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# THE STIMULATING ACTION OF ACETYLCHOLINE ON THE HEART

By R. J. S. McDOWALL, *King's College, London*

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In 1882 Gaskell, when studying the action of the vagus on the heart of the frog, found that the cardiac slowing produced by stimulation of the nerve was commonly followed by an increased activity, and, in some cases, this increase might be the only result seen. Because of the discovery of the sympathetic fibres by Schmiedeberg (1871), Gaskell (1900) was disposed to conclude that the increased action was due to stimulation of sympathetic fibres passing with the vagus. The after-action he considered to be evidence for an anabolic action of the vagus. The use of acetylcholine, which is liberated by the vagus, appeared to offer a method of further investigation of the problem.

The only other experiments in this direction appear to be some by Feldberg & Minz (1931), who, in studying the effects of acetylcholine on ganglia, noted that, after intravenous injection of the drug, there was occasionally an acceleration of the heart *in situ*, even after the vagi had been cut and the stellate ganglia removed.

## METHODS

The experiments were carried out on the isolated hearts of cats, rabbits and rats. The coronary arteries were perfused with Ringer-Locke's solution by the usual Langendorf-Locke modification of the Martin method. In several experiments the calcium chloride concentration of the Ringer-Locke solution was lowered from 0·024 to 0·016%. The drugs to be tested were dissolved in the perfusion fluid and injected in a volume of 0·5 c.c. into the tubing close to the heart. In each case the 0·5 c.c. were injected at a standard rate calculated to give an approximately tenfold dilution of the drug by the perfusion fluid. In some experiments the auriculo-ventricular (A.V.) bundle was cut by inserting a narrow knife through the left ventricle between the coronary arteries; auricular and ventricular contractions were then recorded separately. In all tracings the upstroke is due to systole, the downstroke to diastole.

## RESULTS

In hearts in good condition the injection of 5$\mu$g. acetylcholine causes slowing and weakening of the heart which, however, is almost invariably followed by a period of increased activity (Figs. 1, 2). The stimulating phase sometimes lasts for more than 15 min. During this phase extra systoles are commonly

seen (Fig. 1). In a few hearts the slowing is preceded by a few very forcible contractions. If, in fresh hearts, the injections of 5$\mu$g. acetylcholine are

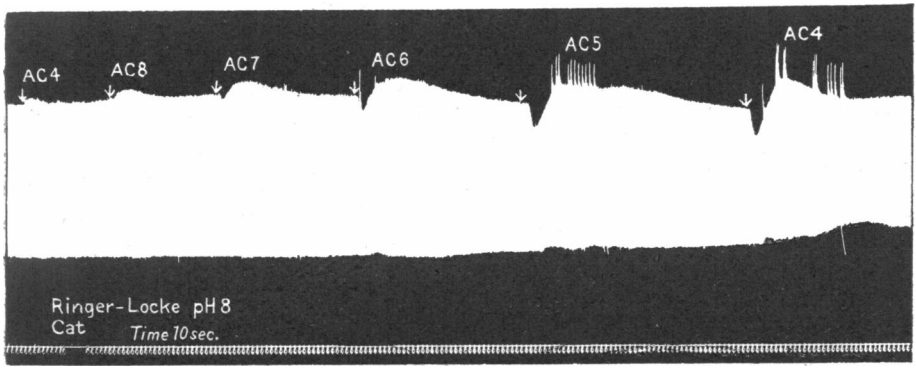


Fig. 1. Cat. The effect of increasing doses of acetylcholine. 0·5 c.c. of AC6 is the equivalent of 0·5$\mu$g. and the other dilutions are corresponding multiples of divisions of 10. Note the increasing frequency of extra systoles during the stimulative phases.

