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ACTION OF SOME SYMPATHOMIMETIC AMINES ON THE CAT'S IRIS, *IN SITU* OR ISOLATED

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Dale & Laidlaw (1912) noted that a number of sympathomimetic amines dilated the pupil of the cat's isolated iris. Alles (1927) found that phenylethylamine, phenylethanolamine or ephedrine dilated the pupils in guinea-pigs. Amines such as ephedrine (Chen & Poth, 1929) and hydroxyamphetamine (Abbott & Henry, 1937) have been used to produce mydriasis in man. The normal mydriatic action of some sympathomimetic compounds seems to depend on the integrity of the sympathetic post-ganglionic nerve endings in the iris, since the denervated radial muscle of the cat's iris becomes supersensitive to adrenaline (Meltzer, 1904) and to noradrenaline (Burn & Hutcheon, 1949), but is virtually insensitive to ephedrine or tyramine (Burn & Tainter, 1931; Burn, 1932).

The present study was undertaken to determine the mode of action of the sympathomimetic amines on the iris. These amines have previously been classified into three groups by their effect on the cat's denervated nictitating membrane (Fleckenstein & Burn, 1953) and by their pressor action in dogs after reserpine (Maxwell, Povalski & Plummer, 1959). A classification of these amines into three groups relating structure and activity is also proposed from the present studies on the iris after chronic sympathetic denervation and after reserpine. A preliminary account of the work has been communicated to the Physiological Society (Marley, 1960).

METHODS

Experiments were performed on fifty-eight cats, anaesthesia being induced with ethyl chloride and ether, and maintained with chloralose (80 mg/kg I.V., but 60 mg/kg in animals pre-treated with reserpine). In two chloralosed cats the mid-brain was transected through a frontoparietal craniotomy (Bradley & Elkes, 1957). Subsequent examination of the formalin-fixed brains of these animals confirmed that the transections were complete. Section of the spinal cord at the level of the C1 vertebra was performed through the

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approach for the *encéphale isolé* (Bremer, 1936). Spinal cats were prepared in the manner described by Kosterlitz, Krayer & Matallana (1955) or by exposing the atlanto-occipital membrane as for section of the spinal cord. In some experiments a portion of the post-ganglionic sympathetic trunk with the superior cervical and vagal nodose ganglia was removed acutely. In others these tissues had been removed aseptically (under pento-barbitone sodium 40 mg/kg) 10–21 days previously to allow for degeneration of the sympathetic post-ganglionic fibres to the iris. The vagal nodose ganglion was extirpated because sympathetic fibres may run in the cervical vagus (Jones, 1932; Agostini, Chinnock, Daly & Murray, 1957). In other experiments the ciliary ganglion was excised acutely (Shen & Cannon, 1936), or aseptically 7–10 days being allowed for degeneration of the ciliary fibres. In some animals the superior cervical and vagal nodose ganglia and the ipsilateral ciliary ganglion were removed so that both sympathetic and parasympathetic post-ganglionic fibres to the iris might degenerate; the orbital contents were subsequently dissected to confirm complete removal of the ciliary ganglion. Both adrenal glands and any adrenal accessory tissue were removed in the majority of animals because of the sensitivity of denervated irides to circulating adrenal catechol amines. Anoxic pupillary dilatation was avoided by artificial ventilation of the animal throughout the experiment; this precluded any effect on pupil size due to respiratory variation as a result of stimulation of the respiratory centre by the sympathomimetic amines.

The cat's head was fastened in a holder and the eyelids and nictitating membrane were tied aside; in a few experiments the nictitating membranes were removed with a cautery. The eyes were equally illuminated and in the threshold-dose experiments the light was just sufficiently bright to constrict the pupil to a narrow slit. To prevent drying, the cornea was irrigated with NaCl solution, 0·9 g/100 ml. Photographs of the pupil were taken at a constant close distance. Observations on the responses of the pupils to near-threshold doses of amines were usually checked by another observer. Drugs were injected into the cannulated right femoral vein. Blood pressure was recorded from the left femoral artery. Drugs were infused with a Palmer slow-infusion pump. Rectal temperatures were maintained at 37° C.

The standard pre-treatment with reserpine was 0·5 mg/kg intraperitoneal for 2 days, followed by 1·0 mg/kg the third day. Variations from this *standard dose regime* are mentioned in the text.

Intact isolated iris. Preparations were made as described by Dale & Laidlaw (1912). In spinal cats the eyes were enucleated and further dissection was carried out in Krebs's solution. The sclera was cut through parallel to, and 5 mm behind, the corneal margin. The vitreous humour was removed, the cornea was cut away, the lens was removed and the eye with its iris was placed in a shallow bath of 30 ml. Krebs's solution at 37° C bubbled with 95% O_2 and 5% CO_2. Drugs were added for a period of 2 or 3 min and changes in the pupil diameter were measured with calipers.

Drugs. The drugs tested were (−)-adrenaline bitartrate; atropine SO_4; (+) and (±)-amphetamine SO_4; bretylium bromide; bromlysergic acid diethylamide (BOL); the hydrochlorides of cocaine, (±)-cobefrine, (−)-ephedrine, epinine (3,4-dihydroxyphenyl-ethylmethylamine), (±)-metanephrine (3-methoxy 4-hydroxyphenyl β-hydroxyethyl-methylamine), (±)-methoxamine, (±)-methyl phenidate, (2,5)- and (3,5)- dimethoxyphenyl-ethylamine; β(3,5-dimethoxy) phenyl-β-hydroxyethylamine; (±)-normetanephrine (3-methoxy, 4-hydroxyphenyl-β-hydroxyethylamine); (±)-phenmetrazine; (−)-phenyl-ephrine; (±)-pipadrol; β-phenylethylamine and tyramine; (±)-isoprenaline SO_4; (−)-noradrenaline bitartrate; *o*-carbomylcholine Cl (carbachol); (±)-oxedrine tartrate (synephrine); (±)-pholedrine SO_4; reserpine in stabilized aqueous solution (Serpasil, Ciba), and tryptamine HCl. The doses of adrenaline, noradrenaline, and isoprenaline are given as base; the rest, as salt. Signs of the optical activity of the compound are only given subsequently if more than one isomer was used.

RESULTS

The mydriatic action of sympathomimetic amines is unaffected by acute nervous lesions

All the sympathomimetic amines which were tested dilated the pupil of the normally innervated iris; the threshold doses for dilatation of the constricted pupil are shown in Table 1. As expected, these amines produced mydriasis after the acute removal of (1) the preganglionic cervical sympathetic nerve, (2) the superior cervical and vagal nodose ganglia together with a portion of the post-ganglionic cervical sympathetic trunk, (3) the ciliary ganglion, and (4) after lesions of, or total destruction of the brain and/or spinal cord.

Chronic sympathetic denervation

The sympathomimetic amines could be classified into three groups both according to their structure and to their action on the denervated iris (Table 2), as found on other tissues by Fleckenstein & Burn (1953) and by Maxwell *et al.* (1959).

