Brit. J. Pharmacol. (1947), **2**, 207

SOME ACTIONS OF β-HYDROXY-αβ-DIPHENYLETHYLAMINE

BY

C. B. B. DOWNMAN

From the Sherrington School of Physiology, St. Thomas's Hospital, London, S.E.1

(Received March 12, 1947)

Morphine being a pharmacologically active derivative of phenanthrene Dodds, Lawson, and Williams (1944a) sought a morphine analogue on the same lines as their discovery of stilboestrol—an intensely active analogue of another phenanthrene derivative, oestradiol. They found that diphenylethylamine and seventeen related compounds possessed in differing degrees some of the properties of morphine ; these were depression of righting reflex in rats, elevation of blood sugar in rabbits, and hyperexcitability, pupil dilatation, and vomiting in cats. Some compounds of this series also possessed some analgesic properties when tested clinically. Of these the most promising was diphenylethanolamine or β-hydroxy-αβ-diphenylethylamine, which may be considered to have the same structural relation to morphine as stilboestrol has to oestradiol. Later (Dodds *et al.*, 1944b) it was shown to be effective only in cases where the pain was associated with pressure on nerves. The possible therapeutic uses of this substance, called M4 in their series, justified an investigation of its general pharmacological actions.

Tiffeneau, Levy, and Boyer (1928) found that diphenylethanolamine caused weakening of the beat and slowing of the isolated snail and frog heart, and also of the exposed heart of chloralosed dogs. The substance also caused relaxation of isolated intestine, transient fall of arterial pressure in chloralosed dogs, but gave a vasoconstriction of the perfused isolated frog leg. Hasama (1930) showed that the fall of blood pressure induced by the substance in urethanized rabbits was still obtained after vagotomy or atropine. The vessels of the isolated rabbit ear were dilated by diphenylethanolamine, even in the presence of atropine ; since the dilator action could antagonize barium chloride vasoconstriction Hasama suggested that the activities of the substance were the result of a direct toxic action on smooth muscle. Tainter (1933) and Dodds,

Lawson, and Williams (1944a) also noted the depressor action and the latter authors commented on the dilatation of the pupil and general hyperexcitability of unanaesthetized cats following intramuscular injection of the substance.

In this paper is presented confirmation and extension of the previous work with an analysis of the site of action of the substance. The actions of β-hydroxy-αβ-diphenylethylamine hydrochloride, which will be referred to as M4, were tested on the following mammalian tissues: (*a*) small intestine, (*b*) cardiovascular system, (*c*) the pupil of the eye.

METHODS

For studies on isolated intestines and heart, rabbits were killed by stunning. Pieces of small intestine were suspended in a bath of oxygenated Tyrode solution (formula in Bayliss, 1924) at 37–38° C. and *p*H 7.4, and the contractions of the longitudinal muscles recorded. The coronary arteries of the heart were perfused with oxygenated Ringer-Locke solution (formula in Bain, 1938) at 37–38° C. and *p*H 7.4, through a cannula in the aorta; movements of the right ventricle were recorded, the heart being steadied by pinning the apex. For perfusion of the hind quarters, rabbits and cats were used; the former were killed by stunning, the latter had been anaesthetized with chloralose (60–80 mg./kg. intravenously) for other experiments. A cannula was inserted in the lower aorta and the legs were perfused with Ringer-Locke solution at 38° C. and *p*H 7.4 by a Dale-Schuster perfusion pump, the inflow pressure being recorded with a mercury manometer.

Blood pressure changes were recorded in cats anaesthetized with chloralose (60 mg./kg. intravenously in preliminary ether anaesthesia) or with nembutal (0.5 c.c. nembutal solution (Abbott)/kg. intraperitoneally). Carotid arterial pressure was recorded by a mercury manometer. To assess the action of M4 on the vessels of different tissues the volumes of a hind paw, of the opposite skinned hind leg, and of a 2-in. length of small intestine were recorded optically on photosensitive paper (Downman, Goggio, McSwiney, and Young, 1943); in these experiments the arterial pressure was also recorded optically on the same paper. The organs were enclosed in plethysmographs, the leg plethysmograph enclosing the skinned leg from upper thigh to ankle, but the skin of the same paw and its venous drainage were left intact outside the plethysmograph.

