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THE ACTION OF A SINGLE VAGAL VOLLEY ON THE RHYTHM OF THE HEART BEAT.

By G. L. BROWN and J. C. ECCLES.

(*From the Physiological Laboratory, Oxford.*)

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It may now be taken as established that the vagus exerts its inhibitory action on the heart by liberating a substance which biological tests have been unable to distinguish from acetylcholine and which in this paper will be called A.C. substance (see Feldberg and Krayer [1933] for a recent survey of the evidence). Three problems arise out of this first important step towards a solution of the vagal action on the heart:

(1) How do impulses in the postganglionic nerve fibres of the vagus liberate A.C. substance?

(2) What factors govern the transport of A.C. substance from the region of its liberation to the site of its action?

(3) How does A.C. substance exert its inhibitory effect on the heart, *e.g.* how does it act on the rhythmic mechanism of the pacemaker?

This paper and the next are for the most part concerned with the third problem, but some of the evidence also bears on the first and second problems. In the present paper a detailed study has been made of the effect on heart rate produced by a single volley of impulses down either the right or left vagus of the cat. A preliminary account of some of this work has already been published [Brown, Eccles and Hoff, 1932].

The inhibitory effect of single stimuli applied to the vagus was first described by Donders [1868], who found that the slowing of the heart rhythm persisted for several beats. Nüel [1874] stated that a single stimulus to the frog's vagus had no effect on the heart, but Heidenhain [1882] found that a very definite slowing was produced. Gaskell [1883] observed that a single shock applied to the vagus produced a prolonged diminution in the contraction of the tortoise heart, and recently Gilson

[1932] has subjected this action of the vagus to a detailed investigation. Trendelenburg [1902] found that a single shock to the frog's vagus had only a very small effect in slowing the heart rate. These observations of Trendelenburg together with those of Nüel provided an experimental basis for the now discredited idea that the vagus is an iterative nerve [Lapicque, 1912; Chauchard and Chauchard, 1922].

I. Method.

Decerebrate cats were used in all experiments. The heart was exposed at its base and the action potentials of the pacemaker were led off to an amplifier and Matthews oscillograph through two silver wires fixed to the surface of the heart, one being in the sino-auricular node and the other in the auricle. The vagus was dissected in the neck, divided and the peripheral end was stimulated by glass shielded silver electrodes. A detailed description of the experimental technique is given by Eccles and Hoff [1934*a*].

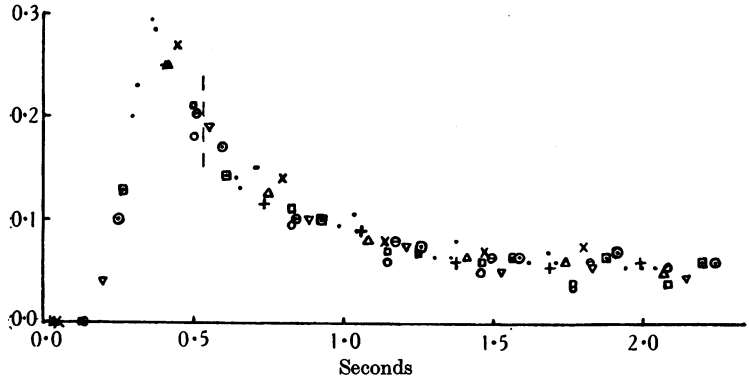
II. Experimental results.

(1) *Introduction.*

Pl. I, fig. 1, shows a series of observations in which a single induction shock (signalled by the stimulus artefact) has been applied to a vagus nerve. In each observation it is evident that there has been a lengthening of the cardiac cycles following the stimulus. Similar results have been obtained throughout each of our twenty-eight experiments, thus confirming Donders [1868]. In our experiments the induction shocks were delivered from coreless coils and were much too weak to have set up more than one impulse in any nerve fibre, a conclusion which was confirmed by records of the action potential of the vagus. Therefore a single volley of impulses passing down the vagus nerve—henceforth called a vagal volley—inhibits the rate of the heart beat, *i.e.* in so far as its negative chronotropic action is concerned, the vagus is not an iterative nerve.