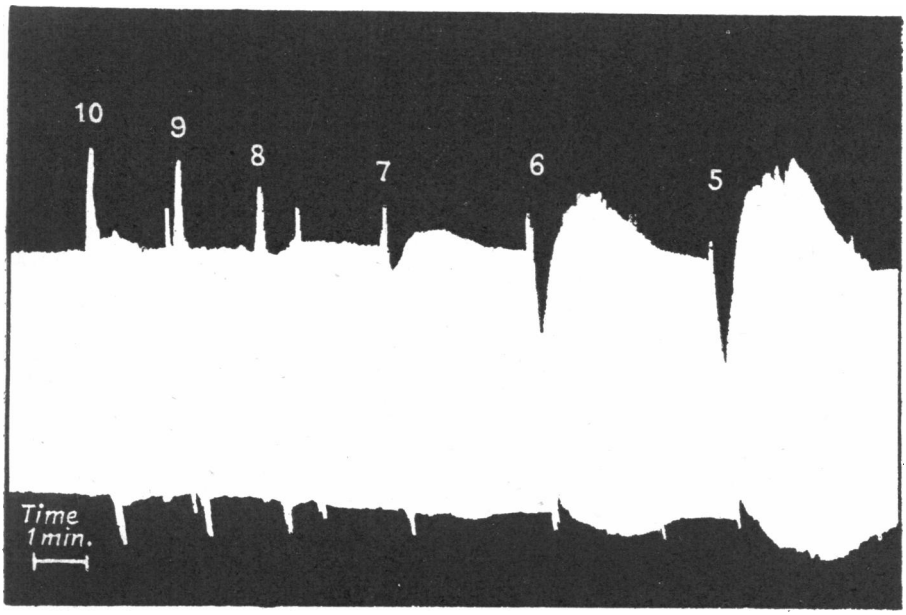


Fig. 2. Cat. The effect of increasing doses of acetylcholine, as in Fig. 1, in an instance in which the initial stimulating effect was particularly well marked. This is an unusual response.

repeated during the stimulating phase of the previous acetylcholine response, the inhibiting effect of acetylcholine gradually diminishes and eventually disappears.

With smaller doses of acetylcholine the response of the heart varies greatly and is dependent on the season. During the months of May and June, when sympathetic activity is known to be good (Armitage, McDowall & Mathur, 1932), small doses of acetylcholine (0·00005–0·05$\mu$g.) usually have a stimulating action on the heart; on the other hand, during the winter months such action cannot always be observed with these small doses of acetylcholine. But even in May and June it is essential, in order to demonstrate the stimulating action of small doses of acetylcholine, that the preparation should be from an animal in good condition, fresh and not exhausted from a previous stimulation by adrenaline or high temperature. A further advantage is afforded by the use of the Ringer-Locke solution with the low calcium content (see Methods).

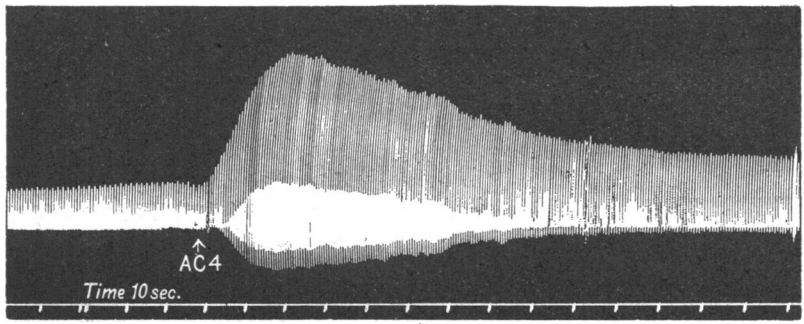


Fig. 3. Cat. The effect of a large dose of acetylcholine (50$\mu$g.) after atropine (16·6$\mu$g.).

Typical results are shown in Figs. 1 and 2. The smallest effective dose causes stimulation only; with increasing dose the inhibiting effect appears first in the tracing as a slight notch superimposed upon the stimulating effect. It is interesting to note that when the inhibiting action of acetylcholine passes off, and particularly towards the end of the period of increased activity by acetylcholine, the response of the heart to adrenaline is increased. As this appears to be a general phenomenon it is made the subject of a subsequent paper.

