J. Pharm. Pharmacol., 1964, **16**, 779–787 Received September 21, 1964

Eserine and autonomic nervous control of guinea-pig vas deferens

D. DELLA BELLA, G. BENELLI AND A. GANDINI

In the presence of eserine, the isolated preparation of guinea-pig vas deferens responds to electrical stimulation of the hypogastric nerve with enhanced contractions, which are no longer antagonised by adrenergic blocking agents but are strongly inhibited by atropine. Similarly, in preparations from reserpine-pretreated animals which have become unresponsive to the hypogastric stimulation, eserine elicits full-size responses which are almost completely abolished by atropine and not affected by adrenergic blocking drugs. Direct stimulation with acetylcholine contracts the vas deferens both of normal and reserpinised animals: the responses are antagonised by atropine and enhanced by eserine. It is concluded that there are grounds for inferring that eserine acts not by enhancing an adrenergic mechanism, but by uncovering a parasympathetic cholinergic component in the autonomic nervous control of the preparation.

IN agreement with previous observations (Boyd, Chang & Rand, 1960), Burn & Weetman (1963) advanced the hypothesis that the enhancing effect of eserine on the responses of the guinea-pig vas deferens to electrical stimulation of the hypogastric nerve is due to a reinforcement of a non-synaptic action of acetylcholine promoting a greater release of adrenergic transmitter.

In line with this assumption we made experiments to establish whether an analogous effect was exerted by eserine *in vivo* at the level of the peripheral sympathetic structures and which could thus account for its hypertensive action in the urethanised rat (Della Bella, Gandini & Preti, 1964). Whilst doing this work, some observations made on the isolated vas deferens aroused our interest and seemed worthwhile investigating more thoroughly so that the mechanism whereby eserine potentiates the responses of the preparation to electrical stimulation might be better understood, and additional information about the autonomic nervous control of the vas deferens could be collected.

Methods and materials

HYPOGASTRIC NERVE—VAS DEFERENS PREPARATION

Guinea-pigs weighing 400–500 g were used. The preparation was set up according to Huković (1961) in a 100 ml organ bath, containing Krebs solution gassed with 5% carbon dioxide and 95% oxygen, at 32°. The hypogastric nerve, about 3 cm, was placed on shielded platinum electrodes submerged in the bath, 2 cm from the vas deferens and connected to an electronic stimulator. Rectangular pulses, 200 of 1 msec duration, were applied at 2 min intervals, at the alternate frequency of 10 and 50 shocks/sec, from a constant voltage source at 2–3 V. Contractions were recorded by an isotonic writing lever (load 2 g) with a magnification of four times.

From the Department of Pharmacological Research, ZAMBON S.p.A., Bresso-Milan, Italy.

For the experiments using direct chemical stimulation, the vas deferens was removed from the body without the hypogastric nerve, and the mesenteric investment was carefully stripped away so as to give a more sensitive preparation (Bentley & Sabine, 1963). In this work a 20-ml organ bath was used. Some preparations were made from reserpine-pretreated animals, which had received reserpine, 0·5 and 1 mg/kg intraperitoneally, for the 2 days previously as described by Huković (1961).

The following drugs were used: acetylcholine chloride, carbachol, acetyl-β-methylcholine chloride, noradrenaline, atropine sulphate, dihydroergotamine methansulphonate, dibenamine chloride, phenoxybenzamine chloride, phentolamine methansulphonate, veratrine, hexamethonium bromide, propantheline bromide, diphemanil methylsulphate. With the exception of noradrenaline and veratrine the concentrations in the text refer to the salts.

Results

INTERACTION BETWEEN ESERINE AND ATROPINE

In agreement with Boyd & others (1960) and Burn & Weetman (1963), we found that the addition of eserine to the bath at doses ranging from

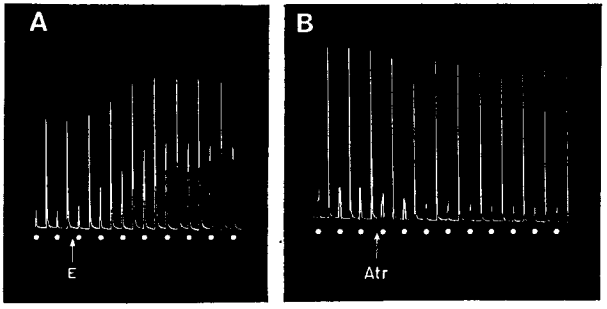

FIG. 1. Contractions of guinea-pig isolated vas deferens in response to hypogastric nerve electrical stimulation. Each stimulation consisted of 200 shocks, applied alternately at the frequency of 50 and 10 shocks/sec (at dots), every 2 min. A. Addition of eserine to the perfusion bath (at E, 2·5 μg/ml) evokes an immediate and progressive increase in the responses of the preparation, more evident at the low frequency stimulation. B. The responses appear scarcely affected by atropine (at Atr, 1 μg/ml): a slight reduction, more marked for the contractions at the low rate of stimulation, is observable.