The time course of this inhibition has been depicted by plotting the lengthening of each cycle (expressed as a fraction of the normal cycle) against the interval that lies between the vagal stimulus and the end of that cycle [cf. Donders]. This particular method of plotting will be justified later. In this way a series of points is obtained for any one observation. But the intervals between these points are too long to allow the details of the course of the inhibition to be determined. This disadvantage is overcome by plotting the points from several observa-

tions, as is done for example in Text-fig. 1, which is typical of the simple type which has been obtained in many experiments. After the latent period the curve rises rapidly to a sharp summit, after which it falls off, at first rapidly and then more and more slowly. This decline has in some experiments been followed over several seconds. The height of any part of the inhibitory curve constructed in this way is obviously related to the intensity of the inhibition at that point, and so the curve gives some idea of the time course of the inhibition. Similar curves have been obtained by Gilson [1932] for the negative inotropic effect of a single vagal volley on the tortoise auricle.

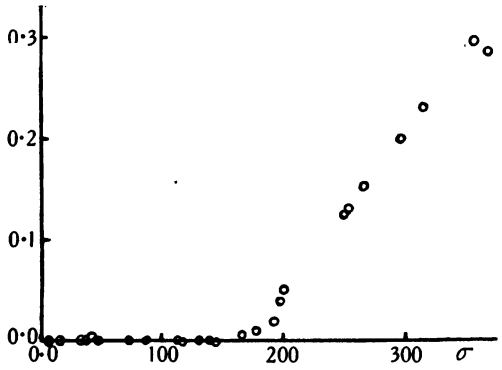


Text-fig. 1. The inhibitory curve produced by applying a single stimulus to the left vagus. The lengthening of each cycle, *i.e.* the amount by which it exceeds a normal cycle, is expressed as a fraction of a normal cycle (305σ), and is plotted against the interval between the vagal stimulus and the end of that particular cycle. In this way a series of points is obtained for each observation. Some observations are reproduced in Pl. I, fig. 1. Text-fig. 1 comprises series of points from thirteen observations, nine series being marked in a characteristic way. With all points to the left of the perpendicular broken line the previous cycle was not inhibited, with points to the right it was.

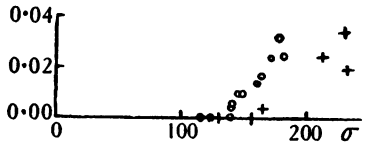
With a submaximal vagal volley the inhibitory curve is lower, *i.e.* the intensity of the inhibition is less, and at least several grades of this submaximal effect may be obtained by varying the size of the submaximal volley. Since this happens even when the action potential indicates that there has been no displacement of the pacemaker, several preganglionic fibres must be in physiological connection with the postganglionic fibres distributed to the pacemaker. This indicates that the pacemaker probably has a significant extensity, a conclusion previously reached in discussing the disturbance of rhythm produced by early premature beats [Eccles and Hoff, 1934*c*].

(2) *Latent period.*

Consideration of Pl. I, fig. 1, and Text-fig. 1 shows that when the stimulus falls late in any given cardiac cycle, that cycle is not lengthened: whereas a stimulus falling early in a cycle produces a lengthening of the cycle in which it falls. Text-fig. 2 shows clearly that there is no lengthening of those cycles which end at intervals shorter than 160σ after the vagal stimulus (cf. observations 1, 2 and 3, Pl. I, fig. 1). It will be seen that



Text-fig. 2. A series of observations plotted as in Text-fig. 1, but with a more extended time scale, the rising part of the inhibitory curve being shown. Length of normal cycle $=305\sigma$.



Text-fig. 3. As in Text-fig. 2 in another experiment (right vagus stimulated), the crosses representing points obtained with normal rhythm (cycle $=350\sigma$) and the circles points obtained during tetanic stimulation of accelerantes (cycle $=275\sigma$). The latent period is shortened from about 156 to 136σ.

an accurate evaluation of this latent period is difficult on account of the gradual onset of the inhibition, but in fourteen experiments the latent period has been determined with an error of not more than 10σ. In twelve its duration has been between 100σ and 160σ (see Text-figs. 3, 19 and 20), but in the remaining two experiments it had a duration of about 200σ, the cardiac cycle itself being exceptionally long—500σ or more (Text-fig. 18). In the twelve other experiments there was no correlation between the latent period and the duration of the cardiac cycle.

The values given by Donders [1868] for the latent period were rather longer, but he used the mechanical response of the ventricle to signal the instant of the heart beat.