*After atropine.* A dose of 15$\mu$g. atropine, which abolishes the inhibiting action of acetylcholine on the heart, has no effect on the stimulating action. After atropine it is possible to discern a stimulating effect of acetylcholine on the force of the contractions and on their frequency. The effect on the force is constant and usually the sole effect. Acceleration occurs only rarely and is best seen at the commencement of an experiment when the preparation is fresh. In the experiment of Fig. 3 the effect of acetylcholine on the atropinized heart is on the force only; during the stimulating phase the heart not only contracts more forcibly in systole, but also relaxes more completely in diastole. This is not always the case. In a number of experiments, especially in summer,

the heart, during the stimulating phase, was seen to beat in a more systolic position. Such an experiment is illustrated in Fig. 4.

Some atropinized hearts show the stimulating effect of acetylcholine better than others under apparently similar conditions, and on repeated injections of acetylcholine some continue to show the stimulating effect much longer than others. In six out of eighty atropinized hearts no stimulating action of acetylcholine could be observed.

With doses of atropine much larger than 15$\mu$g., the stimulating effect of acetylcholine on the heart is also affected and sometimes even abolished.

It is possible to unmask the stimulating action of acetylcholine by methylene blue which abolishes the inhibiting action, but this atropine-like action may be abolished by the injection of lactic acid or by stopping the coronary perfusion for a short period.

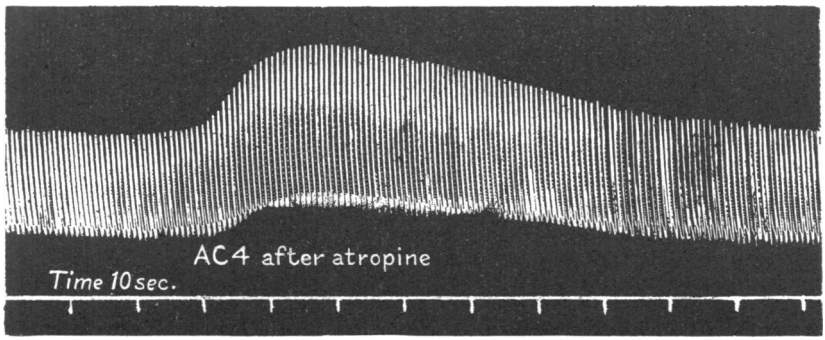


**Fig. 4. Cat. The effect of a large dose of acetylcholine (50$\mu$g.) after atropine (16·6$\mu$g.). Note the increase of tone. This latter only occurs occasionally.**

The effects of acetylcholine on the heart are not due to the slight variation in coronary flow produced by acetylcholine. Similar changes in coronary flow produced by other means did not affect the rate or force of the heart.

*After eserine* the stimulating action of acetylcholine on the atropinized heart is enhanced. The action of acetylcholine may simulate that produced by adrenaline in affecting the rate and the force of the contractions simultaneously (Fig. 5).

*After ergotoxine* the stimulating action of acetylcholine on the atropinized heart is abolished or may even be reversed (Fig. 6).

*Nicotine*, in doses which paralyse autonomic ganglia, as indicated by the failure of a second dose to cause slowing, does not abolish the stimulating action of acetylcholine on the atropinized heart. This action, therefore, must be due to an effect of acetylcholine on the heart muscle; it cannot be due to an action on aberrant sympathetic ganglia in the heart itself. The presence of such ganglia is, in fact, somewhat doubtful. The effects of nicotine itself on the heart

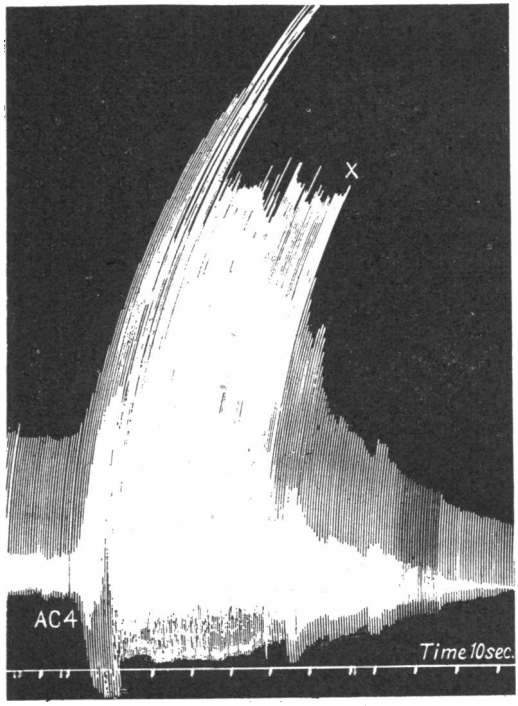


Fig. 5. Rabbit. The effects of a large dose of acetylcholine (50$\mu$g.) after atropine (16·6$\mu$g.) and eserine. The drum was stopped at *X* to facilitate reproduction of the record.