Group 1: *the phenylethylamines without –OH groups* (*inactive after denervation*). Fleckenstein & Burn (1953) showed that amines of this structure lost their sympathomimetic activity on the cat's nictitating membrane 10–14 days after its sympathetic denervation. In the present experiments it was found that 10–14 days after chronic denervation the phenylethylamines still retained much of their mydriatic activity, which only disappeared 21 days or longer after denervation (Pl. 1, fig. 1).

In previous studies on mydriasis the pupil was initially constricted (Burn, 1932; Burn & Rand, 1959). In spinal cats and in some chloralosed cats the pupils are almost maximally dilated; 21 days after iris denervation the phenylethylamines not only failed to dilate the pupil but actually produced a miosis (Pl. 1, fig. 1) persisting after atropine (0·2–1·0 mg/kg I.V.). This resembled the effect of tryptamine (0·5–1·0 mg I.V.) which, however, contracted the innervated as well as the denervated iris. The miotic action of tryptamine was extremely powerful, for it was able to overcome the dilatation of the denervated iris produced by large doses of adrenaline (25 μg/kg I.V.) or of the innervated iris by tyramine (0·5–1·0 mg/kg I.V.).

Conscious animals. The action of amphetamine differed in the conscious animal, for it *dilated* the pupil of the denervated iris. This action disappeared when the animal was anaesthetized, suggesting that part of the mydriatic action of amphetamine is central and is due to inhibition of parasympathetic tone (Marley, 1961). Similar results were found with β-phenylethylamine.

TABLE 1. Effect of chronic sympathetic denervation (10–14 days) on mydriatic thresholds of cats' irides to some sympathomimetic amines *in vivo*. Molar potencies of the bases are given relative to adrenaline = 1

Expt. no.	Adrenaline (μg/kg)	Noradrenaline (μg/kg)	Isoprenaline (μg/kg)	Cobefrine (mg/kg)	Epinine (mg/kg)	Dopamine (mg/kg)	Phenylephrine (mg/kg)	Oxedrine (mg/kg)	Methoxamine (mg/kg)	(−)-Ephedrine (mg/kg)	Tyramine (mg/kg)	Pholedrine (mg/kg)
Innervated iris												
18	—	—	—	—	—	—	—	0·30	—	—	—	—
21	—	—	—	0·200	0·100	—	0·1000	—	0·300	—	—	0·3
23	3·00	50·0	—	0·100	—	—	0·0500	0·20	0·050	—	0·2	—
29	0·75	3·5	3·0	0·025	0·025	0·050	0·0125	0·15	0·025	0·100	—	—
36	0·10	10·0	9·0	0·025	0·075	0·200	—	—	0·200	0·150	0·2	—
41	0·50	2·5	—	—	—	0·04	—	0·15	0·050	—	—	—
Mean	1·09	16·5	6·0	0·088	0·067	0·097	0·0542	0·20	0·125	0·125	0·2	0·3
Relative molar potency	1	16·4	4·8	67·8	55·7	86·6	45·1	138·8	84·8	104·4	194·1	235·3
Denervated iris												
18	—	—	—	—	—	—	—	0·060	—	—	—	—
21	—	—	—	0·010	0·005	—	0·0050	—	0·050	—	—	0·6
23	0·0500	2·00	—	0·004	—	—	0·0050	0·050	0·010	—	2·0	—
29	0·0250	0·90	1·0	0·003	0·003	0·0125	0·0025	0·050	0·005	0·25	—	—
36	0·0125	0·03	2·0	0·001	0·005	0·0500	—	—	0·025	0·50	1·5	—
41	0·02	0·05	—	—	—	0·0400	—	0·15	0·050	—	—	—
Mean	0·0269	0·74	1·5	0·0045	0·0043	0·0342	0·0042	0·044	0·030	0·38	1·75	0·6

Group 2a: *amines with an –OH only on the phenyl ring* (*less active after denervation*). As with group 1 the mydriatic effect of tyramine and pholedrine was reduced 10–14 days after iris denervation (Table 1) and that of tyramine disappeared after 17 days. At this time tyramine had an effect which was biphasic or purely miotic (Pl. 1, fig. 2), even after atropine (0·2–1·0 mg/kg I.V.).

Group 2b: *amines with an –OH on the β-carbon atom of the side chain only* (*activity variable after denervation*). In this group there was considerable variation in response after denervation. Thus pipadrol resembled group 1, 15 mg/kg producing no mydriasis 21 days after denervation. Ephedrine behaved differently. Its action appeared to resemble that of group 2*a*, since denervation raised the threshold for mydriasis. However, unlike tyramine, which contracted the iris 17 days after denervation, ephedrine always retained some mydriatic activity.

Methoxamine was different again, as it was more active on the denervated than on the innervated iris 10–14 days after denervation (Table 1). The response of the denervated iris to methoxamine exhibited three aspects of supersensitivity described by Cannon & Rosenblueth (1949), namely, augmentation of action, prolongation of action and reduction in threshold.

TABLE 2. Functional and structural classification for the effect of some sympathomimetic amines on the cat's chronic sympathetic-denervated iris. Note homogeneity of response to groups 1 and 3 amines, but functional overlap of response with group 2. With groups 2 and 3 adrenaline-like activity on denervated iris is greatest for compound on right; thus that of (–)-ephedrine is greater than that of tyramine, that of epinine is greater than that of dopamine. Molecules drawn to emphasize phenylethylamine skeleton

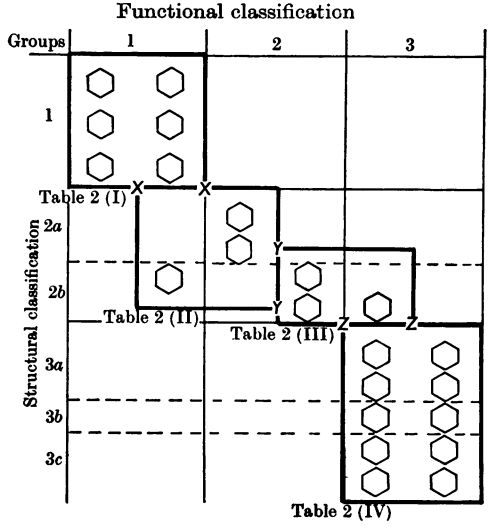


Diagram showing positions of blocks as one table (Table 2).