For experiments on the pupils of cats, slit-like pupils were produced in two ways. First, the animals were anaesthetized with chloralose (80 mg./kg. intravenously) given in preliminary ether anaesthesia (McDowall, 1925; Bain, Irving, and McSwiney, 1935). Secondly, under ether anaesthesia cats were decerebrated through a trephine opening in the cranium, the plane of section curving downwards and forwards from the upper edge of the inferior colliculi to the sphenoid eminence, leaving the hypothalamus and adjoining structures intact. The carotid arteries were temporarily occluded by clips and the vertebral arteries by digital pressure, but they were released as soon as possible. As the animal excreted its ether the pupils closed down to slits. If restoration of blood flow to the brain stem was delayed too long the pupils might not constrict. The occulomotor nerve could be exposed by tearing the dural covering over it on its way to the orbit.

Stock solutions of M4 were made by dissolving the hydrochloride in distilled water, without heat, to give 2 or 5 per cent (w/v) solutions. Dilutions were made from the stock solution into the appropriate physiological salt solution. It should be noted that solutions of M4 are acid, and simple neutralization throws the base out of solution. All doses are in terms of the hydrochloride. Injections into the cat were made into the superficial vein of the right foreleg.

RESULTS

(a) Small intestine

Isolated small intestine of rabbit was relaxed by M4 and the rhythmic movements diminished in amplitude. The lowest concentration found effective was 1 in 20,000. The loss of tone was rapid at first, but was followed by prompt recovery of tone and activity on washing (Fig. 1). M4 was active on the atropinized intestine and antagonized the spasm produced by acetylcholine, eserine, prostigmine, and barium chloride. Whereas M4 relaxed the gut, similar concentrations of morphine sulphate produced a slow increase of tonus.

FIG. 1.—Action of hydroxydiphenylethylamine upon longitudinal movements of rabbit duodenum suspended in Tyrode solution. M4= addition of 2 per cent solution to give a final solution of 1:5,000. W= wash. Time signal = 30 sec.

Aqueous solutions of M4 being acid—e.g., *p*H 5.3 for 2 per cent (w/v) solution—it might be argued that the spasmolytic action represents no more than the action of an acid solution. Indeed there was a noticeable fall of *p*H, shown by adding phenol red to the Tyrode solution, after addition of an active quantity of M4. Advantage was taken of the buffering power of plasma. The *p*H of heparinized rabbit plasma was 7.40. After the addition of 1 volume of 2 per cent (w/v) aqueous solution of M4 to 4 volumes of plasma the *p*H of the mixture, measured with a glass electrode *p*H meter, was the same as that of the original plasma. The buffered M4 produced the same changes of intestinal activity as unbuffered M4, when each was added to the Tyrode bathing the intestine to the same final concentration of M4.

(b) Cardiovascular system

Isolated rabbit heart.—0.1 to 0.2 c.c. of 2 per cent (w/v) solution of M4 injected quickly into the perfusion cannula produced a sharp decrease of amplitude, with not more than 15 per cent slowing of the beat. The heart recovered steadily in the next 5 minutes. Similar results were recorded when the same doses of M4 were diluted with 0.8 c.c. rabbit plasma before injection, whereas plasma alone usually produced a slight increase in the amplitude of beat.