0·5 to 5 μg/ml significantly enhanced the responses of the vas deferens to the electrical stimulation of the hypogastric nerve in 11 out of 14 preparations (Fig. 1A). Potentiation was most consistently seen with the 5 μg/ml dose and was more pronounced for responses to 10 than to 50 shocks/sec. Doses of eserine of 0·5–2 μg/ml elicited fewer potentiated responses. In contrast to the observations by Burn & Weetman (1963), only in two of the eleven experiments did we note a progressive decline of the contractions at high frequency.

The results obtained with atropine agreed with the previous findings: 0·05 to 2 μg/ml concentrations only slightly reduced the responses of the electrically-stimulated preparation, the inhibition concerning mainly the contractions at the low rate of stimulation (Fig. 1B).

When eserine was added to the bath in high doses, the presence of atropine slightly inhibited, but did not abolish, the typical potentiation

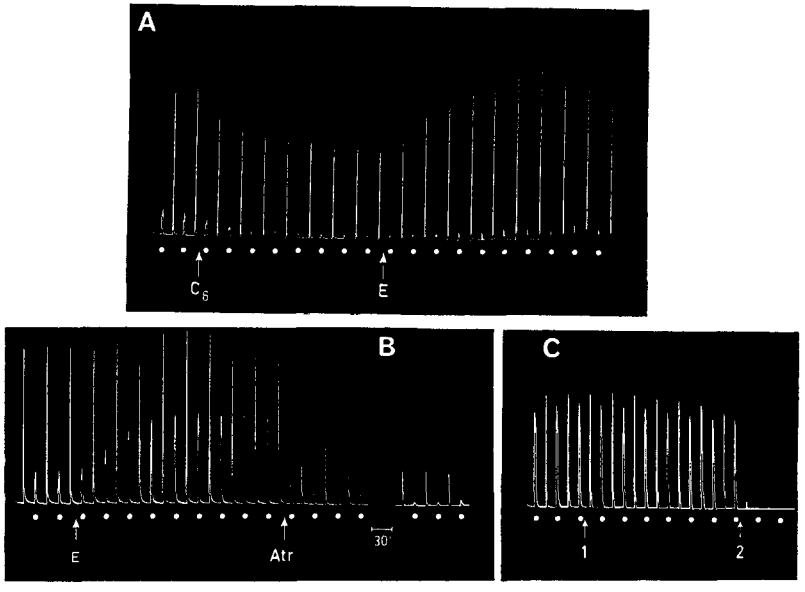

FIG. 2. Preparations and parameters as in Fig. 1. A. At C_6, hexamethonium, 10 μg/ml. At E, eserine, 3 μg/ml. Addition of eserine to a preparation partially blocked by hexamethonium does not exert the typical enhancing effect at the low frequency stimulation. The potentiation by eserine develops as usual for the responses at high frequency. B. At E, eserine, 5 μg/ml. At Atr, atropine, 0·25 μg/ml. Unlike the experiment in Fig. 1B, a small dose of atropine, given after the enhancing effect of eserine has developed, causes an immediate strong reduction of the responses. The inhibitory effect becomes progressively more intense, but even 30 min later the responses are not abolished. C. Eserine has been added to the bath at the concentration of 5 μg/ml 20 min previously. At 1, phentolamine, 0·5 μg/ml. At 2, veratrine, 1 μg/ml. Phentolamine leaves the height of contractions unimpaired while veratrine almost immediately abolishes the responses.

induced by eserine. On the contrary, doses as low as 0·5–2·5 μg/ml in the presence of atropine occasionally failed to exert any potentiating effect; at this dose level we obtained contrasting results from different experiments and enhancement, when present, varied from one preparation to another.

When the preparation was pretreated with hexamethonium, the eserine-potentiating effect appeared only at the higher frequency of stimulation (Fig. 2A).

