Brit. J. Pharmacol. (1956), **11**, 1.

A SERIES OF FULLY SUBSTITUTED ETHYLENEDIAMINES

BY

J. D. P. GRAHAM AND R. S. TONKS

From the Department of Pharmacology, Welsh National School of Medicine, Cardiff

(RECEIVED FEBRUARY 18, 1955)

N-dimethylaminoethyl-*N*-benzylaniline (2339 RP or "Antergan") has long been known as a histamine antagonist (Halpern, 1942). It is five to fifteen times more potent than its *N*-ethylaniline analogue (2325 RP).

Nickerson and Gump (1949) demonstrated a more potent antiadrenaline activity in certain compounds after increasing the size of one aryl residue. Accordingly a series of analogues of 2339 RP and 2325 RP was synthesized (Chapman, James, and Williams, 1952) in which the size of one aryl nucleus was increased by replacing the phenyl by a naphthyl group. The ethylenediamine side chain can be introduced in the 1- or 2-positions of this nucleus (see Table I). A brief report of certain activities has been made (Tonks, 1953). The neuromuscular blocking activity of these substances, which resembles that of decamethonium, has been described by Graham and Tonks (1954). We now describe the antihistamine, antiadrenaline, local anaesthetic, and other properties of these compounds. For convenience they are labelled T1 to T8.

METHODS

Comparison was made with 2339 RP and 2325 RP throughout the tests. The naphthyl analogues (T1–8) of the RP compounds are oily bases. They were dissolved in acetic acid (33%) and the volume was adjusted with citric acid/phosphate buffer of *p*H 6.5.

Antihistamine activity was investigated on the response of the blood pressure of atropinized and anaesthetized cats, dogs, and rabbits injected intravenously with histamine (1–4 μg./kg.) before and 5 min. after graded doses of T1–T8. The mode of action was studied by the method of Chen and Russell (1950), together with the course of activity with time. Guinea-pigs of about 300 g. were exposed individually to an aerosol of histamine solution (0.5%) and the time taken to collapse from asphyxia noted. The animals were resuscitated and used in groups of 10 at intervals of 7 days, when graded doses of T1–T8 were injected subcutaneously 1 hr. before exposure. The effect of T1–T8 on the vascular reactions of guinea-pig skin was investigated by the method of Miles and Miles (1952). In addition their effect on smooth muscle (guinea-pig ileum, rat uterus, perfused vessels of rabbit ear) and cardiac muscle (perfused cat and rabbit heart) was examined. Gastric juice was collected from 8 cats under chloralose by cannulation of the pylorus after ligation of the oesophagus and wash out. Histamine (15 μg./0.5 ml./min.) was infused intravenously for 45 min. and the gastric juice collected until flow ceased. One hour later T1 (10 mg./kg.) was injected intravenously and after 15 min. the histamine infusion was repeated. One hour after flow ceased the infusion was repeated for the third time. T1 was omitted in 3 cats. Histaminase was prepared from fresh hog-kidney and its activity on the substrate was tested alone and in the presence of T1 and 2339 RP (2×10^{-3} to 7×10^{-3}) according to Kapeller-Adler (1951). Antiadrenaline activity was examined on the blood pressure responses of atropinized spinal cats, cats anaesthetized with chloralose, rabbits anaesthetized with urethane, and dogs anaesthetized with sodium pentobarbitone or with sodium thiopentone followed by chlorbutol (1 ml. 40% w/v soln. in 70% ethanol/kg. intraperitoneally). The technique of Chen, Nash, and Russell (1950) was applied, and the duration of action of effective doses noted. The effect of T7 (7.5 mg./kg. intraperitoneally) on the hyperglycaemic response to adrenaline (40 μg./kg. subcutaneously) was observed in 6 rabbits. These were separately caged for 24 hr. without food before individual fasting blood-sugar levels were determined by the modified Folin-Malmros method (Landgrebe and Munday, 1954). They were injected subcutaneously at 7 day intervals with 0.5 ml. saline, adrenaline, T7, or T7 followed 30 min. later by adrenaline, and hourly individual blood-sugar levels were estimated for 5 hr. The effect of the compounds on the responses of smooth muscle (gut, pregnant and non-pregnant uterus of cat and rabbit, perfused vessels of rabbit ear) and cardiac muscle (cat and rabbit heart, rabbit auricles) to adrenaline was also tested. Homogenized guinea-pig liver was used as a source of amine oxidase and the action of the compounds on this enzyme was examined manometrically with adrenaline as substrate. Lineweaver and Burk's (1934) method was used to investigate the type of inhibition exerted. The concentrations of adrenaline substrate were 0.025 M to 0.001 M and those of the inhibitors 2×10^{-4}, 10^{-4}, and 2×10^{-5}.