Faradizing a vagus nerve supplying the isolated heart caused slowing and weakening of the beat. At the height of the M4 action vagal stimulation produced no or but slight slowing of the beat. It was noticed also that the amplitude of the beat might be a little increased during the stimulation

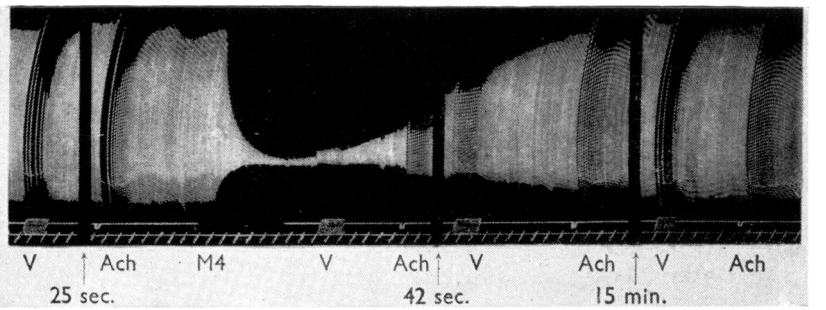

FIG. 2.—Action of hydroxy-diphenylethylamine on the isolated perfused rabbit heart. Tracing shows movements of right ventricle. M4 = injection of 2 mg. hydroxy-diphenylethylamine HCl into aortic perfusion cannula, buffered by mixing 1 vol. 2 per cent solution of M4 with 4 vol. rabbit plasma. Ach = injection of 2 μg. acetylcholine in 0.2 c.c. Ringer-Locke solution. V = faradic stimulation of vagus nerve on the oesophagus. Time tracing = 5 sec.

(Fig. 2). The vagal inhibitory action returned as the heart itself recovered from the influence of the M4, but the full return of vagal response was not seen until some 5 minutes after the amplitude of the heart beat had fully recovered. The response to acetylcholine—e.g., 1 μg. in 0.1 c.c. Ringer Locke—was also reduced, and vagal and acetylcholine action was recovered at about the same speed. M4 action on the heart was not influenced by prior atropinization. Following a depressant dose of M4 the action of adrenaline was reduced but not abolished.

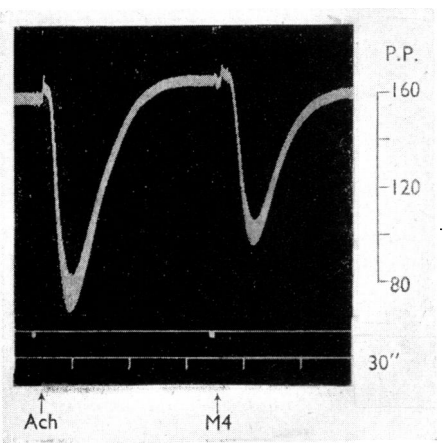

FIG. 3.—Vasodilator action of hydroxy-diphenylethylamine and of acetylcholine compared in cat hindquarters perfused Ringer-Locke solution, *p*H 7.5, containing 1 : 500,000 adrenaline. Ach = 3 μg. acetylcholine. M4 = 2 mg. hydroxy-diphenylethylamine HCl. Time signal = 30 sec. Perfusion pressure (P.P.) in mm. Hg.

Perfused hind limb.—In order to demonstrate the action of M4 or any vasodilator drug the tone of the vessels was raised by adding adrenaline to the perfusion fluid in a concentration of 2×10^{-6} to 3×10^{-7}. M4 now produced a transient dilatation of the limb vessels, shown by a fall of the perfusion pressure head (Fig. 3). This dilator response, which was mimicked by 0.05 to 0.1 c.c. $N/10$ hydrochloric acid, cannot be attributed solely to the injection of an acid solution. It was obtained when the M4 was buffered adequately with plasma, as described above, although plasma injected alone produced a small rise of perfusion pressure.

The dilator action of M4 was seen when the limb vessels were constricted not only by adrenaline but also by posterior pituitary extract or barium chloride.