When atropine was added to the bath after the enhancing effect of eserine had developed, different results were obtained: concentrations of

atropine as low as 0·05–0·5 μg/ml strongly reduced the potentiated responses of the preparation in all instances (Fig. 2B). Atropine mainly reduced the responses to the low-frequency stimulation. These were almost completely abolished; the high frequency responses declined to 10–25% of their previous height. Further addition of atropine, even up to 1–2 μg/ml did not abolish them.

Hexamethonium (10 μg/ml), given after the potentiating effect of eserine had developed, was responsible for a progressive reduction of the responses ranging between 20 and 50%; a partial block, more pronounced than that by atropine, was obtained with 0·2–0·5 μg/ml of diphemanil and propantheline, which are parasympatholytic drugs endowed with both atropine-like and ganglion blocking properties (Margolin, Doyle, Giblin, Makovsky, Spoerlein, Stephens, Berchtold, Belloff & Tislow, 1951; Johnson & Wood, 1954). Similarly, as already reported for normal preparations (Della Bella & Benelli, 1964), 1–2 μg/ml veratrine abolished the responses potentiated by eserine (Fig. 2C).

INTERACTION BETWEEN ESERINE AND ADRENERGIC BLOCKING DRUGS

The reported effects of pretreatment with adrenergic blocking drugs on the responses of the vas deferens to the electrical stimulation of the hypogastric nerve are not in agreement. Ohlin & Strömblad (1963) showed that the effect of the hypogastric stimulation is enhanced by dihydroergotamine and phenoxybenzamine; they excluded sensitisation due to the anticholinesterase activity of the drugs, as suggested by Boyd & others (1960), who described the inhibitory properties of other adrenergic blocking agents. Inhibitory effects by dihydroergotamine and phentolamine on the transmurally-stimulated preparation were clearly demonstrated by Birmingham & Wilson (1963). In our experience, dibenamine, phentolamine, phenoxybenzamine and dihydroergotamine, given at the concentrations of 0·05–0·1 μg/ml, reduced the responses of the preparation by 50–90%. The effect of higher doses was investigated only for dihydroergotamine and phenoxybenzamine: paradoxically, lower responses were sometimes obtained.

The addition of eserine, 2·5–5 μg/ml, to preparations blocked by an adrenergic blocking agent, evoked normally potentiated responses in seven of nine preparations. The responses were particularly evident at the lower rate of stimulation (Fig. 3A & B). Further addition of the adrenergic blocking agent at this point left the height of contractions unaffected (Fig. 3A): occasionally some enhancement was observed.

Analogous results were obtained when eserinised preparations were treated with adrenergic blocking agents: no modifications of the eserine-enhanced contractions were observed (Fig. 2C).

EFFECTS OF ESERINE ON VAS DEFERENS PREPARATIONS FROM RESERPINE-PRETREATED GUINEA-PIGS

Our findings on preparations from reserpine-pretreated animals were consistent with those of Huković (1961): after a few normal responses to electrical stimulation, the responsiveness of the preparation became

progressively less, without becoming completely abolished; 15–30 min after beginning stimulation, the height of contractions varied from 8 to 19% of the initial responses. In line with previous suggestions by Huković (1961), the phenomenon has been ascribed by Birmingham & Wilson (1963) to a progressive reduction of noradrenaline tissue stores.

Addition of eserine after the preparation had become almost unresponsive to the electrical stimulation, evoked immediately increasing

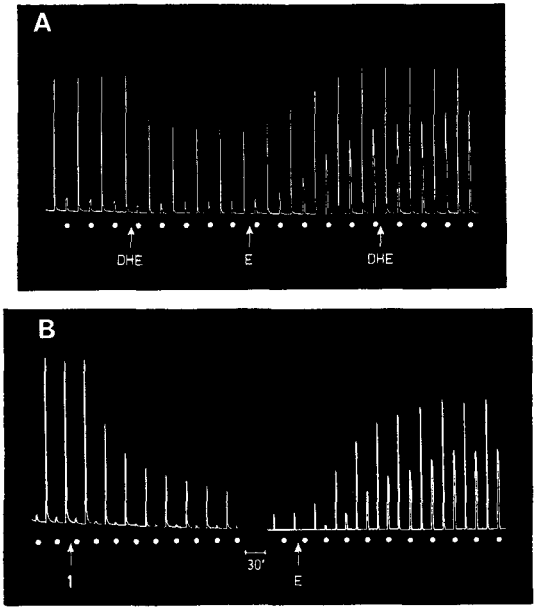

FIG. 3. Preparations and parameters as for Fig. 1. A. At DHE, dihydroergotamine, 0·5 μg/ml initially, then 10 μg/ml. At E, eserine, 2·5 μg/ml. Addition of eserine to a preparation partially blocked by dihydroergotamine evokes normally potentiated responses. A further higher dose of dihydroergotamine is completely ineffective on the eserine-potentiated contractions. B. At 1, dibenamine, 0·25 μg/ml. At E, eserine, 5 μg/ml. Addition of eserine to a preparation under prolonged block by dibenamine, evokes the typical enhanced responses.