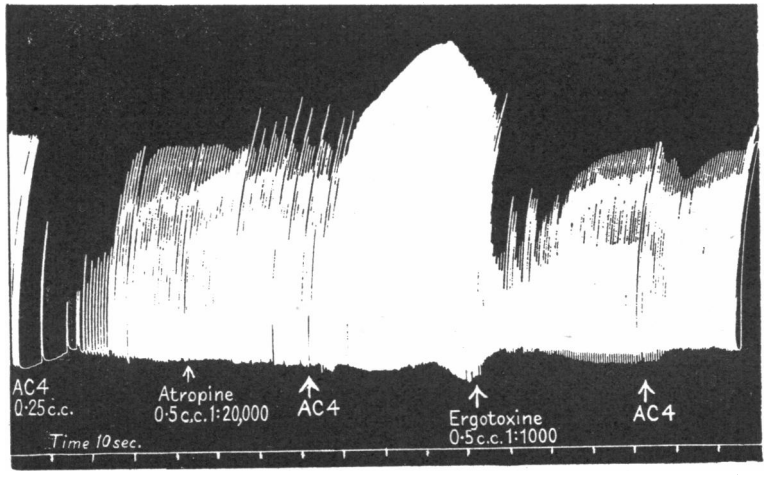


Fig. 6. Cat. At the beginning of the record the effect of a large dose of acetylcholine (25$\mu$g.) is shown, and later the effect of the same dose after atropine (25$\mu$g.). This effect was abolished by an injection of ergotoxine (500$\mu$g.).