Since the vagus was stimulated in the neck, part at least of the latent period of its effect must be due to conduction time from the point of stimulation to the endings in the pacemaker. The rate of conduction in the preganglionic pathway has been measured directly by stimulating the cardiac branches of the vagus in the thorax and recording the action potential from the vagus in the neck. By using stimuli just above and below the threshold for the inhibition of the heart rate, the corresponding wave in the complex nerve action potential may be identified, and so its conduction rate may be directly measured. The average rate of conduction found in this way is 30 metres a second, and since the total length of the preganglionic pathway is about 11 cm., only about 4σ would be occupied in the preganglionic conduction time. Heinbecker [1931] states that the negative chronotropic fibres of the turtle vagus belong to the *C* group and on analogy it has been suggested that the negative chronotropic fibres in the cat belong to the *C* group also [Heinbecker and O'Leary, 1933], but determinations of conduction velocity, threshold and refractory period [Brown and Eccles, 1934] show that in the cat these fibres form a fast component of their B_2 group. The short synaptic delays in the superior cervical ganglion, about $2–6\sigma$ [Brown, 1934; Eccles, 1934], and in the spinal cord for the simple flexor reflex, $3–4\sigma$ [Eccles and Sherrington, 1931] make it unlikely that more than a small part of the latent period could be accounted for as a synaptic delay between the pre- and postganglionic fibres. (Lawrentjew [1929] confirms the existence of this synapse in the vagal pathway.) Moreover, the postganglionic fibres are probably so short (Woollard [1926] and Anufriew [1928] describe ganglia just over the sino-auricular node) that the conduction time in them could only be a few σ at the slow conduction rate of the *C* group fibres. Hence only a fraction of the latent period is occupied by the time of travel of impulses from the point of stimulation to the fibre terminals in the pacemaker.

This conclusion is supported by those experiments in which direct electrical stimulation of the pacemaker was complicated by inhibition presumably arising from stimulation of the terminal vagal fibres, of the ganglia, or of the postganglionic fibres [Eccles and Hoff, 1934*b*]. The latent period of this inhibition was only about 20σ shorter than the latent period following stimulation of the vagus in the neck. This value

of 20σ is in agreement with the duration which has just been calculated for the total nerve conduction time.

That part of the latent period not explicable as nerve conduction time is most probably occupied in the liberation of the A.C. substance, in its diffusion to the pacemaker, and in its action on the rhythmic mechanism. There is no concensus of opinion with regard to the relation of the nerve fibres to the muscle fibres of the sino-auricular node [Meiklejohn, 1913; Fukutake, 1925; Woollard, 1926; Lawrentjew, 1929], though Boeke [1932] strongly advocates the existence of intramuscular nerve endings. The paucity of well-defined nerve endings in heart muscle makes it seem likely that A.C. substance is liberated from the whole length of the ramifying nerve fibres by an impulse passing along them. In this connection it is of interest to note that Witanowski [1925] and Chang and Gaddum [1933] found that both sympathetic nerves and sympathetic ganglia were very rich in an A.C. substance, and recently Feldberg and Gaddum [1934] have shown that during stimulation of the cervical sympathetic an A.C. substance is liberated into fluid perfused through the superior cervical ganglion.

There is no appreciable difference between the latent periods of the inhibitory effects produced by maximal or submaximal vagal volleys. The latter have been set up by submaximal stimulation of the whole nerve or by maximal stimulation of the nerve above a region where it is partly transected. Moreover, there is no appreciable difference between the latent periods for the right and left vagi.

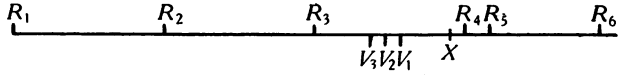
Shortening the cardiac cycle by a background tetanic stimulation of the nervi accelerantes was accompanied by a relatively smaller shortening of the latent period in three of the four experiments (Text-fig. 3). Conversely lengthening of the cardiac cycle by a background tetanic stimulation of the other vagus was associated with a relatively smaller lengthening of the latent period in two of the three experiments. In the two exceptional experiments the latent period was unaltered.

(3) *The rising phase of the inhibitory curve.*

At the beginning of the inhibitory curve the rise is slow, but it is always followed by a steeper ascent. With intense inhibitions this ascent may be very steep and the first inhibited cycle may be more than twice the duration of a normal cycle. Pl. I, fig. 2, shows a series of such strong inhibitions, and in Text-fig. 4 is a diagrammatic representation of the conditions. $R_1R_2R_3R_4$ is the normal rhythm, and V_1 is a vagal

stimulus which just fails to lengthen the cycle R_3R_4 (cf. observation 1, Pl. I, fig. 2). V_1R_4 is therefore the latent period.