contractions at both rates of stimulation (Fig. 4A & B): the enhancing effect of the drug developed progressively and lasted throughout the experiment, in the same manner observed in normal preparations.

Treatment with an adrenergic blocking drug did not impair the eserine enhancement but this was diminished by hexamethonium and abolished promptly by atropine (Fig. 4A & B).

PHARMACOLOGICAL ANALYSIS OF THE RESPONSES OF THE VAS DEFERENS TO NORADRENALINE AND ACETYLCHOLINE

We had observed previously that the responsiveness of the vas deferens to the chemical stimulation is greatly increased when the organ is carefully

isolated from the mesenteric investment without the hypogastric nerve. Similar observations were made by Bentley & Sabine (1963).

With this preparation, direct chemical stimulation was applied at 3–5 min intervals using noradrenaline and acetylcholine at concentrations ranging from 0·1–0·5 μg/ml. In all instances the preparation responded immediately with regular contractions, which reached a peak in a few sec and then rapidly declined.

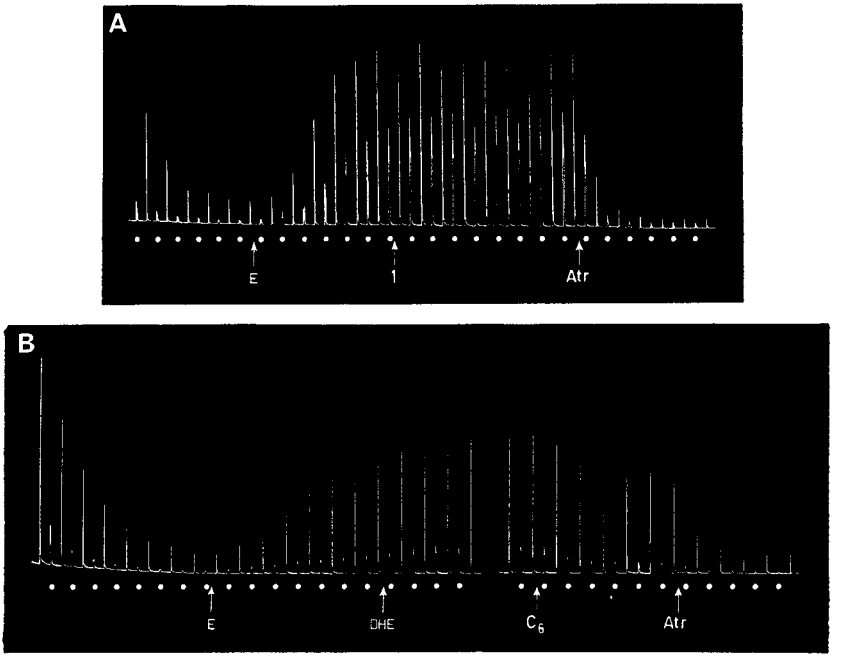

FIG. 4. Preparations and parameters as for Fig. 1. Preparation made from a reserpine-pretreated animal. A. At E, eserine, 2·5 μg/ml. At 1, phenoxybenzamine, 0·25 μg/ml. At Atr, atropine, 0·01 μg/ml. Note the initial spontaneous progressive reduction of the responses to the electrical stimulation. Addition of eserine then elicits enhanced responses which are unaffected by phenoxybenzamine and, on the contrary, are almost completely abolished by atropine. B. At E, eserine, 2·5 μg/ml. At DHE, dihydroergotamine, 1 μg/ml. The addition of eserine when maximal spontaneous reduction of the responses to the electrical stimulation has occurred, evokes strongly potentiated responses. No modification appears upon addition of dihydroergotamine. Note also the inhibitory effect of hexamethonium (at C_6, 5 μg/ml) and subsequently that of atropine (at Atr, 0·01 μg/ml).