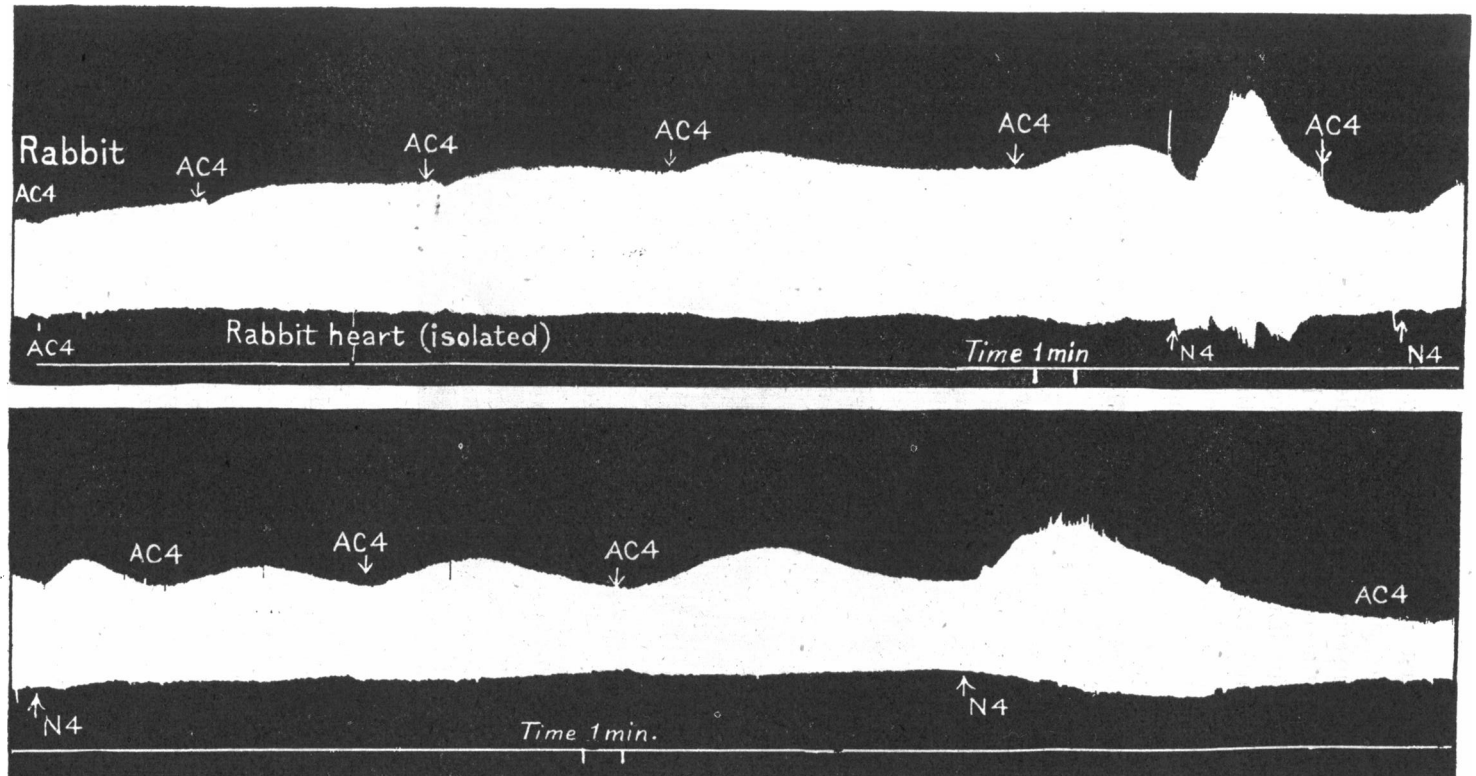


Fig. 7. Rabbit. Showing the effect of acetylcholine (50$\mu$g.) after a dose of atropine (16·6$\mu$g.) and of nicotine (50$\mu$g.) sufficient to paralyse the ganglia, as indicated by the absence of preliminary weakening of the heart. The immediate effect of the nicotine is toxic and decreased the response to acetylcholine but there was a slow recovery. A subsequent dose of nicotine again abolished the response to acetylcholine and to nicotine itself and again there was recovery.

are complex. A first injection of 0·005$\mu$g. nicotine causes inhibition, which is presumably due to stimulation of the parasympathetic ganglia, and this is followed by cardiac acceleration. When the ganglia are paralysed, subsequent injections of nicotine only stimulate the heart. At this stage, acetylcholine still slows the heart if no atropine is given, but stimulates it after atropine (Fig. 7). After further injections of nicotine, the heart muscle itself becomes poisoned, and the stimulating action of either nicotine, acetylcholine or adrenaline is abolished or very much reduced.

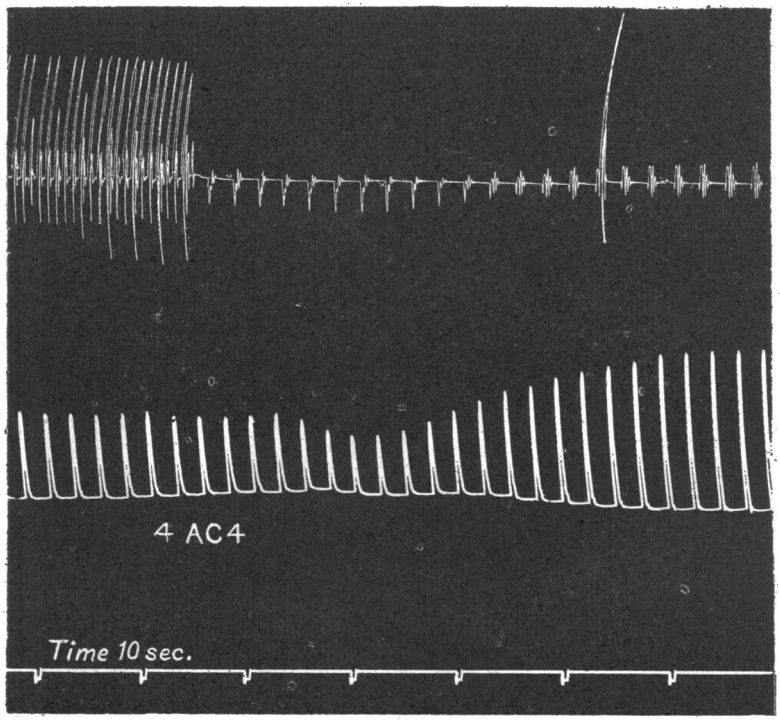


Fig. 8. Cat. The effects of acetylcholine (200$\mu$g.) in heart block produced by section of the A.V. bundle. When the auricle stopped beating the ventricular contractions continued to be transmitted slightly to the auricular lever. Upper record, auricle; lower record, ventricle.