TABLE 2 (*cont.*)

Table 2 (I)

Functional classification

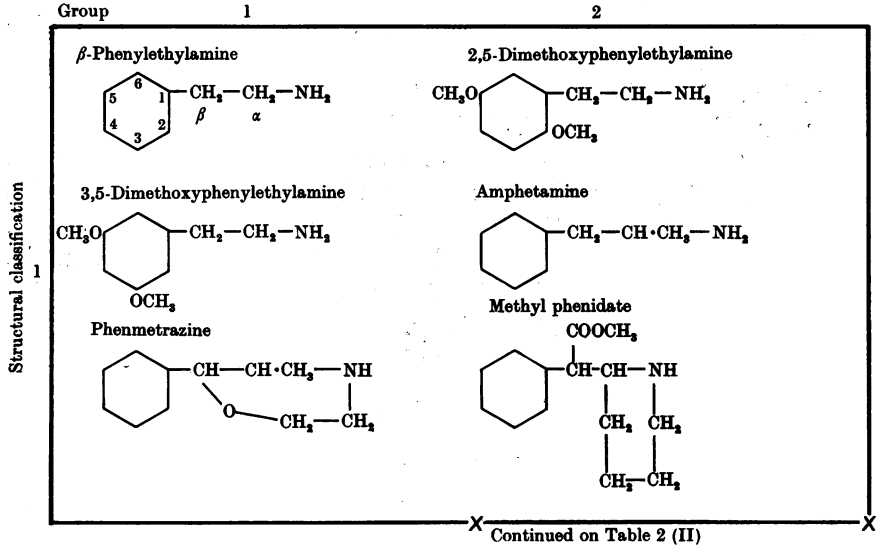


Table 2 (II)

Functional classification

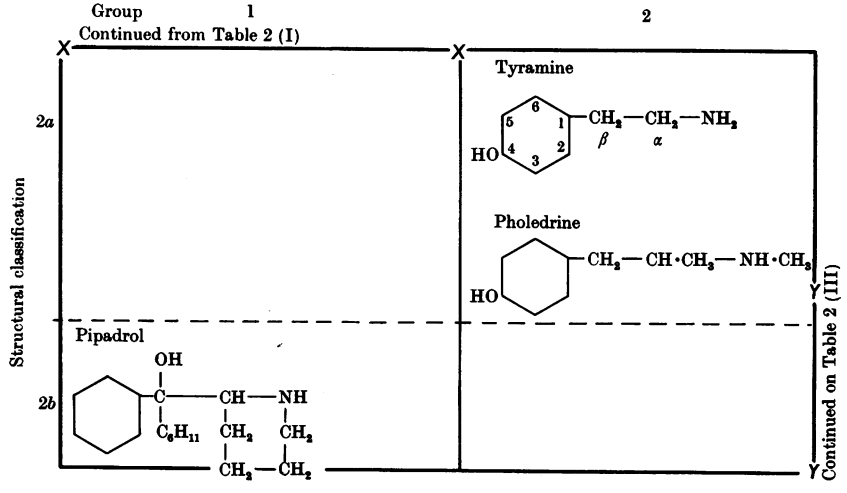


TABLE 2 (*cont.*)

Table 2 (III)

Functional classification

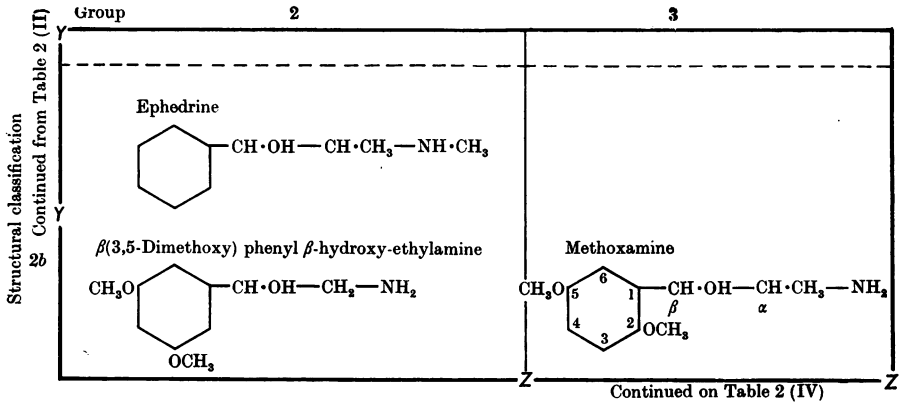
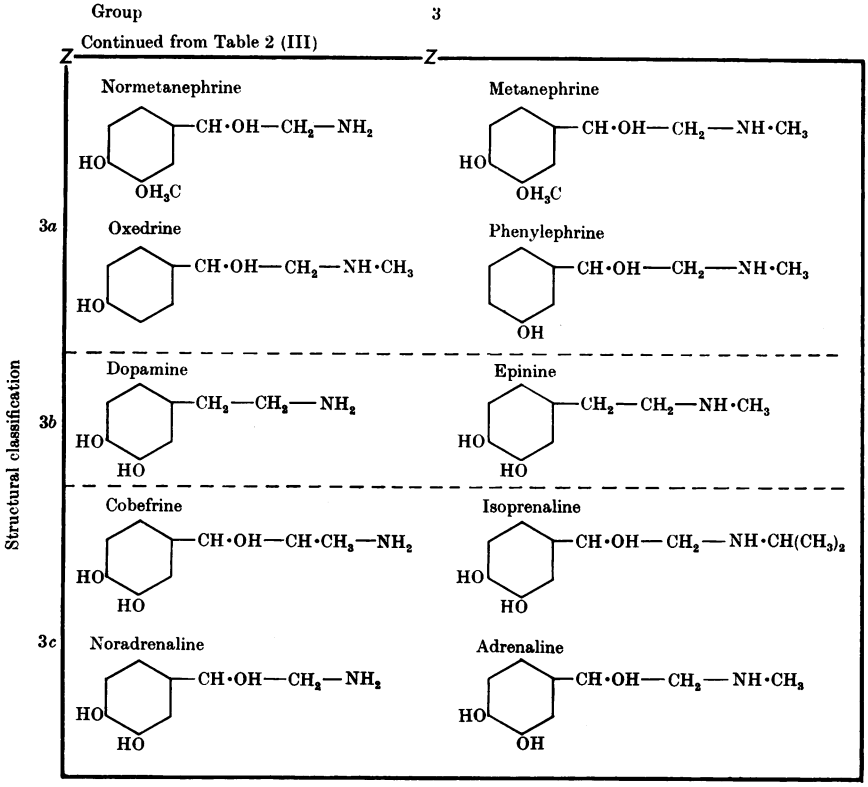


Table 2 (IV)

Functional classification



Methoxamine and 3,5 dimethoxy-β-hydroxyethylamine, which dilated the denervated iris in 50 μg/kg I.V. doses, have a β-OH group and methoxy groups in the 2,5 and 3,5 positions on the phenyl ring. This β-OH must be important for mydriatic activity, as 2 mg/kg I.V. of (2,5) and (3,5) dimethoxyphenylethylamine, which are chemically related to the above, but which lack the β-OH, were ineffective on either denervated or innervated irides.

In summary, a single –OH group on the otherwise unsubstituted β-carbon atom of the side chain, even when a CH_3 group is present on the α-carbon atom, confers more adrenaline-like activity than a single –OH group in the *p*-position on the benzene ring.

Group 3a: amines with one –OH on the phenyl ring and one–OH on the β carbon atom of the side chain. The denervated iris was supersensitive to phenylephrine (*m*-hydroxy), confirming Drake, John, Renshaw & Thienes (1939) that the catechol nucleus is not essential for sensitization. The *m*-hydroxy compound was about three times more potent on the innervated and ten times more potent on the denervated iris than the *p*-hydroxy compound oxedrine (Table 1). A facet of supersensitivity not mentioned by Cannon & Rosenblueth (1949) appeared with phenylephrine, namely that the response to the amine occurred earlier in the denervated than in the innervated iris. This was also seen with groups 3*b* and 3*c*.

