J. Physiol. (1949) 110, 164–172 546.431:612.891

THE NICOTINIC ACTION OF SUBSTANCES SUPPOSED TO BE PURELY SMOOTH-MUSCLE STIMULATING.

(B) EFFECT OF $BaCl_2$ AND PILOCARPINE ON THE SUPERIOR CERVICAL GANGLION

By N. AMBACHE

From the Department of Physiology, University College, London, and The Ophthalmological Research Unit (Medical Research Council), Institute of Ophthalmology, Judd Street, London, W.C.1

(*Received* 30 *April* 1949)

This paper is concerned with the ganglionic actions of two substances ($BaCl_2$ and pilocarpine) which are usually assumed to be purely smooth-muscle stimulating. It was pointed out previously (Ambache, 1946) that a number of drugs may owe at least part of their action on the gut to an indirect effect upon the intestinal innervation. Some of the substances used in the experiments described in that paper were known to affect nerve cells and their fibres or endings in other parts of the body. For instance, KCl releases acetylcholine from a wide variety of cholinergic nerve endings, and stimulates ganglion cells and nerve fibres as well. It was known, also, that $BaCl_2$ exerts a veratrine-like action on motor nerve fibres and endings in the toad (Dun & Feng, 1940), and Feng (1937) has suggested, further, that barium may induce a leakage of acetylcholine at motor endings. Lastly, it had been shown by Chou & Chin (1943) that minute amounts of $BaCl_2$ (0·0001–0·0018 mM/kg.), introduced into the cerebrospinal fluid, give rise to tetanic spasms and to convulsive seizures as a result of stimulation of the central nervous system. In agreement with all this, evidence was produced (Ambache, 1946) to show that both these substances, and possibly histamine too (which is capable of stimulating certain types of sensory nerve endings in the skin, and of eliciting axon reflexes) could not be regarded as purely smooth-muscle stimulating substances.

In repeating some of these experiments, Emmelin & Feldberg (1947) used three drugs, in crucial 'control' experiments, on the assumption that they were purely smooth-muscle stimulating substances, namely, choline, pilocarpine, and 2268F. These drugs were believed to be free from any action on the ganglion cells. Their argument was largely based upon this assumption, which for 2268F

has been disproved in the preceding paper, and for choline and pilocarpine was anyway incorrect. For it was well known, and from evidence provided by one of these authors, that choline stimulates ganglion cells in small doses, e.g. 25 μg. injected into the perfusion system of the superior cervical ganglion (Feldberg & Vartiainen, 1934); the dose used by Emmelin & Feldberg (1947) was 300 μg. into a 10 c.c. organ bath. Dale & Laidlaw (1912) had already shown that pilocarpine can stimulate the superior cervical ganglion when applied externally. Later, Bacq & Simonart (1938) described the nicotinic action of pilocarpine on the blood pressure; and Marrazzi (1939) found electrical evidence of an increase in ganglionic activity produced by the same drug, an effect which was antagonized by atropine. Lastly, an analogous action of pilocarpine upon the suprarenal medulla had been recognized previously by Feldberg, Minz & Tsudzimura (1934), who found that pilocarpine brought about a release of adrenaline which could be abolished by atropine.

In the course of the perfusion experiments described in the previous paper, the opportunity was taken to verify the fact that pilocarpine can stimulate the superior cervical ganglion, and it has also been found that $BaCl_2$ does the same. Since it shows that $BaCl_2$ is capable of exciting nerve cells, this finding confirms the point of view taken previously that the action of $BaCl_2$ on the gut is, at least in part, due to a stimulation of the motor innervation within it.

The method is the same as in the preceding paper and needs no further description.

RESULTS

Pilocarpine.

The observations of Dale & Laidlaw (1912), and of Marrazzi (1939), were confirmed in three different experiments, with doses of pilocarpine ranging from 0·25 to 10 μg. One of these is illustrated in Fig. 1, which is the continuation of the experiment described in Fig. 6 of the preceding paper.