Local Anaesthesia.—The ethylenediamines were compared with procaine, with and without adrenaline, and with cocaine as infiltration anaesthetics by Bülbring and Wajda's method (1945). Duration of surface anaesthetic activity was measured on rabbit corneas (6 rabbits/compound) with the aid of a light von Frey bristle. The solution under test was kept in contact with the cornea for 1 min. The eye was then flushed with saline and touched thereafter four times every 2 min. until the corneal reflex reappeared. The right eyes of 3 rabbits and the left eyes of another 3 rabbits were used for each compound. After recovery, cocaine was tested in the unused eyes. Three concentrations of each compound were tested at intervals of 7 days, the highest used for T1–8 being 0.125 to 0.5% w/v; for cocaine 2% w/v, and for 2339 RP and 2325 RP 10% w/v.

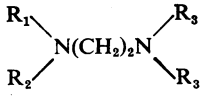
Results

Toxicity

Table I shows the structures of the compounds and the toxicity of 2339 RP, 2325 RP and their naphthyl analogues estimated in mice and rats by Karber's method. The initial effects of a lethal dose of any of the compounds injected intraperitoneally are observed within 5 min. and death occurs within 45 min., whereas it occurs within

TABLE I

THE STRUCTURE AND TOXICITIES (MG./KG.) OF 2339 RP, 2325 RP, AND THE ANALOGOUS SERIES OF NAPHTHYL DERIVATIVES



Code No.	R_1	R_2	R_3	LD50 Mice i.p.	LD50 Mice s.c.	Min. LD i.p. Mice	Min. LD i.p. Rats
2339 RP (antergan)	Benzyl	Phenyl	Methyl	175	400	110	120
2325 RP	Ethyl			500	1150	350	350
T1	Benzyl	1-Naphthyl	Methyl	135	463	75	90
T2			Ethyl	63	300	35	45
T3	Benzyl	2-Naphthyl	Methyl	265	740	180	135
T4			Ethyl	128	459	100	90
T5	Ethyl	1-Naphthyl	Methyl	121	443	80	80
T6			Ethyl	65	306	40	45
T7	Ethyl	2-Naphthyl	Methyl	53	287	32	50
T8			Ethyl	35	172	25	40

12 to 24 hr. after subcutaneous injection. Stimulation of the central nervous system is observed in all animals. Tremor and hyperpnoea may be followed by tail stiffening, clonic convulsions and terminal asphyxia. The stimulant action is most pronounced in the *N*-ethyl analogues; mice surviving the acute toxicity test convulse for 3 to 4 hr. The RP compounds are less active in this respect. The growth of litter-mate groups of rats is unaffected by the compounds in maximal tolerated doses (T1 20 mg./kg., T7 10 mg./kg. subcutaneously, or 40 mg./kg. of either orally) and blood tests after 6–7 weeks' administration reveal no abnormality.

All the compounds when injected intradermally in high concentrations (0.5% (w/v)) produce an area of inflammation which ulcerates in 48 hr. Microscopically the ulcers show a more or less complete destruction of the epidermal architecture with infiltration of the dermis by polymorphs in dogs and by eosinophils in rabbits. The relative potencies of the compounds in producing ulceration in the skin of guinea-pigs at 48 hr. were measured by determining minimal effective concentrations. The 1-naphthyl compounds are less effective than their 2-naphthyl isomerides in the benzyl series, while the converse is true for the ethyl series. The influence of the side-chain position on general toxicity is the reverse of that observed for tissue damage.