It has hitherto been assumed that the adrenaline and noradrenaline metabolites metanephrine and normetanephrine (*o*-methylated in the 3 position) are inactive; yet in the present experiments weak mydriatic activity could be demonstrated with metanephrine (15 μg/kg I.V.) and normetanephrine (30 μg/kg I.V.) on the sensitized denervated iris, but metanephrine (150 μg/kg I.V.) and normetanephrine (300 μg/kg I.V.) were inactive on the innervated iris.

Group 3b: amines with –OH groups in the 3,4 *positions on the benzene ring but without an –OH on the β carbon atom of the side chain.* All aspects of supersensitivity were seen on the denervated iris. The threshold dose of epinine was only slightly lower than that of dopamine on the innervated iris, but about nine times lower on the denervated iris (Table 1).

Group 3c: amines with –OH groups in the 3,4 *positions on the benzene ring and an –OH on the β carbon atom of the side chain.* The denervated iris displayed all features of supersensitivity towards adrenaline, noradrenaline, isoprenaline and cobefrine (Table 1).

In summary, the mydriatic activity of all group 3 amines on the denervated iris was greater than on the innervated, the more so when the terminal amino group was methyl-substituted (adrenaline, epinine, metanephrine) than when unsubstituted (noradrenaline, dopamine and normetanephrine, respectively).

Chronic parasympathetic denervation

Unilateral removal of the ciliary ganglion. In these cats the pupil was not maximally dilated, as found by Lowenstein & Loewenfeld (1950) after acute removal of the ciliary ganglion, and the iris dilated further after intravenous injections of amines from all three groups. Thus the integrity of parasympathetic endings in the iris appears to be less important for the normal mydriatic action of these amines than the integrity of the sympathetic.

Total autonomic (double) denervation of one iris, compared with contralateral parasympathetic denervation alone. After sympathetic denervation alone there is no appreciable difference in the resting size of the pupils. It was therefore surprising to find (Pl. 1, fig. 2*A*) that the doubly denervated iris was more dilated at rest than the contralateral iris deprived only of its parasympathetic supply. Although the doubly denervated iris is supersensitive to circulating adrenal catechol amines, the excessive dilatation of the doubly denervated iris cannot be explained on the basis of supersensitivity, as it was found in both adrenalectomized and non-adrenalectomized animals.

After double denervation, as after sympathetic denervation alone, the mydriatic action of group 1 (amphetamine, β-phenylethylamine) or group 2*a* (tyramine) drugs was not lost for at least 17 days. Subsequently, the action of these amines was reversed, and they then produced contraction of the iris on the doubly denervated side, but dilated the iris on the side retaining its sympathetic innervation (Pl. 1, fig. 2*B*). This appears to resemble the effect of tryptamine, which always contracts the doubly denervated iris, since the miosis produced by tryptamine and (after 17 days) by β-phenylethylamine, amphetamine or tyramine was substantially reduced but not abolished by the tryptamine-antagonist BOL (2–6 mg I.V.).

Animals treated with reserpine

The response of the iris to amines of groups 1–3 has been also studied after reserpine, the effect of which has been likened by Burn & Rand (1959) to that of adrenergic denervation.

Acute administration of reserpine. It is known that reserpine contracts the pupil by stimulation of third nerve centres (Bein, Gross, Tripod & Meier, 1953). In the present experiments, in fact, mydriatic thresholds were raised within 2 hr of an intravenous dose of reserpine. For instance, in one animal reserpine increased the threshold (*a*) for adrenaline from 1·0 μg to 2·5 μg/kg I.V., (*b*) for noradrenaline from 7·5 to 10 μg/kg I.V., (*c*) for epinine from 0·05 to 0·1 mg/kg I.V., and (*d*) for tyramine from 0·3 to 0·5 mg/kg I.V. Similar results were obtained with dopamine and cobefrine.

Likewise, any miotic drug would be expected to raise the mydriatic threshold and in fact, carbachol infused I.V. raised the threshold for all three groups of amines.

Chronic administration of reserpine. Absence of pressor response to tyramine (2 mg/kg I.V.) was used as a criterion of full reserpine effect in the following experiments after chronic reserpine treatment (standard dose given in Methods). When this criterion was satisfied, the iris was also found to have become less sensitive to most of the mydriatic amines. Thus, in one animal 100 mg (±)-amphetamine (group 1) given in divided doses over 140 min was without effect on the iris. In another a single injection of 20 mg/kg I.V. (±)-amphetamine had no mydriatic effect, but after the infusion of 0·125 mg noradrenaline, 2·5 mg/kg I.V. (±)-amphetamine given slowly over 5 min produced moderate increase in pupil size. In contrast to the abolition of the mydriatic and pressor responses of these amines by reserpine treatment, the central effects of amphetamine and phenmetrazine were unaltered (see also Tripod, Bein & Meier, 1954) with the production of non-conjugate eye movements and myoclonic jerking in the face and limbs.

Even after the smaller doses of reserpine (Table 3) the mean thresholds for groups 2*a* and 2*b* were much raised, except for methoxamine. The effect of large doses of reserpine on the iris closely resembled that of chronic sympathetic denervation, for not only was there loss of mydriatic activity of tyramine and the group 1 amines but also the mydriatic action of tyramine was lost before that of β-phenylethylamine. Once their mydriatic action had disappeared these amines could also contract the iris if initially dilated.

The mean thresholds for group 3*c* were, however, normal after the standard dose of reserpine, whereas the thresholds were slightly raised for groups 3*a* and 3*b* (Table 3). Further doses of reserpine (0·5 mg/kg I.V.) in these chronically treated animals did not alter the threshold for group 3 amines, suggesting that the miosis due to reserpine was already maximal.

In contrast to the above results, Burn & Rand (1959) have described supersensitivity of the iris to catechol amines after reserpine, similar to that after denervation, but they used much larger doses of reserpine. Some of the present experiments were therefore repeated with larger doses of reserpine (e.g. Expt. 40, Table 3). Increased sensitivity was noticed, but this was limited only to a lowering of threshold dose for group 3 amines.