Denervated ganglion. The action of pilocarpine was present in a ganglion 18 days after preganglionic denervation, and is shown in Fig. 9*C* of the preceding paper. The threshold dose in this particular experiment lay between 0·1 and 0·5 μg., and the latency of the response shown in Fig. 9*C* was 6 sec. The effect of the same dose of pilocarpine was abolished, at *I*, by 0·2 μg. of atropine administered to the ganglion 5 min. earlier.

$BaCl_2$.

It has been found that $BaCl_2.2H_2O$, injected into the perfusion system in doses ranging from 50 to 500 μg., exerts a definite stimulating action upon the ganglion. In all of four experiments 200 μg. (and in a fifth, 500 μg.) of $BaCl_2.2H_2O$ produced marked contractions of the nictitating membrane after the usual latent period inherent in the perfusion system.

It was noticed in one of these experiments (that of Fig. 2) that the pupil dilated simultaneously on the homolateral, but not on the contralateral, side. In this experiment also, a paralytic effect was observed after a second dose of $BaCl_2.2H_2O$ (100 μg. at *D* in Fig. 2). This was administered 5 min. later and elicited a slightly smaller, but equally prolonged, response, which was followed by a reduction in the response to preganglionic stimulation 4 min. later, to $\frac{1}{4}$ of

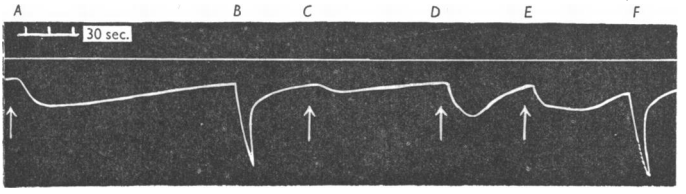

Fig. 1. (continuation of Fig. 6 of preceding paper). Effect of pilocarpine upon the superior cervical ganglion. At *B* and *F*, maximal preganglionic stimulation for 5 sec. Injections of pilocarpine nitrate at *A*, 1 μg.; at *C*, 0·25 μg. *D* shows effect of 10 μg. of acetylcholine (containing also 0·5 mg. of NaH_2PO_4); and *E*, of 0·2 μg. of 2268F.

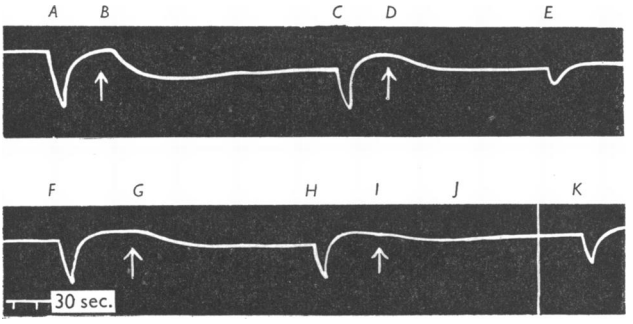

Fig. 2. Cat, 2·75 kg., pregnant. Effect of $BaCl_2$ on superior cervical ganglion by perfusion. At *A*, *C*, *E*, *F*, *H*, *J*, and *K*, maximal preganglionic stimulation; at *B* and *I*, injections of 200 μg. of $BaCl_2$; at *D*, 100 μg. $BaCl_2$; at *G*, 50 μg. $BaCl_2$. The drum was stopped between *J* and *K*; the actual time interval between them was 7 min.

its previous size. After a further 3 min. the response to preganglionic stimulation had recovered to $\frac{3}{4}$ of its original size. Later, 200 μg. of $BaCl_2.2H_2O$ (at *I*) had a much smaller stimulant effect upon the ganglion, and was followed by an absence of response to preganglionic stimulation at *J*, and its restoration 7 min. later at *K*.