Vascular responses in anaesthetized cats.—Intravenous injection of M4 dissolved in 0.9 per cent sodium chloride solution produced a temporary fall of general arterial pressure (Fig. 4). With small doses arterial pressure recovered

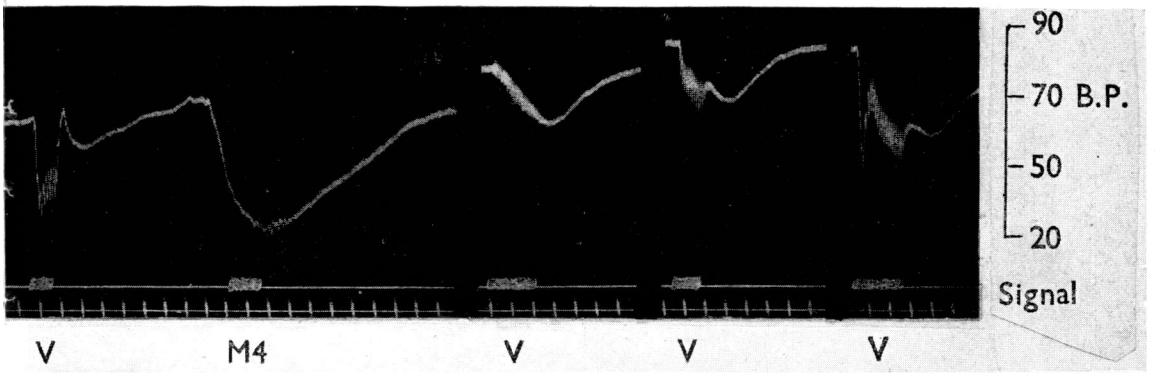

FIG. 4.—Depressor action of hydroxy-diphenylethylamine, 20 mg. i.v., upon carotid arterial pressure of chloralosed cat. (Signal for injection is 10 sec. late.) V=faradic stimulation of peripheral end of cut right vagus nerve in neck. Time tracing=10 sec. B.P. calibration in mm. Hg.

quickly and was usually followed by a small but prolonged hypertension. In chloralosed cats 10 mg. M4/kg. produced about a 40-mm. Hg fall of blood pressure with recovery in two minutes, while 50 mg./kg. led to a 75-mm. Hg fall of pressure with cardiac irregularity. Doses over 50 mg./kg. caused cessation of breathing for periods up to 25 minutes.

Accompanying the fall of arterial pressure there was a rapid decrease of volume of the paw, skinned limb, and intestine. At the same time the pulsations in these organs decreased in amplitude. As the arterial pressure recovered the organ volume and the amplitude of the pulsations returned. Similar changes were seen after bilateral vagotomy in the neck and inactivation of both carotid sinuses by tying the arterial trunks close to the sinus where they entered and left the structure. The shrinkage of the peripheral organs started at the same time in each of them and did not commence until the central arterial pressure had already fallen a little (Fig. 5).

The loss of vagus action in the presence of M4 could be shown in the intact animal by comparing the depressor response to faradizing the peripheral end of the cut right vagus nerve in the neck before and after injection of a dose of M4 which produced a prolonged action. The fall of pressure became smaller and the vagal inhibition of heart rate was much reduced; as in the isolated

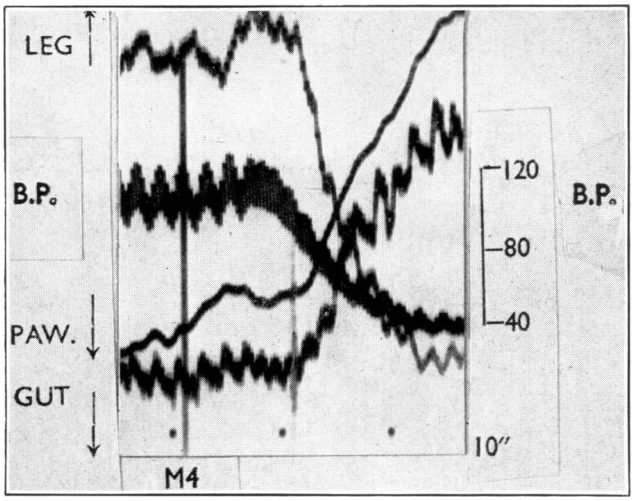

FIG. 5.—Optical record of carotid arterial pressure, with volumes of left hind paw, skinned right hind leg, and segment of jejunum. Cat, nembutal i.p. At signal 30 mg. hydroxy-diphenylethylamine i.v. Time marking = 10 sec. B.P. calibration in mm. Hg. Arrows show direction of vasodilatation.