If, in fact, reserpine produces all the changes of chronic sympathetic denervation, there should be no difference between normal and denervated irides after reserpine. Experiments were therefore performed in reserpinized cats with unilateral sympathetic denervation of the iris. In one animal (given 3 mg/kg reserpine one day and 2 mg/kg the second) the threshold doses of the group 3 amines for the innervated iris were in fact

TABLE 3. Effect of chronic reserpine dosage on mydriatic thresholds of cats' irides to some sympathomimetic amines *in vivo*. (Cats 19, 20, 22, standard dose of reserpine. Cat 24, 0·5 mg/kg for 2 days; cat 27, 0·5 mg/kg first day, 1 mg/kg second day; cat 40, 3 mg/kg first day, 2 mg/kg second day, 1 mg/kg third day)

Expt. no.	Adrenaline (μg/kg)	Noradrenaline (μg/kg)	Isoprenaline (μg/kg)	Cobefrine (mg/kg)	Epinine (mg/kg)	Dopamine (mg/kg)	Phenylephrine (mg/kg)	Oxedrine (mg/kg)	Methoxamine (mg/kg)	(−)-Ephedrine (mg/kg)	Tyramine (mg/kg)	Pholedrine (mg/kg)
19	—	33·0	—	0·200	—	—	0·10	1·00	0·200	—	2·5	—
20	—	—	—	0·200	0·750	—	0·10	—	0·400	—	—	2·0
22	—	—	—	0·200	0·030	—	0·01	0·25	0·060	—	—	2·0
24	2·00	7·5	10·0	0·050	0·030	0·3	0·02	0·50	0·075	—	3·0	—
27	1·50	5·0	7·5	0·100	0·400	0·6	0·03	0·60	0·150	1·5	2·0	—
40	0·30	—	0·5	0·005	0·075	—	—	—	0·100	—	—	—
Mean	1·27	15·2	6·0	0·096	0·214	0·45	0·25	0·59	0·148	1·5	2·5	2·0
27 (after cocaine 0·2 mg/kg I.V.)	0·45	—	—	0·01	0·03	0·50	—	0·30	0·150	—	—	—

TABLE 4. Effect of combined chronic reserpine and chronic sympathetic denervation (10–14 days) on mydriatic thresholds of cats' irides to some sympathomimetic amines *in vivo*. Cat 25, reserpine intraperitoneal 0·5 mg/kg first and second days, 1 mg/kg third day. Cat 38, 3 mg/kg first day, 2 mg/kg second day

Expt. no.	Adrenaline (μg/kg)	Noradrenaline (μg/kg)	Isoprenaline (μg/kg)	Cobefrine (mg/kg)	Epinine (mg/kg)	Dopamine (mg/kg)	Phenylephrine (mg/kg)	Oxedrine (mg/kg)	Methoxamine (mg/kg)
Innervated iris									
25	2·00	4·00	—	0·02	0·030	0·50	—	0·3	0·150
38	0·03	0·25	0·03	0·004	0·025	0·02	0·002	0·075	0·040
Denervated iris									
25	0·1	0·05	—	0·002	0·005	0·20	—	0·1	0·075
38	0·01	0·10	0·03	0·004	0·025	0·02	0·002	0·075	0·020

almost identical with those for the denervated iris (Table 4, expt. 38); but with doses above threshold the degree of mydriasis was still greater and more prolonged in the denervated than in the innervated iris. In another cat, given 2 mg/kg reserpine for 2 days, tyramine (2 mg/kg I.V.) had no effect either on the two irides or on the blood pressure; but the innervated iris responded without evidence of supersensitivity to phenylephrine (Pl. 2), although the denervated iris showed all aspects of supersensitivity. Moreover, catechol amines were still considerably potentiated in the innervated iris 60 min after cocaine (0·2 mg/kg I.V., Pl. 2*B*). Another difference due to the fourth facet of denervation supersensitivity was shown in a further animal which had been given the smaller standard dose of reserpine; as is shown in Pl. 3*B*, the denervated iris dilated earlier than the innervated iris to cobefrine. Thus the effect due to reserpine on the innervated iris was not equal to that of denervation (plus reserpine) on the opposite side. Moreover, the innervated iris was not otherwise supersensitive (for threshold doses see Table 4, expt. 25), even though the pressor response to tyramine was absent, indicating full peripheral reserpinization.

These results supplement those of Burn & Rand (1959), who were the first to describe a lowering of the threshold of the iris to adrenaline and noradrenaline after chronic administration of reserpine. It is clear also from the present experiments that increased sensitivity in terms of the threshold dose can be observed. On the other hand, it is much harder to produce in the iris than in the vascular system. With a dose of 1–2 mg reserpine supersensitivity of the iris was never seen, although the pressor responses to the group 3 amines were enhanced. With 4–6 mg reserpine increased sensitivity in terms of threshold dose occurred in two experiments, although it was verified in all three that supersensitivity due to denervation was well developed. If the full picture of supersensitivity is taken into account (decreased threshold dose, shorter latency for response, greater amplitude and duration of response) the supersensitivity found with reserpine appears to be incomplete.

Other drugs

Cocaine (0·2 mg/kg I.V.) produced changes in iris response to amines of groups 1–3, similar to those seen after chronic sympathetic denervation.

Bretylium, on the other hand, increased the sensitivity to groups 1–3. Thus after bretylium 6 mg/kg I.V., when post-ganglionic sympathetic nerve stimulation ceased to be effective on the iris, the iris became more sensitive to all mydriatic amines. The threshold mydriatic dose was reduced for epinine from 0·04 to 0·01 mg/kg, and for tyramine from 0·15 to 0·015 mg/kg; the mydriatic effect of (+)-amphetamine 1·0 mg/kg was much prolonged. Increased sensitivity of the nictitating membrane to tyramine and adrenaline was noticed by Green (1960) in cats given bretylium daily for two weeks. These findings suggest that either the stores of noradrenaline are not available for release by group 1 and 2 amines after cocaine

but are after bretylium, or that the receptors on which the sympathomimetic amines act are modified or occupied in different ways by cocaine and bretylium.

Isolated iris

The isolated iris preparation was not entirely satisfactory because the resting diameter of the pupil increased progressively from the time the tissue was excised. This may be due to swifter deterioration (see Walter, 1958) of the sphincter than of the dilator pupillae muscle, which would favour mydriatic responses as the experiment proceeds and produce an apparent lowering of mydriatic thresholds.

In contrast to the results *in vivo*, with 'non-denervated' irides *in vitro* the mydriatic effect of group 1 amines was inconsistent. For example, even 1 mg amphetamine/ml. might produce only slight mydriasis on its first addition to the bath; thereafter, subsequent doses of the amine even with long rest intervals had no effect or produced pupil constriction. The results with the isolated iris otherwise corroborated the experiments *in vivo*. The mydriatic thresholds *in vitro* of chronic sympathetic-denervated irides were lower than those of 'non-denervated' preparations for adrenaline, noradrenaline, epinine and oxedrine, but higher for tyramine and ephedrine (Table 5). All aspects of supersensitivity to group 3 amines found with denervated irides *in vivo* were observed *in vitro*. There was earlier, greater and more prolonged dilatation of the pupil of denervated than of 'non-denervated' irides after an identical dose of the amine.

Isolated iris preparations were also made from a cat given large doses of reserpine. The threshold mydriatic doses were normal (for adrenaline 0·5 μg/ml.; for epinine 0·006 mg/ml.).