In another of these experiments the effect of 500 μg. of $BaCl_2.2H_2O$ was reduced by 50% after a dose of 1 μg. of atropine administered to the ganglion $2\frac{1}{2}$ min. previously.

Denervated ganglion. As shown in Fig. 3 below, and in Fig. 9 of the preceding paper, the denervated ganglion responded to doses of $BaCl_2.2H_2O$ ranging

from 50 μg. to 1 mg. This action was reversibly abolished by nicotine (Fig. 3 *L* and *M*, with recovery at *Q*), and by atropine (Fig. 9 of preceding paper, *J* and *L*). The latency of the response was 8, 7·5 and 13 sec. for three doses of 50 μg., 9 sec. for 200 μg., and 8 and 5 sec. for two consecutive doses of 1 mg. (at Fig. 3*E* and *F*). The effect of the second dose of 1 mg., at *F*, was smaller than that of the first at *E*.

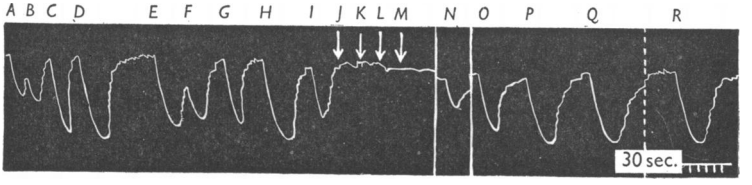

Fig. 3. Cat, 3·2 kg. (♂). Perfusion of the superior cervical ganglion decentralized 18 days previously. Continuation of Fig. 9 of the preceding paper. Showing ganglionic effects of barium and their abolition by nicotine. Injections of $BaCl_2.2H_2O$: at *A*, *L*, and *Q*, 200 μg.; at *B*, 250 μg.; at *E*, *F*, and *M*, 1 mg. Injections of 0·05 μg. acetylcholine at *C*, *G*, *J*, *N*, and *O*. Injections of 0·5 μg. 2268F at *D*, *H*, *K*, *P*, and *R*. 20 μg. of nicotine hydrogen tartrate at *I*, followed by paralysis of the responses to A.Ch. (*J*), 2268F (*K*), and barium (*L*, 200 μg., and *M*, 1 mg.), with recovery of all three later at *O*, *P*, and *Q*. Between *Q* and *R*, 3 mg. of atropine intravenously produced dilatation of the pupil but did not affect the response to 2268F at *R*, 2 min. later. Drum stopped 6 min. between *M* and *N*, and 5 min. between *N* and *O*.

DISCUSSION

The present findings provide direct evidence that $BaCl_2$ has, as postulated previously, an action on nervous tissue.

The experiments on the denervated ganglion show that the action of barium is still present after the preganglionic nerve endings have degenerated, and the fact that this action is abolished by nicotine suggests that the effects observed were due to a stimulation of the ganglion cells proper. These findings confirm the interpretation of the mode of action of barium on the gut which was put forward in an earlier paper. This point of view now receives further support from the recent observation of Collins (1948), who finds that the effect of $BaCl_2$ on the gut is depressed (in four out of five experiments) by the tetra-ethyl-ammonium ion, which is a ganglionic poison. Also, if the earlier work of Berkson (1933*a*, *b*) on the electrical activity of the isolated gut is consulted, one is struck by the correspondence between the type of electrical change immediately consequent upon the administration of $BaCl_2$ (Berkson, 1933*a*; Fig. 2*C*), and that seen after nicotine (Berkson, 1933*b*; Fig. 3*A*). The responses are identical and quite unlike anything else shown in his records.

A curarizing effect of $BaCl_2$ is shown in Figs. 2 and 3F, and may account for the variability in its action on the gut found by Emmelin & Feldberg (1947, fig. 7*A*), particularly if the $BaCl_2$ is administered repeatedly at short intervals,

as in their experiments. Also, it is known clinically that in barium poisoning, which occurs in some parts of China, and is known as Paping, 'occasionally the limbs are found to be spastic, but, when this is overcome, they remain flaccid, or complete paralysis is present' (Allen, 1943, p. 248).