Antagonism to Histamine, Adrenaline, etc.

Antihistamine activity is slight in the naphthyl derivatives except for T1, which is the most active member of the series. The results are given in Table II. The antihistamine activity is specific, whereas this is not so with 2339 RP and 2325 RP, in which an atropine-like activity is present. The onset of antihistamine activity is rapid—within 5 min.—and the duration of activity of T1, 10 mg./kg., injected intravenously in dogs is 2.5 hours, whereas T7, 20 mg./kg., is effective for one hour. Application of Gaddum's equation (1943) to the

TABLE II

THE ED50 OF THE ETHYLENEDIAMINES AS INHIBITORS OF (1) THE DEPRESSOR ACTION OF HISTAMINE (2 μG./KG.) IN THE CAT, (2) ASPHYXIATION OF GUINEA-PIGS WITH HISTAMINE AEROSOL, (3) CONSTRICTION OF PERFUSED RABBIT EAR VESSELS BY 0·5 μG. HISTAMINE, AND (4) THE CONTRACTION OF ISOLATED GUINEA-PIG ILEUM TO HISTAMINE 10^{-8}

Compound	(1) Blood Pressure (mg. kg., i.v.)	(2) Bronchi (mg./kg. s.c.)	(3) Ear Vessels (μg.)	(4) Ileum (μg./100 ml.)
N-benzyl series:				
2339 RP	1·4	0·6	0·0125	0·065
T1	3·3	5·6	0·280	3·4
T2	17·0	0*	—	4·0
T3	20·0	0*	—	5·0
T4	24·0	0*	—	16·0
N-ethyl series:				
2325 RP	8·0	9·8	0·16	2·5
T5	11·0	0*	2·0	20·0
T6	13·0	0*	—	24·0
T7	15·0	0*	—	31·0
T8	18·0	0*	—	51·0

* Less than 50% effect after maximal tolerated doses.

determination of equi-effective amounts of histamine in the presence of increasing amounts of T1 indicates that the antagonism is competitive at all doses.

Only compound T1 displays notable activity in protecting guinea-pigs exposed to histamine spray. Maximum tolerated doses of the remaining naphthyl derivatives (16 mg./kg. subcutaneously) prolong the time to asphyxiation by 15–28%, whereas 2339 RP gives a figure of 100%, T1 81% and 2325 RP 64% at this dose.

The results of the assays on isolated guinea-pig ileum are shown in Table II. T1 is 30 times more effective in antagonizing the response to histamine than the response to ACh and barium, while T2–8 are only ten times more effective in this respect. 2339 RP shows atropine-like activity in addition to being a more potent antihistamine. The inhibition of the response of the perfused vessels of the isolated rabbit ear to histamine by T1 and its *N*-ethyl analogue T5 is also shown in Table II.

From the ED50's, 2339 RP is seven times more active than T1 in inhibiting the flare produced in guinea-pigs by intradermal histamine. The volume of gastric juice in cats after intravenous histamine is increased fourfold by a dose of T1 (10 mg./kg.) which completely inhibits the vasodepressor action of histamine. The acidity is approximately the same as for the histamine controls. The stimulant action of histamine on isolated perfused hearts is readily abolished by these compounds; thus the ED50 for this action on the Langendorff preparation is 6 μg. for 2339 RP, 9 μg. T1, 12.5 μg. 2325 RP and 21 μg. T5. As with all antihistamines so far tested the response of the rat uterus to histamine is unaffected.

T1 is a weak inhibitor of histaminase, a concentration of 10^{-3} reducing activity by 40%. 2339 RP is inactive.