*Section of the auriculo-ventricular bundle* greatly reduces the inhibiting action of acetylcholine on the ventricle but not on the auricle. In some hearts, as much as 500$\mu$g. acetylcholine have a negligible weakening effect only on the ventricle. This difference in the sensitivity of the auricle and ventricle to the depressant action of acetylcholine is evident from Figs. 8 and 9. The stimulating action of acetylcholine in suitable dosage, on the other hand, may affect the ventricle only. In the experiment shown in Fig. 8 heart block had been produced by section of the A.V. bundle; the injection of 200$\mu$g. acetylcholine

produced stoppage of the auricular contractions; the ventricular contractions, however, after an initial short period of slight depression, became much stronger. No atropine had been given in this experiment. The inhibiting action of acetylcholine on the ventricle is so small that it does not mask the stimulating effect. Before section of the bundle the effect of a similar dose of acetylcholine was strong inhibition of the ventricle. In the experiment of Fig. 9, section of the A.V. bundle had caused cessation of ventricular action; no atropine was given; the ventricle was caused to contract rhythmically by

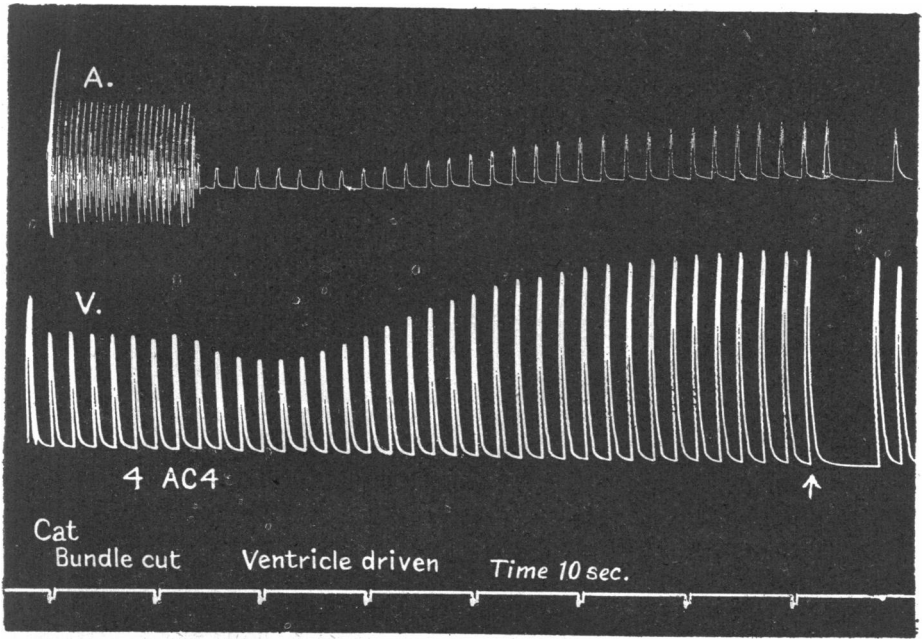


**Fig. 9. Cat. The action of acetylcholine (200 $\mu$g.) immediately after section of the A.V. bundle, the heart being driven electrically. At the arrow the electrical drive was omitted for three stimulations. Upper tracing, auricle; lower tracing, ventricle.**

electrical stimulation. The tracing again illustrates the difference in the action of acetylcholine on ventricular and auricular contractions. This difference may be seen even in hearts without section of the A.V. bundle. In weakly atropinized hearts acetylcholine sometimes still depresses the auricle but stimulates the ventricle (Fig. 10).