It has also been reported by Thienes (1927) that $BaCl_2$ (1:100,000 to 1:20,000) elicited, in seven out of 100 experiments, *inhibitory* responses in the rabbit gut, an effect which it might be easier to explain on the basis of an action on the innervation of the gut rather than directly on the muscle fibres.

Lastly, the fact, which is illustrated in Fig. 9 of the preceding paper, that the ganglion stimulating action of barium may be sensitive to small doses of atropine, could perhaps explain some of Emmelin & Feldberg's findings with these two drugs in combination. They state that in some of their experiments, for example that of fig. 7 *b* of their paper, the barium contractions were definitely reduced by atropine. Moreover, in other experiments, the barium contractions were not restored, when, after washing the atropine out, the muscle was again sensitive to acetylcholine. As shown above, in Fig. 9 of the preceding paper, the ganglionic response to A.Ch. (at *K*) may recover before that of barium (at *L* and *N*), suggesting a slower rate of recovery for barium after atropine, which may explain their findings.

SUMMARY

1. The existence of a ganglion-stimulating action of pilocarpine (Dale & Laidlaw, 1912) has been verified. It was present in a preganglionically denervated ganglion and was abolished by atropine.

2. $BaCl_2$ is capable of stimulating the superior cervical ganglion. This action was present in a decentralised ganglion, and was reversibly abolished both by nicotine and by atropine.

Note added in proof. The author's attention has been kindly drawn by Prof. J. H. Burn to a thesis written in his department by Dr T. C. Chou (1947), which includes a description of some experiments with barium on the perfused superior cervical ganglion. Special mention must be made of the two experiments illustrated in the figures, which show that the same dose of $BaCl_2$ may produce stimulation of one ganglion, and depression of another—a result somewhat analogous to that shown in Fig. 2 above (*B* and *I*), obtained in one and the same ganglion.

I wish to thank Miss Sheila Stennett for her assistance.

APPENDIX

In this appendix it is proposed to discuss the relevance of the findings described in this and the preceding paper to present-day conceptions of drug action on smooth muscle. For the sake of clarity, it is necessary to summarize as briefly as possible both my previous results (1946) and those of Emmelin & Feldberg (1947).

First, it had been found by Dikshit (1938) that cooling abolishes the production of acetylcholine in the gut. This method was therefore used by the

present author as a means of 'denervating' the gut. After cooling, it was found that the response to doses of KCl and $BaCl_2$, which had been previously effective, was considerably reduced, and later absent. This change was attributed to a functional 'denervation' produced by the cooling, and was taken as confirming the hypothesis that these substances must owe at least part of their effect to an action on the nervous apparatus in the gut. As regards the effect with $BaCl_2$, these results were confirmed by Emmelin & Feldberg, who also found that the sensitivity of the gut to KCl decreased (at first) to a greater extent than to acetylcholine.

Use of pilocarpine in cooling experiments. In controlling these particular experiments, Emmelin & Feldberg used pilocarpine, and they found that cooling diminished the response of the gut to pilocarpine to a much greater extent than was expected. Because of this, they concluded that the principal effect of cooling was due to a depression of the muscle fibres themselves since, it was argued, pilocarpine was a 'purely smooth-muscle stimulating substance'. However, the use of pilocarpine provides no control; it is, as we have seen, as though one were to use nicotine itself, because pilocarpine possesses a nicotinic action (Dale & Laidlaw, 1912). This is also shown by the present experiments on the ganglion, in which 0·25–1 μg. of pilocarpine had distinct effects on the ganglion; the dose used by Emmelin & Feldberg was 2·5 and 10 μg. Now, the action of nicotine on the gut is reduced by cooling, and then abolished (Vogt, 1943); and that component in the response to pilocarpine which is due to the stimulation of ganglion cells would also be removed by cooling.

