. (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 201–208. London: Churchill.

LOCKETT, M. F. (1950). The effect of denervation on the responses of the cat's nictitating membrane to sympathomimetic amines. *Brit. J. Pharmacol.*, **5**, 485–496.

LOCKETT, M. F. & EAKINS, K. E. (1960). The release of sympathetic amines by tyramine from the aortic walls of cats. *J. Pharm. Pharmacol.*, **12**, 720–725.

MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. & DANIEL, A. I. (1960a). Pharmacology of [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate (SU-5864). *J. Pharmacol. exp. Ther.*, **128**, 22–29.

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

NASMYTH, P. A. (1960). *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 337–344. London: Churchill.

SCHÜMANN, H. J. (1961). Weitere untersuchungen zum Mechanismus Freisetzung von Brenzcatechinaminen. *Arch. exp. Path. Pharmak.*, **241**, 200–201.

TAYLOR, S. H. & DONALD, K. W. (1960). The circulatory effects of bretylium tosylate and guanethidine. *Lancet*, **ii**, 389–394.

VERNIKOS-DANELLIS, J. & ZAIMIS, E. (1960). Some pharmacological actions of bretylium and guanethidine. *Lancet*, **ii**, 787–788.

ZAIMIS, E. (1960). Parallelism of changes produced by cooling and by drugs known to affect adrenergic mechanisms. *Nature (Lond.)*, **187**, 213–216.