Responses to noradrenaline. Noradrenaline-evoked contractions appeared to be abolished completely by the adrenergic blocking drugs tested. Fig. 5A illustrates the antagonistic effect of dihydroergotamine at the dose of 0·2 μg/ml. No modification was observed with atropine, eserine, hexamethonium and bretylium. Veratrine and occasionally bretylium caused some enhancement.

Responses to acetylcholine. Contractions in response to acetylcholine were effectively antagonised by atropine at 0·01 μg/ml: the inhibitory

effect was immediate and was slowly reversible (Fig. 5B). Some enhancement, which progressively disappeared over 3–4 responses, occurred upon addition of 0·25 μg/ml of eserine (Fig. 5C).

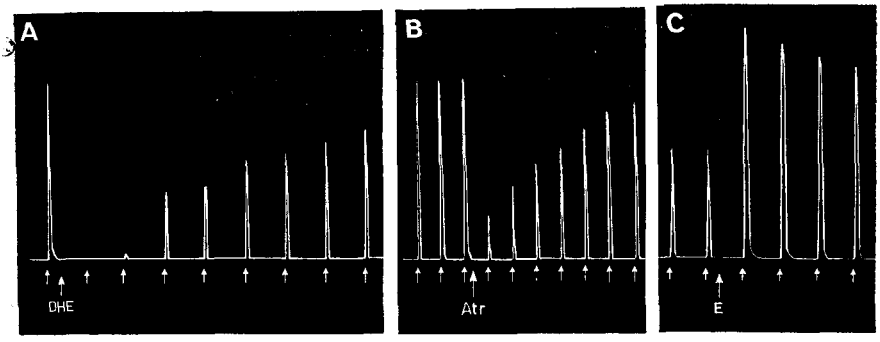

FIG. 5. Isolated, stripped vas deferens preparation. Recording of contractions in response to (A) noradrenaline, (B, C) acetylcholine (1 μg/ml at arrows). Time of contact, 45 sec. Interval between stimulations, 5 min. Note the pronounced and long-lasting inhibitory effect of dihydroergotamine (at DHE, 0·2 μg/ml) and the marked and slowly reversible inhibitory effect of atropine (at Atr, 0·01 μg/ml). Addition of eserine (at E, 0·25 μg/ml) is responsible for a strong and long-lasting potentiation of the responses.

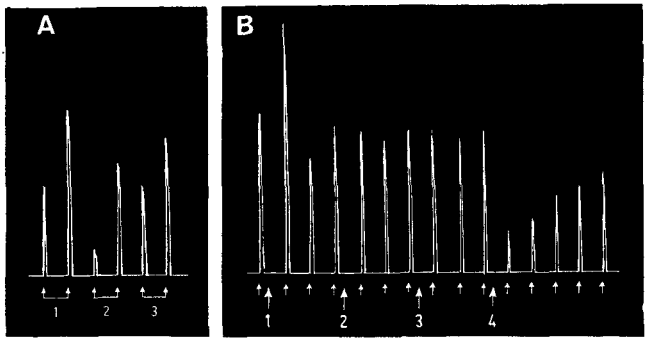

FIG. 6. Isolated, stripped vas deferens preparation. A. Recording of contractions in response to direct stimulation with two successive doses (0·05 and 0·1 μg/ml) of acetyl-β-methylcholine (at 1), carbachol (at 2) and acetylcholine (at 3). Time of contact for each drug, 45 sec. Then, thorough washing out of the preparation. Interval between stimulations, 5 min. B. Recording of contractions in response to acetylcholine (1 μg/ml at arrows). Time of contact, 45 sec. Interval between stimulations, 5 min. At 1, dihydroergotamine, at 2, phentolamine, at 3, dibenamine, at 4, phenoxybenzamine, all 2·5 μg/ml. Note the different influence of the same dose of four adrenergic blocking agents: phentolamine and dibenamine leave the responses of the preparation unimpaired, dihydroergotamine induces a clear but immediately reversible potentiation, phenoxybenzamine is responsible for a strong and prolonged reduction of the responses, similar to that induced by atropine.

Acetyl-β-methylcholine and carbachol were also tested on the preparation, which proved more responsive to acetyl-β-methylcholine than to carbachol (Fig. 6A), probably because of the stronger muscarinic properties of the former drug (Goodman & Gilman, 1955).

Amongst the adrenergic blocking agents tested, phentolamine and dibenamine proved practically ineffective towards the responses to acetylcholine; dihydroergotamine caused some potentiation, while phenoxybenzamine exhibited antagonistic properties at 2–4 μg/ml (Fig. 6B).