TABLE 5. Threshold mydriatic doses to some sympathomimetic amines for denervated and 'non-denervated' irides *in vitro*. (Drug contact 3 min)

Iris	Adrenaline (μg/ml.)	Nor-adrenaline (μg/ml.)	Epinine (mg/ml.)	Oxedrine (mg/ml.)	(−)-Ephedrine (mg/ml.)	Tyramine (mg/ml.)
'Non-denervated'	0·17	0·33	0·004	0·017	0·08	0·008
Denervated	0·08	0·10	0·001	0·003	0·20	> 0·33

DISCUSSION

The case for a triple rather than a dual classification of the sympathomimetic amines is strong. These amines have been divided into three groups when studied *in vivo* on the cat's denervated nictitating membrane (Fleckenstein & Burn, 1953), on the cat's blood pressure after reserpine (Maxwell *et al.* 1959), on the central nervous system of some young animals (Key & Marley, 1961), and *in vitro* on the rat's isolated stomach strip (Vane, 1960). The present comparison of the study of their effects on the innervated and on the chronic sympathetic denervated cat's iris also suggests a classification into the following three groups: (1) the phenylethylamines without –OH groups, the normal mydriatic action of which was lost after denervation; (2) substances with an –OH on either the phenyl ring or on the β carbon atom of the side chain—with these the mydriatic threshold dose for the denervated iris was usually raised; and (3) substances with –OH groups in the 3,4 position, with or without a β-OH on the side chain, or with one –OH on the phenyl ring together with an

–OH on the β carbon atom; towards these the denervated iris was supersensitive.

There are several stipulations to make with regard to this classification. Response to group 3 amines was most consistent, supersensitivity being found to all except oxedrine. The classification for groups 1 and 2 was less satisfactory; these compounds release noradrenaline (Burn & Rand, 1958*b*) and so indirectly affect receptors. If their action depended solely on this mechanism this would vitiate any structure–activity study, for the sympathomimetic amines would fall into two groups, those of group 3 acting directly on receptors and those of groups 1 and 2 acting indirectly. There is evidence against this dichotomy.

The mydriatic action of the group 1 amines amphetamine and phenylethylamine persists, but in attenuated form, for about 21 days after removal of the superior cervical ganglion. Thereafter these amines have apparently no effect on the constricted iris, but contract the dilated iris. This contraction was found after combined chronic parasympathetic and sympathetic denervation of the iris, and was presumably due to excitation of the sphincter pupillae. With the parasympathetic innervation intact the contraction was not abolished by the prior administration of atropine but was diminished by the tryptamine-antagonist BOL, suggesting that these amines activate tryptamine receptors on the iris. The loss of mydriatic action of group 1 amines was also found with *in vitro* preparations of innervated or chronic sympathetic-denervated irides.

The possibility that the mydriatic action of the indirectly acting amines might be partly central, and mediated through the parasympathetic pathways, has been neglected. Yet in conscious animals, group 1 amines produced marked mydriasis in denervated and innervated irides, which disappeared when the animal was anaesthetized. Mydriasis produced by these amines seems to be the resultant of dilator actions, elicited both by central inhibition of parasympathetic pupilloconstrictor tone and by peripheral excitation of the sympathetic nerve endings in the iris overwhelming a normally undetectable activation of the sphincter pupillae. Mydriasis produced by other amines is possibly a combination of central and peripheral actions.

The group 2 amines, although intermediate in structure, functionally overlapped the other groups. Tyramine, pholedrine and pipadrol behaved with few differences like group 1. Tyramine was the most exhaustively studied. After iris denervation its mydriatic action was lost before that of the group 1 amines and replaced by a biphasic pupil dilatation followed by constriction. This biphasic response was not seen with group 1 amines; but for these discrepancies the phenylethylamines with a *p*–OH on the benzene ring would have been included with them. Of the β-hydroxy

amines, the mydriatic action of ephedrine was reduced after iris denervation, suggesting that normally ephedrine acts partly by the release of a dilator substance in the iris; that the dilator effect could be elicited when tyramine had either no effect, or a biphasic or a miotic action, suggests that ephedrine has also a direct adrenaline-like action on the iris. Of this group the adrenaline-like action was best seen with methoxamine, which resembled group 3.

Vane (1960) was the first to describe an action by certain sympathomimetic amines on tryptamine receptors in the smooth muscle of the rat's stomach strip. Amines acting on these tryptamine receptors were the same as those here designated group 1, which had tryptamine-like effects on the smooth muscle of the denervated iris. Amines which behaved like adrenaline in relaxing the stomach strip were the same as those here classified in group 3, to which the denervated iris was supersensitive. Amines with a biphasic action on the stomach strip were the same as those now classified as the intermediate group 2 because of their mixed action on the iris smooth muscle.

Burn & Rand (1959) pointed to the similar response of tissues denervated by degeneration of their sympathetic nerve supply or treated with large doses of reserpine. Thus, absence of pressor responses to some sympathomimetic amines and supersensitivity to others (Burn & Rand, 1958*a*, *b*) and supersensitivity of the cat's iris and spleen (Burn & Rand, 1959) occurred after chronic administration of reserpine. Muscholl & Vogt (1958) found no, or reduced, iris responses to sympathetic nerve stimulation after reserpine.

In the present experiments there was no similarity to denervation after single intravenous doses of reserpine, for the sensitivity of the iris to all amines decreased. There was closer resemblance between the effects of chronic reserpine administration and of denervation. The mydriatic action of amines of groups 1 or 2 was reduced or absent, except for methoxamine, which elicited pupil dilatation. Moreover, the mydriatic action of tyramine was lost before that of β-phenylethylamine, after which these amines could produce miosis if the pupil was initially dilated. In contrast to the denervation experiments, sensitivity to group 3 amines was not increased after small doses of reserpine. After large doses of reserpine the mydriatic thresholds were in the supersensitive range, but the response to group 3 amines otherwise differed from that after denervation, for the other features of supersensitivity were not found. Because of the miosis produced by reserpine and mediated through parasympathetic pathways, full supersensitivity of the iris to the catechol amines could probably only be elicited with reserpine after ciliary ganglion removal. Other tissues behave dissimilarly to these amines after denervation and reserpinization. Thus

the concentrations of adrenaline or noradrenaline required to produce ED_{50} responses of the isolated nictitating membrane after chronic reserpinization were not different from those of normal muscles (Burn, Leach, Rand & Thompson, 1959).

Central nervous stimulation occurred in the chronically reserpinized animal after amphetamine in the absence of pressor or mydriatic actions. This suggested that the central action of amphetamine may not be entirely dependent on noradrenaline release, although stronger evidence has come from the antipodal effect of amphetamine and noradrenaline on behaviour and electrocortical activity in young animals (Key & Marley, 1961). Vane (1960) postulated that amphetamine may also act on central tryptamine receptors.

It would appear that the most reasonable classification of the action of the sympathomimetic amines on the smooth muscle of the iris is still afforded by denervation studies. The differential response of the denervated iris mirrors with fair fidelity the differential response of certain central and other peripheral receptors to these amines.

SUMMARY

1. By comparative studies on the innervated and on the chronic sympathetic-denervated cat's iris, a number of sympathomimetic amines could be classified into:

Group 1, the phenylethylamines without –OH, the mydriatic action of which was lost after denervation;

Group 2, substances with an –OH group on either the phenyl ring or on the β carbon atom of the side chain—for these the threshold mydriatic dose for the denervated iris was usually raised;

Group 3, substances with –OH groups in the 3,4 position (with or without a β–OH on the side chain), or with only one –OH on the phenyl ring together with an –OH on the β carbon atom—towards these the denervated iris was supersensitive.