Antiadrenaline activity is weak in this series with the exception of T7, of which 2 mg./kg., i.v., reverses the pressor response to 4 μg./kg. adrenaline and 2 μg./kg. noradrenaline in the cat and dog. As a group the compounds may have one or more of three effects on the pressor response to adrenaline and noradrenaline (viz., potentiation, inhibition or reversal). T7 produces potentiation in the spinal cat only, T8 in spinal and anaesthetized cats only and the remaining compounds in the cat, dog, and rabbit. All except T2 inhibit the responses to adrenaline and noradrenaline in the dog, while 2325 RP and T7 also inhibit them in the cat. T7 produces reversal of the pressor responses in the cat and dog and a maximum tolerated dose of T4 (20 mg./kg.) does so in dogs. The potentiating effect is strongest in the compounds which have the weakest inhibitory action. The minimal effective dose of T7 for the reversal of adrenaline is the same in rabbit, cat, and dog (2 mg./kg.). As with the more potent 2-haloalkylamines (Graham and Lewis, 1954) the pressor response to injected adrenaline in the cat and dog is more easily inhibited than that to noradrenaline. Similarly, the contraction of the nictitating membrane of cats after injected adrenaline is more easily inhibited than that caused by injected noradrenaline or stimulation of the cervical sympathetic nerve. Responses to pre- and postganglionic stimulation are identical in the presence of T7. This compound does not exert ganglion blocking action in doses effective at the neuromuscular junction (Graham and Tonks, 1954). The pressor response to occlusion of the carotid arteries is inhibited to a lesser extent, as is the response to stimulation of the splanchnic nerve. The activities of SY28 (haloalkylamine) and T7 are compared in Table III.

TABLE III

ED50 OF SY28 AND T7 (MG./KG.) IN ANTAGONIZING EQUI-PRESSOR RESPONSES TO ADRENALINE AND NORADRENALINE, THE PRESSOR RESPONSE TO CAROTID OCCLUSION, AND THE CONTRACTION OF THE NICTITATING MEMBRANE TO ADRENALINE AND CERVICAL SYMPATHETIC STIMULATION, IN CATS AND DOGS

Preparation	Treatment	SY28	T7
Cat pressor response	Adrenaline	0·04	0·30
	Noradrenaline	0·10	0·90
Cat nictitating membrane	Adrenaline	0·10	0·70
	Stimulation	4·0	—
Dog pressor response	Adrenaline	0 04	0·30
	Noradrenaline	0·10	0·95
	Carotid reflex	0·60	20 0

In the rabbit, cat, and dog the antagonism of the responses to graded equi-pressor amounts of adrenaline and noradrenaline by T7 is competitive at all dose levels. The onset of antagonism with effective doses occurs within 5 min. Potency and duration go together; thus T7, 20 mg./kg., reverses the pressor response to adrenaline and abolishes that to noradrenaline in dogs. The antagonism lasts for 5 hr. and 3.5 hr. respectively. An identical dose of T3 (benzyl analogue of T7) produces a 50–60% inhibition of the pressor response to adrenaline which lasts for 3 hr. This compound displays very little antagonism to noradrenaline.

Compound T7 (7.5 mg./kg.) reduces the hyperglycaemic response of rabbits to subcutaneous injection of adrenaline by 40%. At peak blood sugar levels there was considerable scatter in individual readings, but a "t" test indicated a significant difference ($0.05>P>0.02$).

Non-toxic concentrations of the naphthyl derivatives do not affect the inhibitory action of adrenaline and noradrenaline on rabbit gut or non-pregnant cat uterus. The responses of isolated auricle or perfused heart are not affected. In contrast stimulation of rabbit uterus, pregnant cat uterus and perfused rabbit ear vessels by both amines is inhibited. In the perfused vessels, T7 is ten times more active than T3 in antagonizing adrenaline, but less active against histamine. The ratio of the ED50 as an antihistaminic to that as an antiadrenaline agent is 12.5 for T7 and 0.5 for T3 in this preparation.