Potentiations by eserine; use of choline and of 2268F. If we think of a drug action as having possible muscular and nervous components, then it should be possible to distinguish the presence of the latter with the help of eserine. Thus it had been suggested that any drug which is potentiated by eserine must owe at least part of its action to a nervous or cholinergic component. Conversely, any drug which is endowed with only a muscular component should fail to be potentiated by eserine (unless it is hydrolysable by cholinesterase). Emmelin & Feldberg (1947) tested the second half of this proposition, but they chose for their 'controls' two drugs, choline and 2268F, both of which possess a nervous (ganglionic) component. Both drugs were potentiated by eserine, which led Emmelin & Feldberg to conclude that the criterion of eserine-potentiation was non-specific. Since they departed from a false premise, this, their conclusion, would appear to be invalidated.

There is one difficulty in applying this criterion, namely that eserine, administered by itself, slowly causes the gut to contract and then throws it into rhythmic contractions. It is evident then that any apparent potentiation of another drug by eserine might be due to the summation of this slow rise in base line produced by eserine, together with the motor effect of the drug proper. However, the effect of a small dose of eserine can be graded, and it usually begins after a latent period of 30–60 sec. or more (see, for example, Ambache, 1946, fig. 16 *C* 3), and rises slowly at first. In all the other experiments of that paper the precaution had been taken of injecting the 'test'

drug whose potentiation was being studied, 5–10 sec. after the eserine, i.e. well before the beginning of any eserine contraction. Moreover, it is the fact that the immediate response to the 'test' drug shows potentiation, which is significant. This response occurs between the 10th and 30th sec. (usually maximal by the 15th–20th sec.) after the first injection of eserine, i.e. during the latent period.

Action of acetylcholine after nicotine; 'controls' with 2268F *and pilocarpine.* In considering the action of acetylcholine itself, I had suggested that, in view of its well-known nicotinic action, part of the response of the gut to this drug might be due to an indirect effect upon the ganglion cells, and that this part was removable both by nicotine paralysis of the ganglion cells, and by cooling. Emmelin & Feldberg 'controlled' this experiment with 2268F and pilocarpine. Their results are shown in fig. 1 of their paper. In this, equivalent doses of 2268F and acetylcholine were administered in alternation throughout the experiment. After the onset of nicotine-paralysis of the ganglion cells, the response to acetylcholine is reduced at first, but recovers later, *although the nicotine is left in the bath.** The initial reduction, which had been reported before, had been attributed (Ambache, 1946) to a removal of a 'nicotinic' component in the action of acetylcholine on the gut. Such an action is denied by Emmelin & Feldberg on the grounds that the action of 2268F is also reduced by nicotine, as shown in their figure. It is quite probable, however, that this reduction by nicotine of the response to 2268F, and to pilocarpine, is itself due to the removal of the ganglionic component of these two drugs.

The more recent experiments of Collins (1948) show that the action of acetylcholine on the gut is reduced (in ten out of thirteen experiments) by another ganglion-cell poison, namely tetraethyl ammonium bromide.

Experiments on acetylcholine production. Emmelin & Feldberg also studied the effect of KCl, $BaCl_2$, and histamine upon the acetylcholine production in the gut. They claim that these substances fail to increase the amount of acetylcholine released within the gut. If this were true, it would indeed be surprising, since it is well established that KCl releases acetylcholine from a wide variety of cholinergic nerve endings, and $BaCl_2$ has, as we have seen, an action upon ganglion cells, and also upon nerve fibres and endings (Feng, 1937; Dun & Feng, 1940), all of which would be expected to result in an increased release of acetylcholine. However, if we analyse Emmelin & Feldberg's results (p. 498) we see that their statement that 'potassium was ineffective or accelerated synthesis in one out of five experiments only' is inaccurate. The amounts of acetylcholine produced in their experiments have been calculated from their table and are set out in Table 1 below.