2. Although, after denervation, supersensitivity to group 3 amines was well developed within 10 days, 21 days elapsed before loss of the mydriatic action of group 1. In groups 1 and 3 responses were consistent within the group, but in group 2, tyramine, pholedrine and pipadrol resembled group 1, whereas methoxamine behaved like group 3. Ephedrine was intermediate in activity but, like group 3, dilated the pupil of the denervated iris although the threshold was raised.

3. When the mydriatic action of group 1 amines was lost, they elicited contraction of the denervated iris; similarly, tyramine (group 2*a*) produced either contraction or dilatation followed by contraction. The miotic action

of these amines was not abolished by atropine nor by combined sympathetic and parasympathetic denervation of the iris, but was partly reduced by the tryptamine antagonist BOL.

4. The mydriatic action of group 1 was due to activation of sympathetic nerve endings in the iris, and central inhibition of parasympathetic pupilloconstrictor tone overwhelming activation of the sphincter pupillae.

5. In group 2 a single –OH group on the β carbon atom of the side chain, in the absence there of other substituents, conferred more mydriatic potency on the denervated iris than a single –OH group in the p-position on the phenyl ring. In group 3 the catechol nucleus was not essential for sensitized responses of the denervated iris, and mydriatic activity was greater in the presence of a $NH.CH_3$ terminal group than if the terminal amino group was unsubstituted.

6. The mydriatic potency of all amines tested was increased by bretylium but declined after intravenous reserpine.

7. In chronic experiments with massive doses of reserpine the iris dilator muscle developed limited sensitivity to group 3 amines. With smaller doses of reserpine the iris responded normally to group 3, even though the mydriatic action of groups 1 or 2 (except methoxamine) was lost.

8. There were similarities between the response of the iris after chronic reserpine and chronic sympathetic denervation. These included the loss of mydriatic and development of miotic properties by certain amines, and the same time sequence of loss of response, the mydriatic effect of tyramine disappearing before that of β-phenylethylamine. Less striking was the development of increased sensitivity to group 3.

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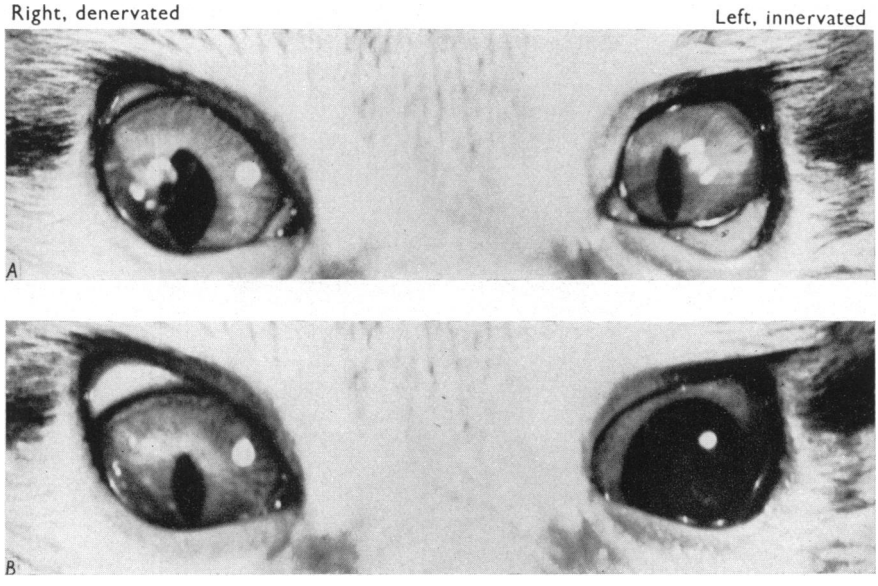