All the compounds investigated inhibit the activity of amine oxidase on adrenaline *in vitro*. The ED50 for T2, the most active potentiator of the vasopressor action of adrenaline, is 10^{-4}, and for T7, the most active inhibitor, 2×10^{-4}. The nature of this action was examined according to Lineweaver and Burk (1934). The velocity constant for each of three concentrations of the compound under test (2×10^{-4}, 10^{-4}, 2×10^{-5}) was determined by multiplying the factor 2.303 by the slope of the curve relating log rate of uptake of oxygen to time for several concentrations (0.025M to 0.001M) of the substrate. The relation between the reciprocals of the velocity constants and the reciprocals of the substrate concentrations is linear, and the intercept of the ordinate axis (velocity constant) increases with the concentration of inhibitor, as does the slope. This illustrates the non-competitive nature of the antagonisms.

Local Anaesthesia

The potencies of these substances as infiltration anaesthetics determined in guinea-pigs relative to procaine are given in Table IV. All the naphthyl derivatives are more active than procaine and T1 is more potent than 2339 RP. Adrenaline increases the potency of procaine tenfold and that of T1 twofold. In view of the necrosis produced when these compounds are injected into the skin, the animals were sacrificed immediately after the test and histological examinations made. No oedema or other evidence of damage was detected with the concentrations used (maximum 0.5% w/v). The surface anaesthetic activity of the ethylenediamines was compared with cocaine, using the corneal reflex in the rabbit. The mean and standard error of 60 readings for each concentration of cocaine (with the range and standard errors of individual sets of readings in parentheses) determined in different groups of rabbits was as follows: 0.5% = 13 ± 1.1 (12.0 ± 1.5 to 13.5 ± 3.4), 1.0% = 28 ± 0.98 (21.5 ± 1.8 to 36.7 ± 4.0), 2.0% = 43.2 ± 0.96 (34.8 ± 3.1 to 54.5 ± 1.2). The regression coefficients of the dose-response relations are almost identical for each compound, the response being the duration of anaesthesia. The potency figures are given in Table IV, column 3. The inactivity of 2325 RP compared with 2339 RP has been recorded by Halpern, Perrin and Dews (1947). No conjunctivitis results from the use of these drugs. Mydriatic activity was not detected in compounds T1–8.

TABLE IV

THE POTENCIES OF COCAINE AND PROCAINE, 2339 RP, 2325 RP, AND T1–8 AS LOCAL ANAESTHETICS DETERMINED BY INFILTRATION OF GUINEA-PIG SKIN AND SURFACE ACTIVITY (CORNEAL REFLEX IN RABBITS), AS INHIBITORS OF THE ACTION OF ACh ON FROG RECTUS, INHIBITORS OF THE PERISTALTIC REFLEX IN RABBIT GUT, AND AS ANTIHISTAMINES (CAT BLOOD PRESSURE)

Compound	Potencies				
	Anaesthetic		Anti-ACh	Anti-peristaltic	Anti-hist.
	Infiltr.	Surface			
Procaine ..	1·0	0	1·0	0·2	—
Procaine and adrenaline (10^{-5}) ..	10·0	0	—	—	—
Cocaine ..	6·6	1·0	5·0	1·0	—
2339 RP ..	2·0	0·95	1·25	1·4	1·0
2325 RP ..	0·6	0	0·33	0	0·18
T1	2·6	22	2·5	20	0·4
T1 and adrenaline (10^{-5})	5·2	—	—	—	—
T2	2·1	17	2·4	18	0·08
T3	2·1	19	2·0	18	0·07
T4	2·1	16	1·7	18	0·06
T5	1·2	3·3	1·25	5	0·13
T6	1·5	5·9	1·2	7	0·11
T7	1·4	4·8	1·25	5	0·10
T8	1·5	5·7	1·25	6	0·08

Application of a 5% solution of T1 for 1 min. to a localized area of the tibial nerve in cats anaesthetized with chloralose abolishes reflex contraction of the tibialis anticus muscle when suitable stimuli are applied distal to the block. The effect is apparent for at least 2 hr. after washing off the drug. A 20% solution has a similar effect on conduction in the motor nerve.