heart, as blood pressure recovered so the vagus responses recovered, but the latter was not complete until some 1 to 5 minutes after the end of the M4 depressor response (Fig. 4).

(c) Action on the pupil

In the chloralosed or decerebrated cat with slit-like pupils intravenous injection of M4, in doses of 12 mg./kg. or more, produced a rapid wide dilatation of the pupil with slow recovery. The whole effect lasted 10 to 30 minutes, and was not altered by cutting both cervical sympathetic chains in the neck. In cats with one oculomotor nerve severed it was possible to follow the dilator action of M4 upon the contralateral normal eye, and at the same time to test the ability of the oculomotor nerve to constrict the pupil by faradizing the peripheral end of the cut nerve. With the nerve severed the pupil was widely dilated, but constricted to a slit when the nerve was stimulated. At the height of the M4 action, as judged by the dilatation produced in the normally innervated eye, the constrictor effect of oculomotor stimulation was quite absent. As the pupil dilator action of M4 receded, so the ability of oculomotor nerve impulses to produce pupil constriction returned. Even when oculomotor action on the pupil was paralysed, stimulating the nerve still produced the usual rotation of the eyeball and enophthalmos. Stimulating the peripheral cut end of the cervical sympathetic chain in the neck produced a further slight dilatation

of the pupil when the latter was apparently fully dilated by M4, as well as movement of the nictitating membrane.

M4 could antagonize the action of eserine. This was shown by cutting the oculomotor nerve on one side and then constricting the paralysed pupil by instilling into the conjunctival sac 0.65 mg. eserine sulphate (1 Burroughs Wellcome "Tabloid"). Intravenous injection of a dose of M4, which produced full dilatation of the normal pupil, produced one-third dilatation of the eserinized pupil.

Stereoisomers of hydroxy-diphenylethylamine

There being two assymmetric carbon atoms in the molecule two optically inactive stereoisomers are possible, the normal form (M4 itself) and an *iso* form. Each of these can be resolved into two optically active enantiomorphs. A comparison of the activities of these various isomers was attempted, using rabbit jejunum as the test object. More regular responses were produced if the gut was brought into high tone by suspending it in Tyrode solution to which eserine sulphate was added in a concentration of 1 : 4 million. The isomers were added to the gut bath as 2 per cent (w/v) solutions in water in an amount producing about half the maximum relaxation of the gut. The gut was exposed to each isomer for 5 minutes, then washed twice with Tyrode solution in the next 10 minutes. Even with this long exposure some pieces of gut did not reach their final length as the relaxation was rapid for the first two or three minutes and then proceeded very slowly.

Comparing the relaxation produced by similar concentrations of the isomers, 1 : 15,000, the activities of the isomers could be listed as *l-iso*>*d*-normal>*dl-iso* >*dl*-normal>*d-iso*. The *l*-normal form was not available. Equal relaxation was produced by 1 : 20,000 of the *l-iso* and 1 : 12,000 of the *d-iso*. It is difficult to assess the value of these results. Although there is a consistent difference between the *l-iso* and *d-iso* form, the activities of the first four isomers listed are very similar. Comparison of the vasodepressor action in the chloralosed or spinal cat was less successful. The responses to similar doses of the isomers were not always consistent, and might change during the experiment, but the results did not suggest any such difference of activity as shown by the gut.