V_2 represents the position of an earlier vagal stimulus which would delay the next beat from R_4 to R_5 (cf. observations 2 and 3 of Pl. I, fig. 2). Assuming that there is no uninhibitible period at the end of a cycle, V_1R_4 is the time taken by the vagal stimulus to produce an inhibitory effect on the actual rhythmic mechanism. When the vagal stimulus is at V_2, the point X therefore represents the beginning of the inhibition of the rhythmic mechanism, V_2X equalling V_1R_4. The beat is delayed by this inhibition, but eventually the rhythmic mechanism prevails and the next beat is set up at R_5. A gradual shifting of the vagal stimulus from V_1 to V_2 would reveal a progressive increase in the inhibitory effect, *i.e.* in the inhibitory lengthening of the cycle. This could depend on two factors:



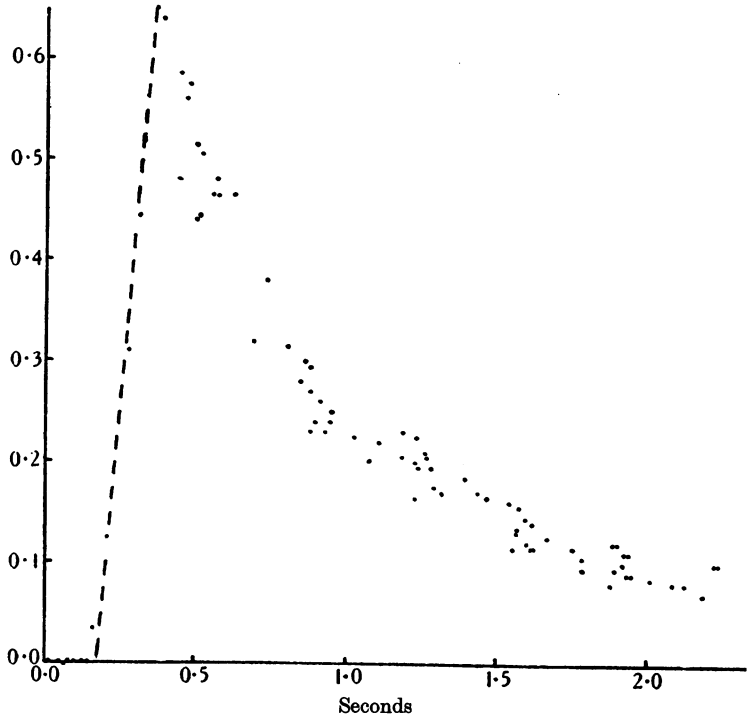
Text-fig. 4. Diagram to illustrate the varying effect of the vagal stimulus according to its position in the cardiac cycle. Full description in text.

(*a*) A progressive increase in the concentration of A.C. substance acting on the rhythmic mechanism. This would be likely to be dependent both on differences between the latent periods of the several vagal fibres acting on the pacemaker due for example to differences in the diffusion time of A.C. substance, and on the initial gradual increase in the effective A.C. substance produced by an impulse in any one of these fibres.

(*b*) The longer the beat is delayed, the longer the rhythmic mechanism would be subjected to the action of the A.C. substance produced by each impulse. It will be seen later that this factor has little if any significance.

If the vagal stimulus were at V_3 instead of V_2 (Text-fig. 4), the next beat might occur at R_6 instead of R_5 (observation 4, Pl. I, fig. 2). This sudden increase in the inhibitory effect would be due to a further development of the conditions obtaining when the vagal stimulus was at V_2. With the stimulus at V_3 the earlier development of the inhibition (relative to the cycle) would serve to postpone the next beat still more (relative to the vagal stimulus), thus allowing the full development of the inhibitory effect to be exerted on the first cycle. Very small alterations in the position of the vagal stimulus in a cycle may therefore be accompanied by a great increase in the inhibitory lengthening of that cycle. Such a

condition may be observed in the steep rising phase of Text-fig. 5. The limiting angle of steepness is indicated as a broken line. It represents the line of the interruption in the curve that would be obtained if during any period the inhibition was so intense that it absolutely prevented the setting up of beats. Such a condition is equivalent to a "breaking" of the inhibitory wave. This condition has only been observed once in our experiments, but it will be seen that in Text-fig. 5 the steepest part of the curve approaches to this limiting slope.