* This recovery from nicotine is not unlike that observed by Thomas & Kuntz (1926), who found that nicotine impaired the effect of vagal stimulation on the gut only at first, and that later, when they had also raised the dose of nicotine (25–50 mg. of nicotine/kg.), vagal function and therefore transmission, recovered: with still higher doses of nicotine the vagal effect was actually potentiated. It would seem that the action of nicotine on the ganglion cells in the gut presents certain anomalies which require further elucidation.

It will be seen that of the total of seven intestinal strips incubated with KCl, four (marked by asterisks) show a distinct increase in acetylcholine production over and above the control strips of the same experiment. Moreover, in this section of their paper, Emmelin & Feldberg did not repeat my original experiments under identical conditions. The main differences in our methods were: (*a*) in the concentration of eserine used, and (*b*) that, whereas I had used pieces of intestine 13–15 cm. in length, in Emmelin & Feldberg's experiments the intestines were cut into small pieces of 1 cm. It is possible that the very much greater injury produced by their procedure, acting itself as a stimulus, was responsible for some of the rather high values, e.g. 3 and 5·2 μg./g. in their control groups (col. 2).

In their experiments on acetylcholine synthesis in the presence of barium, again the conditions of my experiments were not closely observed. The total dose of $BaCl_2.2H_2O$ in my experiments was 2–4 mg. which represents a concentration of 1·7–3·4 mg. of $BaCl_2$ in 6·3 c.c. (i.e. 0·27–0·54 mg./c.c.), whereas Emmelin & Feldberg used a dose about 7–28 *times as large*, i.e. 4 and 8 mg./c.c. of $BaCl_2$. Now, we have seen from the present experiments that a large dose of barium may exert a depressing action upon the ganglion cells: this may account for some of Emmelin & Feldberg's low values. Of the strips incubated with $BaCl_2$, one had a negative value for acetylcholine production (− 2, in

TABLE 1. Production of acetylcholine (μg./g./40 min.) in intestinal strips (compiled from Emmelin & Feldberg, 1947, p. 498)

	Controls		KCl		$BaCl_2$	
Exp.	(1)	(2)	(3)	(4)	(5)	(6)
1	2·5	3·0	4·3*	—	2·6	—
2	2·1	2·7	2·8	—	2·7	—
3	1·8	1·7	5·0*	—	−2†	5·4*
4	1·4	5·2	2·5	3·6	—	—
5 (buffered)	0·9	2·1	3·5*	3·0*	4·0*	3·9*
					3·72 (omitting†)	
Average	2·34		3·53		2·77 (including†)	

Exp. 3) which is difficult to understand. Apart from this probably erratic result, three (asterisks) of the other five strips show a distinct increase in acetylcholine production over and above the control strips of the same experiment. In their last experiment (no. 5) the experimental conditions appear to have been improved by the introduction of a buffer. This experiment shows a clear-cut increase in acetylcholine synthesis produced by *both* KCl and $BaCl_2$.

The conditions of these experiments with KCl and $BaCl_2$ appear to have been so diverse that a statistical analysis on these few results is inconclusive, but 50 % of them appear to show a distinct trend. I am indebted to Mr D. A. Scholl, of the Department of Anatomy, and to Mr D. R. Westgarth, of the Department of Statistics, University College, for an analysis of variance (allowing for the disparity in the number of observations in the two groups) which demonstrated that a significance level of 11 % was associated with the differences between the KCl and control groups of results. This level admittedly does not indicate a marked difference between the two groups on the basis of this data, but is too unusual to dismiss, and it would be unwise to conclude that the observed difference was due merely to chance.