*Comparison with adrenaline.* When the stimulating action of acetylcholine on the atropinized heart is compared with that of adrenaline the following differences are noted: (1) Stimulation by acetylcholine in fresh hearts lasts longer than an equivalent stimulation by adrenaline. (2) On a heart not beating vigorously, adrenaline usually acts to increase the frequency and force,

acetylcholine more commonly affects the force only. Acceleration by acetylcholine may occur occasionally, but it is then more abrupt in onset than the acceleration produced by adrenaline. (3) Acetylcholine is more effective in equalizing irregularities of heart action, especially alternate beats of varying strength.

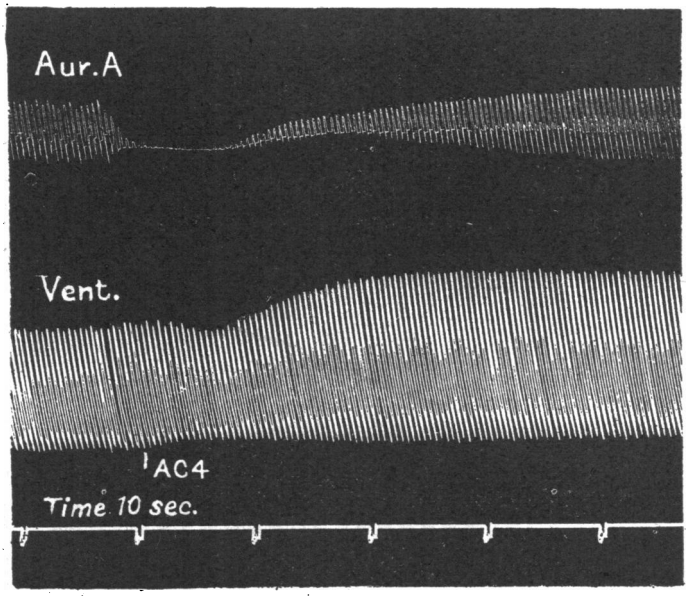


Fig. 10. Cat. The effect of acetylcholine (50$\mu$g.) on the auricle (upper tracing) and ventricle, showing the much greater sensitivity of the auricle after a dose of atropine (16·6$\mu$g.).

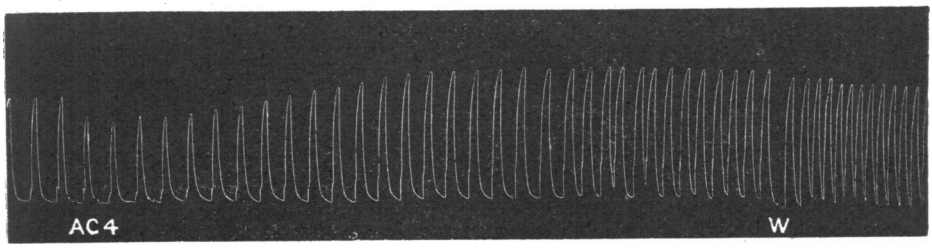


Fig. 11. The effect of acetylcholine (1 : 10,000) dropped on a suspended frog heart *in situ* and washed off at *W*. Note later acceleration.

*Frog's heart.* The stimulating effect of acetylcholine can also be demonstrated in experiments on the frog's heart. It is not, however, obtained so regularly as on the mammalian heart. If a drop of acetylcholine, 1 : 10,000, is applied to the frog's heart attached to a cardiograph in the usual way, slowing and weakening is seen. With smaller concentrations of acetylcholine

there is still slowing, but the heart beats vigorously (Fig. 11). If the acetylcholine solution has been warmed to counteract the slowing, the sole effect may be an increased force of contraction. The stimulating effect can be well demonstrated when the acetylcholine dropped on the heart is washed off a minute later. A heart which, before the acetylcholine treatment, was beating rather weakly may beat vigorously after washing out the acetylcholine. Controls with washing off drops of saline solution have no effect of this kind. Atropine, in doses just sufficient to abolish the inhibiting action, does not abolish the stimulating effect of acetylcholine, eserine enhances it.