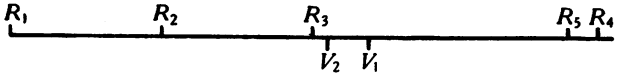
Text-fig. 5. A series of observations plotted as in Text-fig. 1 and from the same experiment, the stimulus being applied to the right vagus. The oblique broken line shows the limiting angle of steepness of the rising phase of the curve.

A very steep ascent is present in all experiments in which the vagal inhibition is large. The smaller the vagal inhibition the less steep is the ascent of the curve, *e.g.* the curve for the left vagus (Text-fig. 1) is less steep than the curve of Text-fig. 5 simultaneously obtained for the right vagus (see also Text-fig. 20, and Text-fig. 2 of the next paper). When the inhibitory effect of a vagal volley is diminished by partly cutting

through the vagus or by using submaximal stimuli, the ascent of the curve also becomes less steep (see Text-fig. 19). The maximal lengthening of cardiac cycle produced by a single vagal volley varies greatly from experiment to experiment. The extreme values for the right vagus have been 130 and 6 p.c., and for the left vagus 68 and 3 p.c.

(4) *Summit and descending curve.*

As a rule the summit of the inhibitory curve is fairly sharp, and usually it is reached by cycles in which the vagal stimulus fell early. When the inhibition is very large, the first inhibited cycles may also form a considerable part of the descending curve. In such cases the inhibition is less when the vagal stimulus is early in a cycle than when it is a little later. Thus in Text-fig. 6 $R_1R_2R_3$ represents the normal

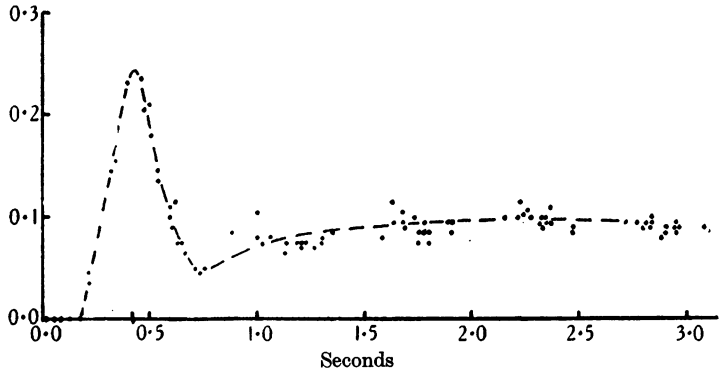


Text-fig. 6. Diagram of the same type as Text-fig. 4. Full description in text.

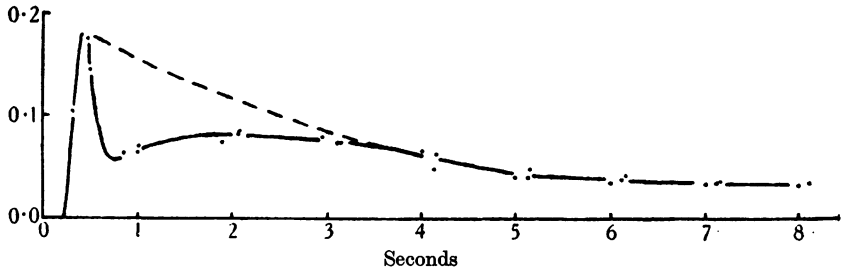
rhythmic beat, and V_1 is a vagal stimulus at the optimal point causing a lengthening of the next cycle to R_4; however, an earlier vagal stimulus V_2 only lengthened the cycle to R_5. Such a condition is illustrated by the series of observations in Pl. I, fig. 3, which are arranged from above downwards so that the vagal stimulus is progressively earlier in the cycle, the beats before the vagal stimulus being synchronized. The significance of this condition will be discussed later. In two experiments the decline was so rapid that over a certain range V_2R_5 was equal to V_1R_4 (Text-fig. 6), *i.e.* the next beat occurred at a constant interval after the vagal stimulus. For such a range in the position of the vagal stimulus the condition of the rhythmic mechanism at the onset of the inhibitory effect had no determining influence on the time of the next beat!