The potencies of the compounds relative to cocaine and procaine in abolishing the response of isolated frog rectus muscle to standard doses of ACh are recorded in Table IV, column 4, and potencies in abolishing the peristaltic reflex in isolated segments of rabbit gut (Feldberg and Linn, 1948) in column 5. Cocaine, procaine, and the ethylenediamines were in contact with the cornea, frog rectus, and rabbit gut for 1 min. The antihistamine potencies determined on the blood pressure of cats are given in column 6 for comparison.

The ethylenediamines stimulate the central nervous system at all levels. In low doses (0.0625 of the LD50 administered subcutaneously to mice)

T1 is less potent than leptazole as an analeptic, whereas T5 is twice as active. In higher doses both compounds are toxic. All the compounds in non-toxic doses produce respiratory stimulation in dogs anaesthetized with pentobarbitone sodium.

Discussion

Increasing the size of 2339 RP and 2325 RP by changing the phenyl nucleus to naphthyl increases the toxicity and the local anaesthetic potency. Replacement of dimethyl by diethyl in the sidechain of this series increases toxicity. This relation does not hold in the benzyl derivatives for local anaesthetic activity. Alteration of the sidechain from the 1-naphthyl position to the 2-naphthyl position increases toxicity and local anaesthetic activity in the ethyl series and decreases both in the benzyl series. By increasing the molecular weight of the aryl residue of 2339 and 2325 RP, antihistamine activity is reduced, 2339 RP being the most active of the series. It is apparent from this investigation that benzyl substitution on the aromatic amino group and dimethyl substitution on the aliphatic amino group give the greatest antihistamine activity. In addition the 1-naphthyl position of the sidechain is superior to the 2 position. *N'*-ethyl-*N'*-2-naphthyl-*N*:*N*-dimethyl ethylenediamine (T7) has much the highest antiadrenaline activity.

Muscular paralysis may be a factor in respiratory failure in cats and dogs following toxic doses of these compounds. The potentiation of pressor responses to adrenaline and noradrenaline is inversely related to their efficiency as antagonists. Thus T7, the active antiadrenaline member of the series, produces little potentiation, whereas the converse is true for T2, the least active. Similar instances were observed by Graham and Lewis (1953) in the related field of 2-haloethylamines. Only very high concentrations of T1–8 act directly on cardiac and smooth muscle cells to impair function and sensitivity. The antagonism to both adrenaline and histamine is peripheral and competitive, and, just as exogenous histamine is more readily inhibited than endogenous histamine, so exogenous or injected adrenaline and noradrenaline are more easily antagonized than are the transmitters released by stimulating adrenergic nerves. Both effects develop quickly, but the duration differs markedly. There is no obvious relation between molecular structure and antihistamine and antiadrenaline activity, the strongest antiadrenaline agent being a feeble, but not the weakest, antihistamine, and vice versa.

It has been suggested (Burn, 1950a and b) that a relation exists between antihistamine and local anaesthetic action on the one hand and anti-ACh action and local anaesthesia on the other. The substituted ethylenediamines T1–8, which conform to the formula aromatic residue (lipophilic)—intermediate chain—amino group (hydrophilic) suggested by Löfgren (1948) are active local anaesthetics, but no relation between potency in this respect and antihistamine activity has been found. Although T1 is the most active local anaesthetic its antihistamine potency is less than one-half that of 2339 RP.