Right, denervated
Left, innervated
A
B

Fig. 1

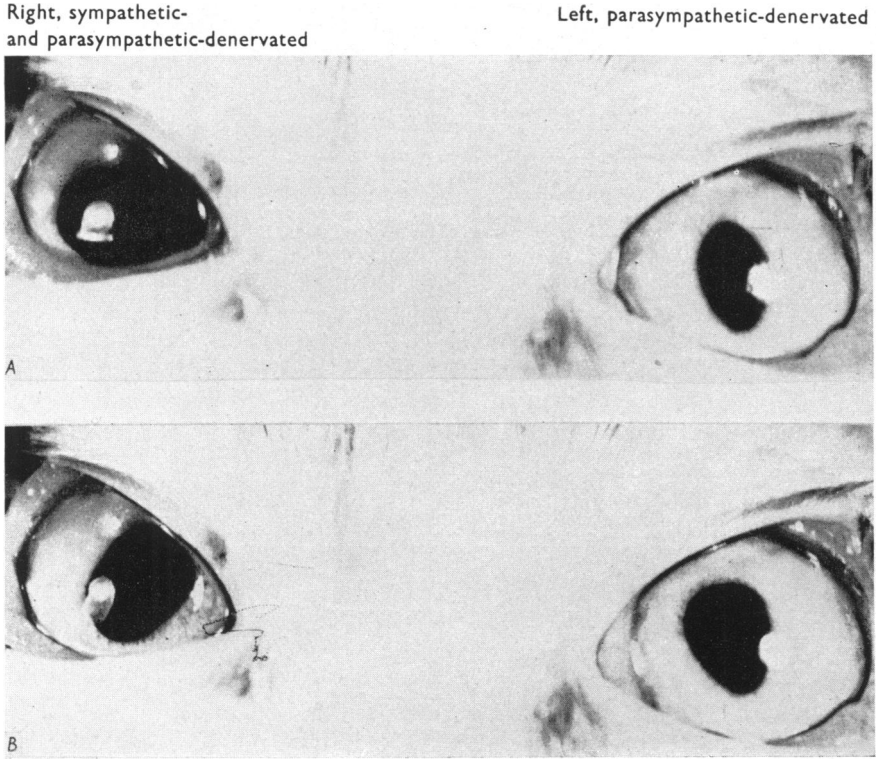
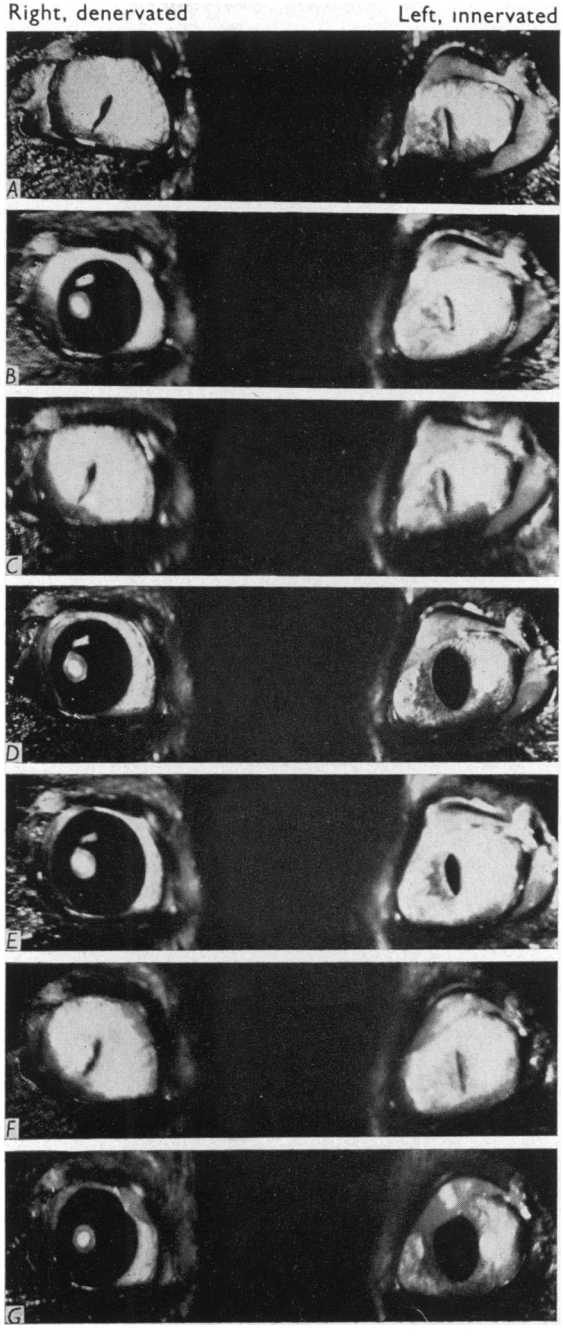
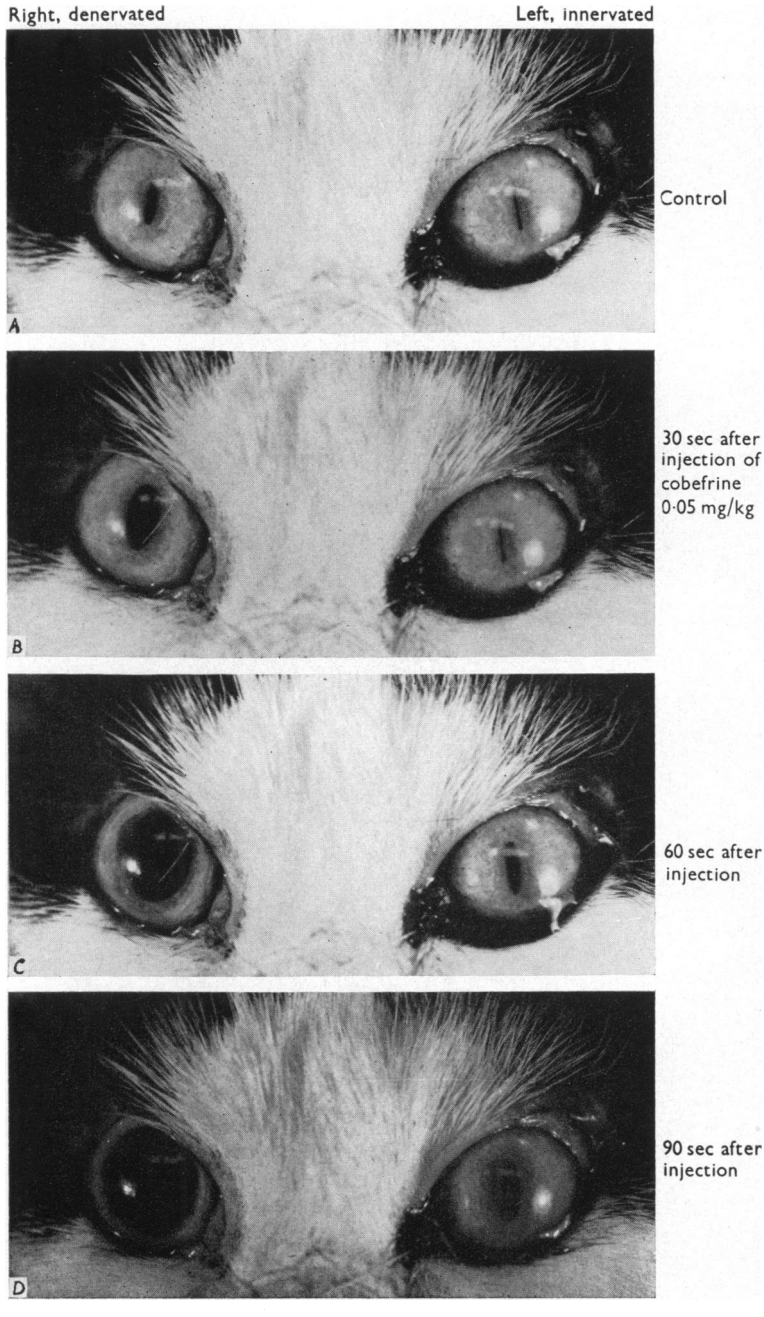

Right, sympathetic-
and parasympathetic-denervated
Left, parasympathetic-denervated
A
B

Fig. 2


Right, denervated
Left, innervated
A
B
C
D
E
F
G


Right, denervated
Left, innervated
Control
A
30 sec after injection of cobefrine 0·05 mg/kg
B
60 sec after injection
C
90 sec after injection
D

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Chloralosed cat, 2·1 kg; acute bilateral adrenalectomy. Right superior cervical and vagal nodose ganglia removed 21 days previously. *A*: Controls. *B*: Dilatation of the innervated, and contraction of the denervated, iris 1 min after β-phenylethylamine 3·0 mg/kg I.V.

Fig. 2. Chloralosed cat, 1·5 kg; acute bilateral adrenalectomy. Right superior cervical and vagal nodose ganglia removed 20 days previously and bilateral removal of the ciliary ganglia 8 days previously. *A*: Controls. *B*: Contraction of the doubly denervated right iris, and dilatation of the parasympathetic-denervated left iris 1 min after tyramine 2·0 mg/kg I.V.

PLATE 2

Chloralosed cat, 3·0 kg; acute bilateral adrenalectomy. Right superior cervical and vagal nodose ganglia removed 12 days previously. No supersensitivity of innervated iris (left) to phenylephrine 2 days after chronic reserpine treatment (2 mg/kg intraperitoneal) but increased sensitivity after cocaine; right, control chronic sympathetic-denervated iris showing usual supersensitivity.

A and *C*: Controls. *B*: Dilatation of denervated, but not of innervated, iris 30 sec after phenylephrine 0·025 mg/kg I.V. *D* and *E*: Greater and more protracted dilatation of denervated iris, 15 and 60 sec, respectively, after phenylephrine 0·075 mg/kg. *F*: Control, 60 min after cocaine 0·2 mg/kg. *G*: Some dilatation now of innervated, but less than of denervated, iris, 30 sec after phenylephrine 0·025 mg/kg (previously ineffective at *B*).

PLATE 3

Earlier dilatation of denervated supersensitive iris. Chloralosed cat, 3·5 kg, pre-treated with reserpine (0·5 mg/kg intraperitoneal for 2 days and 1·0 mg/kg the third day); acute bilateral adrenalectomy. Right superior cervical and vagal nodose ganglia removed 11 days previously. *A*: Controls. *B*, *C* and *D*: 30, 60 and 90 sec, respectively, after cobefrine 0·05 mg/kg I.V.