Hexamethonium, bretylium and guanethidine did not affect the responses of the preparation, occasionally, some potentiation was observed with veratrine.

Experiments on chemically-stimulated preparations of vas deferens from reserpinised animals, gave analogous results to those above.

Discussion

Our results may be summarised as follows:

(i) In contrast to the behaviour of the hypogastric nerve—vas deferens preparation towards atropine and adrenergic blocking agents, the responses after eserine treatment are strongly reduced by atropine and are not modified by adrenergic blocking agents.

(ii) Addition of eserine to a preparation in which the responses have been reduced by an adrenergic blocking agent elicits enhanced responses which are counteracted by atropine but not by further adrenergic blocking drug. Occasionally some enhancement is observed.

(iii) Addition of eserine to a reserpinised preparation, whose responses to electrical stimulation are significantly reduced because of the depletion of adrenergic mediator stores, evokes full-size responses which are unaffected by adrenergic blocking drugs but are abolished by atropine, as also observed by Schümann & Grobecker (1963).

(iv) The responses of the eserinised vas deferens to electrical stimulation are inhibited, although to different extents, by hexamethonium, veratrine, diphemanil and propantheline, which all share the property of affecting ganglionic transmission. The greater antagonistic activity exhibited by the two latter drugs may be accounted for by their also having atropine-like properties.

(v) The direct responses of the vas deferens to noradrenaline are not modified by atropine and eserine but are antagonised by the adrenergic blocking drugs tested.

(vi) The direct responses to acetylcholine of preparations of the vas deferens from normal or reserpinised animals are antagonised by atropine and enhanced by eserine. The finding that, under these experimental conditions and at the doses tested, no antagonism is present either with hexamethonium or veratrine seems to provide evidence for a direct action of acetylcholine on a muscarinic receptor. The greater responsiveness of the preparation to acetyl-β-methylcholine than to carbachol would also be consistent with this view. However, the possibility cannot be ruled out that under other experimental conditions and at higher doses, such as those adopted by Schümann & Grobecker (1963), acetylcholine

might elicit adrenergic responses mediated through either the ganglion or the chromaffin cells.

The analysis of the results provides grounds for thinking that the responses of the vas deferens to electrical stimulation, which are adrenergic under normal conditions, assume after eserinisation cholinergic parasympathetic-like features. Eserine therefore seems able to modify the responses of the preparation not only quantitatively, by promoting a greater release of adrenergic transmitter as postulated by Burn & Weetman (1963), but also qualitatively. Experiences on the reserpinised preparation strongly support this; less reliable, although consistent with our view, are data obtained with the adrenergic blocking agents, which proved far more effective against added noradrenaline than against sympathetic stimulation.

On the basis of the data obtained, we wonder whether a parasympathetic cholinergic mechanism may be playing a role in the nervous control of the vas deferens; if this is so, the problem arises as to why in the eserinised preparation atropine causes not only the disappearance of the potentiation, but also a marked reduction of the responses.

References

Bentley, G. A. & Sabine, J. R. (1963). *Brit. J. Pharmacol.*, **21**, 190–201.
Birmingham, A. T. & Wilson, A. B. (1963). *Ibid.*, 569–580.
Boyd, H., Chang, V. & Rand, M. J. (1960). *Ibid.*, **15**, 525–531.
Burn, J. H. & Weetman, D. F. (1963). *Ibid.*, **20**, 74–82.
Della Bella, D. & Benelli, G. (1964). *Arch. internat. Physiol. Biochem.*, **72**, 301–305.
Della Bella, D., Gandini, A. & Preti, M. (1964). *Brit.J.Pharmacol.*, in the press.
Goodman, L. S. & Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 429, New York: Macmillan.
Huković, S. (1961). *Brit. J. Pharmacol.*, **16**, 188–194.
Johnson, E. A. & Wood, D. R. (1954). *Ibid.*, **9**, 218–223.
Margolin, S., Doyle, M., Giblin, J., Makovsky, A., Spoerlein, M. T., Stephens, I., Berchtold, H., Belloff, G. & Tislow, R. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 576–580.
Ohlin, P. & Strömblad, B. C. R. (1963). *Brit. J. Pharmacol.*, **20**, 299–306.
Schümann, H. J. & Grobecker, H. (1963). *Arch. exp. Path. Pharmak.*, **246**, 215–225.