## DISCUSSION

The results given above may be looked upon as bridging a gap in our knowledge of the action of acetylcholine on the heart and on muscle. They show that, apart from the well-known action of acetylcholine in inhibiting impulse formation and conduction and in weakening auricular contraction, acetylcholine has a stimulating effect, especially on the ventricle. This effect is seen on the non-atropinized heart with minute doses of acetylcholine. Larger doses inhibit the ventricle, and, in order to unmask the stimulating effect of acetylcholine, atropine or methylene blue has to be given. When the A.V. bundle has been severed, however, large doses of acetylcholine may stimulate the ventricle even without atropine. It appears that the main action of acetylcholine on the ventricle is one of increasing the force of contraction.

The stimulating action of acetylcholine appears to be a direct one on the cardiac muscle, for it persists after the administration of nicotine in amounts apparently large enough to paralyse any ganglia which may be present.

Since acetylcholine is normally liberated by the vagus, the extent to which the stimulating action of the substance may be a physiological phenomenon requires consideration. In this connexion the fact that the effect is primarily one on the force of the heart may be considered of special importance, since vagal action on the heart under physiological conditions must always be associated with an increased force of contraction of the heart. It has already been pointed out (McDowall, 1926) that the development of vagus restraint of the heart is of special significance in relation to the output, and that its well-known development during physical training is presumably associated with greater efficiency of the cardiac muscle, which can then deal with the increased filling of a longer diastole. Hitherto, the increased force of contraction has been generally looked upon as purely a function of the cardiac muscle in accordance with Starling's Law of the Heart, and it has been presumed that vagal stimulation has no direct effect on the ventricular muscle, since the post-ganglionic vagal fibres do not innervate the ventricular muscle. If we assume, however, that some of the acetylcholine released by vagal action escapes to the ventricle, it would have a stimulating action on the force of the ventricular contraction.

It is, therefore, possible that the increased force of ventricular contraction during vagal slowing is in part a function of the cardiac muscle in accordance with Starling's Law of the Heart and in part an effect of the released acetylcholine.

What is probably more important physiologically is that when the inhibitory action of acetylcholine passes off, there follows a period during which there is an increased response to adrenaline. Thus it may be considered that when, in emotional stress or severe action, the normal vagus restraint of the heart is reduced, the heart is made all the more sensitive to sympathetic action. The value of such an arrangement is obvious, and how far it may be true that the parasympathetic in general acts as a sensitizer to the sympathetic is a field of further investigation. Such a possibility would explain the value of the apparently very extravagant continued release of acetylcholine by the parasympathetic in various parts of the body. This assumes that the acetylcholine released by the vagus has, like injected acetylcholine, access to the site of action of adrenaline; although this seems probable, it does not, however, exclude the possibility that the parasympathetic nerves may first liberate acetylcholine which subsequently acts on an adrenaline (or sympathin) releasing mechanism.

SUMMARY

1. It is shown that acetylcholine can, in certain circumstances, stimulate the heart. This occurs when it is administered in small doses to suitable hearts or at the commencement of the action of large doses or, with large doses, if the better known inhibiting action of the substance is abolished by atropine or methylene blue.

2. The stimulating action is enhanced by eserine after atropine, but may be abolished by ergotoxine or large doses of atropine.

3. The stimulating action is seen after the administration of nicotine in sufficient dosage to paralyse autonomic ganglia and if the ventricular muscle is driven electrically. It therefore appears to be a direct action on the cardiac muscle.

4. The effect, compared with that of adrenaline, is more on the force of the heart than on the frequency. The possible significance of these facts in the intact animal is discussed.

In conclusion, I should like to thank Dr D. B. Taylor for confirming many of the results in his classes in Pharmacology, and also Mrs Lynn, Miss Lee and Mr Stonard, whose excellent performance of routine duties for two years in a laboratory peculiarly exposed to enemy action, contributed greatly to the success of the work.

*Note added in Proof.* Since this paper was submitted, a paper by Hoffmann, Middleton & Talesnik (*Amer. J. Physiol* (1945), **144**, 189) has appeared confirming the main results and adding the observation that the stimulating

action of acetylcholine is abolished by curare and is accompanied by a release of adrenaline into the coronary outflow. The results on which my paper is based had been communicated to the Physiological Society in June and November 1944.

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