Discussion

Although hydroxy-diphenylethylamine (M4) apparently resembles morphine in having analgesic action, Dodds, Lawson, and Williams (1944) showed that these two substances probably act in different ways. Similarly, the previous results show that the general activities are also different. M4 is in general a depressor of smooth muscle action. Also it has an action which may for the moment be called "atropine-like."

It has been shown that the drug relaxes intestinal muscle and reduces the amplitude of the rhythmic contractions. This action is reversible and can be exerted against substances, such as barium chloride, which raise the tonus of the muscle. A similar spasmolytic action is produced in the blood vessels. These actions might all be explained by a general toxic action which directly reduces the power of the muscle to contract. Such a depressant action is seen in the heart, where M4 reduces considerably the amplitude but not the frequency of the beat. That this effect is not due to a parasympathomimetic action of the drug is shown by its occurrence in the atropinized heart. These findings agree with the reports of previous workers.

In view of the depressor action of M4 it could be argued that the fall of arterial pressure is a consequence of a peripheral vasodilatation, especially as M4 does have such an action in the isolated perfused limb. The simultaneous recording of paw, skinned limb, and intestine volumes shows however that in the whole animal the fall of arterial pressure is accompanied by a shrinkage of these organs. Since M4 is an active vasodilator in the isolated perfused limb, with maintained inflow, it seems that in the whole animal there is produced a passive vasoconstriction consequent upon the fall of arterial pressure. This is confirmed by the fall of pressure starting demonstrably earlier than the volume changes of skin, skeletal muscle, or intestine. The initial rapid fall of blood pressure on injecting M4 seems, therefore, to be due to a reduction of heart output because of the toxic action of the drug on the heart muscle. This toxic action is well shown on the isolated heart preparation.

The dilator action on the pupil in the unanaesthetized cat might be due to some central action in the brain stem or to a peripheral paralysis of the iris. That the neuromuscular mechanism of the iris is influenced directly is shown by the ineffectiveness of the oculomotor nerve stimulation in the presence of a dilator concentration of M4. It is probable, therefore, that a peripheral action would explain the pupil dilatation observed by Dodds and his colleagues. It is to be noted in the paper by Dodds, Lawson, and Williams that a dose of M4 which produced general hyperexcitability (20 mg./kg. intramuscularly) was less than the pupil-dilating dose (50 mg./kg.). A general toxic action on the whole neuromuscular mechanism does not seem to be an adequate explanation of the oculomotor paralysis. The dilator action of the sympathetic fibres was still present, arguing against an inability of the muscle fibres of the iris to react to nerve impulses; further, conduction in the oculomotor nerve was not impaired because it could still carry nerve impulses to the extraocular muscles, producing movement of the eyeball. The action of M4 on the pupil seems to be of an atropine-like nature, blocking conduction at the parasympathetic terminals. This action is, however, of short duration and is produced only in concentrations which have direct toxic actions on other smooth muscle.

The ability of M4 to block the action of parasympathetic nerve impulses

is seen also in its effect upon the vagal control of heart rate. This action has been observed only after doses of M4 sufficient to produce severe weakening of the beat, but does persist for a few minutes after the visible signs of this depression have worn off. It will be recalled that certain barbiturates produce a similar transient blocking of parasympathetic impulses and there is some evidence that the site of the block may be either in the ganglion or at the neuromuscular junction. Thus Koppanyi, Linegar, and Dille (1935) showed that some barbiturates, other than thiobarbiturates, produced a transient loss of vagal action and the evidence suggests that the site of action of the drug is mainly at the vagus ganglion; Stravraky (1931) showed that amytal paralyses the submaxillary glands to chorda tympani stimulation, and since the acetylcholine response is also paralysed it appears that the drug acts on the nerve terminals or the secretory cells. Garry (1930) also has shown that amytal temporarily abolishes the action of the vagus nerve on the heart in cats and rabbits, the effect passing off much more rapidly in the rabbits; the action of acetylcholine on the frog heart was unchanged, but vagal activity was not tested at the same time in this animal. In the heart both vagal and acetylcholine activities are paralysed by M4 to the same degree, but whether this should be compared with the action of atropine is debatable, since M4 is so toxic to the heart muscle. It is possible that the effect represents no more than a general toxic action which takes a little more time to recede from the more fragile place where acetylcholine acts. The observations on the pupil, however, suggest that toxic and "atropine-like" action may be separate entities. It is curious that although M4 reduces the force of the heart beat very greatly it has relatively little action on the rate of firing of the pacemaker.