Is there such a thing as a purely smooth-muscle stimulating substance? It seems therefore, that, at the moment, it is difficult to specify a drug of which it can be said with absolute certainty that it is a purely muscle-stimulating substance devoid of side effects upon the nervous apparatus in the gut. We have seen why choline, acetylcholine, 2268F, pilocarpine, KCl and $BaCl_2$ will not serve the purpose. Muscarine itself deserves investigation, but it is known to have a nicotine-like action on the suprarenal medulla and excitatory effects upon the central nervous system.

As to histamine, it has a well-known action upon certain types of nerve endings. Thus it is capable of exciting axon reflexes in the skin, and the possibility of axon reflexes occurring in smooth muscle has been raised by Fischer 1944; their presence has been observed in the frog's lung by Dikjstra & Noyons (1939), and in the rabbit's lung (pleuro-pulmonary reflexes) by Reinhardt (1933). In the axon reflex of the 'flare' it is believed that A.Ch. is released by the nerve ending in contact with the blood vessels, since this ending, when excited by antidromic stimulation of the posterior roots, is capable of releasing A.Ch. (Wybauw, 1938). This may therefore be considered as a model of a system in which histamine starts off nerve impulses which eventually release A.Ch. Emmelin & Feldberg have found that benadryl suppresses the action of histamine on the gut without interfering with that of A.Ch. Now it has been shown (Parrot & Lefebvre, 1943) that histamine antagonists may prevent only the *initiation* by histamine of axon reflexes, but that they do not interfere with the rest of the reflex mechanism, since it can still be elicited by electrical stimulation of the axons in the skin, or of the posterior roots antidromically. Emmelin & Feldberg's findings with benadryl may be regarded as analogous to these, and are no proof that histamine cannot release A.Ch. in the gut, as in the skin.

REFERENCES

Allen, A. S. (1943). *Chin. med. J.* **61**, 296.
Ambache, N. (1946). *J. Physiol.* **104**, 266.
Bacq, Z. M. & Simonart, A. (1938). *Arch. int. Pharmacodyn.* **60**, 218.
Berkson, J. (1933*a*). *Amer. J. Physiol.* **104**, 62.
Berkson, J. (1933*b*). *Amer. J. Physiol.* **104**, 67.
Chou, T. C. (1947). *D. Phil. Thesis.* Oxford University.
Chou, T. C. & Chin, Y. C. (1943). *Chin. Med. J.* **61**, 313.
Collins, D. A. (1948). *J. Pharmacol.* **94**, 244.
Dale, H. H. & Laidlaw, P. (1912). *J. Physiol.* **45**, 1.
Dikjstra, C. & Noyons, A. K. M. (1939). *Arch. int. Physiol.* **49**, 257.
Dikshit, R. B. (1938). *Quart. J. exp. Physiol.* **28**, 43.
Dun, F. T. & Feng, T. P. (1940). *Chin. J. Physiol.* **15**, 433.
Emmelin, N. & Feldberg, W. (1947). *J. Physiol.* **106**, 482.
Feldberg, W., Minz, B. & Tsudzimura, H. (1934). *J. Physiol.* **81**, 286.
Feldberg, W. & Vartiainen, A. (1934). *J. Physiol.* **83**, 103.
Feng, T. P. (1937). *Chin. J. Physiol.* **12**, 177.
Fischer, E. (1944). *Physiol. Rev.* **24**, 467.
Marrazzi, A. S. (1939). *J. Pharmacol.* **65**, 18.
Parrot, J. L. & Lefebvre, J. (1943). *C.R. Soc. Biol., Paris*, **137**, 316.
Reinhardt, E. (1933). *Virchow's Arch.* **292**, 322.
Thienes, C. H. (1927). *Proc. Soc. exp. Biol., N.Y.* **24**, 327.
Thomas, J. E. & Kuntz, A. (1926). *Amer. J. Physiol.* **76**, 598.
Vogt, M. (1943). *J. Physiol.* **102**, 170.
Wybauw, L. (1938). *Arch. int. Physiol.*, **46**, 324.