The benzyl derivatives as a group are more potent infiltration anaesthetics and produce necrosis more easily than the phenyl compounds, but in half the members of the benzyl series the two properties do not increase together. Concentrations which are only one-eighth the strength required to produce cloudiness of the cornea cause anaesthesia of the surface for one hour. The measure of this activity and of the power to inhibit the peristaltic reflex in segments of gut are almost identical (see Table IV). The latter action is unlikely to be due to necrosis and it is concluded that anaesthesia is not secondary to gross tissue damage. Eerden (1948) suggested, as the result of experiments with 2339 RP, that anaesthesia of the cornea is connected with a reversible coagulation of intracellular proteins. The reversible opacity of the cornea with high concentrations of cocaine and T1–8 indicates that this factor may play a part in their activity. The parallelism between the activity of some infiltration anaesthetics and their power to antagonize the action of ACh on frog rectus muscle was first pointed out by de Elío (1948). It has been suggested that this is a causative relation. Such parallelism is also seen with the ethylenediamines between their potencies as intradermal local anaesthetics and as antagonists of ACh on frog rectus, although they also antagonize the responses to potassium chloride (Graham and Tonks, 1954). There is an appreciable increase in the anaesthetic potencies of the ethylenediamines relative to cocaine on the avascular cornea compared with their potencies intradermally. Cocaine produces vasoconstriction in skin, the ethylenediamines do not. Added adrenaline potentiates the activity of procaine but has less effect on T1–8 presumably because at the concentrations used they are active antagonists of adrenaline.

Summary

1. The properties of 8 compounds related to 2339 RP (Antergan) and 2325 RP have been examined. They are *N'*-benzyl (or ethyl)-*N'*-1-(or

2-) naphthyl-*N*:*N*-dimethyl-(or diethyl)-ethylene-diamines.

2. The LD50's in mice and minimal lethal doses in rats and mice are reported.

3. All the compounds exhibit competitive antihistamine activity which is less than that of 2339 RP. The benzyl-naphthyl-dimethyl derivative is the most active of the series.

4. The ethyl-2-naphthyl-dimethyl derivative is the most active antiadrenaline agent; the action is competitive. Activity is about 15% that of SY28. The effect of noradrenaline and sympathetic activity is less easily inhibited than that of adrenaline.

5. The compounds are more effective than procaine as infiltration anaesthetics and more potent than cocaine as surface anaesthetics.

6. There is no relation between local anaesthetic potency, antihistamine or antiadrenaline activity.

7. They are stimulants of the central nervous system.

This work was done with the aid of a grant from the Medical Research Council. Dr. N. B. Chapman and colleagues provided compounds T1–8.

References

Bülbring, E., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.

Burn, J. H. (1950a). *Brit. med. J.*, **2**, 691.

—— (1950b). *Physiol. Rev.*, **30**, 177.

Chapman, N. B., James, J. W., and Williams, J. F. A. (1952). *J. chem. Soc.*, 4024.

Chen, G., Nash, V. L., and Russell, D. (1950). *Arch. int. Pharmacodyn.*, **84**, 269.

—— and Russell, D. (1950). Ibid., **84**, 176.

Eerden, A. A. J. J. van der (1948). Thesis. Univ. Utrecht.

Elío, F. J. de (1948). *Brit. J. Pharmacol.*, **3**, 108.

Feldberg, W., and Linn, R. C. Y. (1948). *J. Physiol.*, **107**, 10.

Gaddum, J. H. (1943). *Trans. Faraday Soc.*, **39**, 323.

Graham, J. D. P., and Lewis, G. P. (1953). *Brit. J. Pharmacol.*, **8**, 54.

—— —— (1954). Ibid., **9**, 68.

—— and Tonks, R. S. (1954). Ibid., **9**, 379.

Halpern, B. N. (1942). *Arch. int. Pharmacodyn.*, **68**, 339.

—— Perrin, G., and Dews, P. B. (1947). *C.R. Soc. Biol., Paris*, **141**, 1125.

Kapeller-Adler, R. (1951). *Biochem. J.*, **48**, 99.

Landgrebe, F. W., and Munday, K. A. (1954). *Quart. J. exp. Physiol.*, **39**, 17.

Lineweaver, H., and Burk, D. (1934). *J. Amer. chem. Soc.*, **56**, 658.

Löfgren, N., (1948). Studies on local anaesthetics—Xylocaine. Stockholm: Haeggströms.

Miles, A. A., and Miles, E. M. (1952). *J. Physiol.*, **118**, 228.

Nickerson, M., and Gump, W. L. (1949). *J. Pharmacol.*, **97**, 25.

Tonks, R. S. (1953). *J. Physiol.*, **119**, 25P.