Although many of the actions of M4 may be due to non-specific depression of the tissues, there seems to be stimulation of some parts of the central nervous system. General bodily activity is increased (Dodds *et alia*, 1944), an action shown by diphenylethylamine (Tainter, Ludvena, Lackey, and Neuru, 1942); some related compounds will even cause convulsions in cats. Some of the diphenylethylamine compounds also cause a rise of blood sugar (Dodds *et alia*, 1944), but the ability to cause hyperexcitability and raise the blood sugar are dissociated in M4, this substance producing hyperexcitability without hyperglycaemia. Clearly it would be of interest to know more of the cause of the hyperglycaemia provoked by other diphenylethylamine derivatives.

Summary

A morphine analogue, β-hydroxy-αβ-diphenylethylamine, has the following actions:

1. It relaxes isolated intestine, even in the presence of acetylcholine, eserine, prostigmine, or barium chloride.

2. The contraction of the isolated heart is reduced in amplitude, this action being unaffected by previous atropinization. Loss of response to vagal impulses and to acetylcholine is also produced.

3. It causes a fall of arterial pressure, with peripheral vasoconstriction. The latter is a passive effect. In the isolated perfused limb active dilatation is produced.

4. It produces pupil dilatation in chloralosed and decerebrated cats by an action on the oculomotor nerve terminals in the iris.

5. These results might be explained by a direct toxic action of the drug on the active tissue.

This investigation was undertaken after a suggestion by Prof. E. C. Dodds, M.V.O., F.R.S., whom I have to thank for original supplies of M4 and its isomers. The M4 was made by Boots Pure Drug Co., Ltd., to whom thanks are due. During the work much valuable assistance was given by Miss J. G. Emmett, of Boots Pure Drug Co., Ltd., working in this laboratory.

REFERENCES

Bain, W. A. (1938). *Schafers Experimental Physiology*, 6th edition. London: Longmans Green & Co.
Bain, W. A., Irving, J. T., and McSwiney, B. A. (1935). *J. Physiol.*, **84**, 323.
Bayliss, W. M. (1924). *Principles of General Physiology*. London: Longmans Green & Co.
Dodds, E. C., Lawson, W., and Williams, P. C. (1944a). *Proc. roy. Soc.*, **132B**, 119.
Dodds, E. C., Lawson, W., and Williams, P. C. (1944b). *Nature, Lond.*, **154**, 514.
Downman, C. B. B., Goggio, A. F., McSwiney, B. A., and Young, M. H. C. (1943). *J. Physiol.*, **102**, 216.
Garry, R. C. (1930). *J. Pharmacol.*, **39**, 129.
Hasama, B. (1930). *Arch. exp. Path. Pharmak.*, **153**, 161.
Koppanyi, T., Linegar, C. R., and Dille, J. M. (1935). *Science*, **82**, 232.
McDowall, R. J. S. (1925). *Quart. J. exp. Physiol.*, **15**, 177.
Stavraky, G. W. (1931). *J. Pharmacol.*, **43**, 499.
Tainter, M. L. (1933). *Arch. int. Pharmacodyn.*, **46**, 192.
Tainter, M. L., Ludvena, F. P., Lackey, R. W., and Neuru, E. N. (1942). *J. Pharmacol.*, **77**, 317.
Tiffeneau, M., Levy, J., and Boyer, P. (1928). *Paris méd.*, **67**, 553.