(*a*) *The single-wave type of inhibitory curve.* In some experiments the decline in intensity of the inhibitory effect goes on steadily, but at a gradually decreasing rate throughout the succeeding cycles, *e.g.* Text-figs. 1, 5, 10 and 13, the inhibitory effect being a simple wave with a steeper ascent than descent. The descending part of the curve may be approximately exponential in type, the amount of the lengthening being halved in times which in different experiments may be as long as 0·8 sec. or as short as 0·4 sec. It will be seen later that within limits the lengthening of the cycle is approximately proportional to the concentration of

A.C. substance [cf. Brown and Eccles, 1934], and so it appears that the concentration of A.C. substance declines along an exponential curve, being halved in every 0·8–0·4 sec. When acetylcholine was being destroyed by the blood esterase in *in vitro* experiments Galehr and Plattner [1928] and Matthes [1930] found that the concentration of acetylcholine also declined along an exponential curve, but for the



Text-fig. 7. A series of observations as in Text-fig. 1, but in another experiment, the right vagus being stimulated. Normal cycle = 520 σ.



Text-fig. 8. A series as in Text-fig. 7 and later in the same experiment (cycle 500 σ). The second inhibitory wave is followed for a much longer time. The broken line illustrates the true shape of the inhibitory wave, if the trough between the two waves is due to a transient intercurrent acceleration.

esterase of cat's blood the concentration of acetylcholine was halved every 50 sec., *i.e.* about one hundred times slower than the above rate. This difference is of course due to the much larger concentrations of acetylcholine used by Galehr and Plattner. If concentrations of acetylcholine equivalent to those of the A.C. substance liberated in the heart had been used as the substrate, the rate of decline in concentration would probably have been comparable with that indicated by the vagal inhibitory curves.

(*b*) *The double-wave type of inhibitory curve.* Text-fig. 7 is typical of many experiments in which an abrupt descent from the primary wave is followed by a second slow rise and fall in the inhibitory effect. The secondary inhibitory wave in this experiment is followed for a longer time in Text-fig. 8 which is constructed from a series of observations taken a few minutes later than those of Text-fig. 7. This type of curve has been particularly common in hearts in which both the nervi accelerantes and vagi had been cut, being found in fifteen out of nineteen

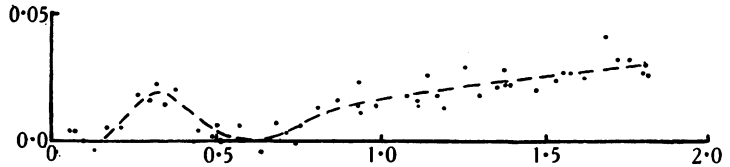


Fig. 9 *a*.

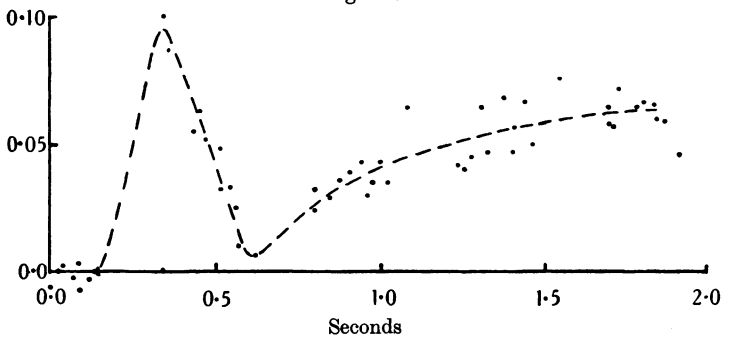


Fig. 9 *b*.

Text-fig. 9. As in Text-fig. 7 in another experiment, *a* being for the left vagus, and *b* for the right vagus. Normal cycle = 460 σ. The observations were not continued sufficiently long for the summit of the second inhibitory wave to be reached.

such experiments. The descent from the primary wave is steeper than with those inhibitory curves which have no secondary inhibitory wave, the lengthening being halved in as short a time as 0·1 sec. (Text-figs. 7 and 9). The secondary inhibitory wave is first perceptible 0·6–1·5 sec. after the vagal stimulus and attains its maximum 2–2·5 sec. after the stimulus. At the summit of the primary wave there may be even more than a doubling of the duration of the cardiac cycle, but with the secondary wave the maximal lengthening is never more than 12 p.c. In some experiments the secondary wave is feebly developed and appears

only as a hump on the descending part of the primary wave (Text-fig. 2 next paper), while in other experiments the secondary inhibitory wave reaches a higher summit than the primary, *e.g.* Text-fig. 9*a*.

(5) *Comparison of the inhibitory curves of the right and left vagi.*

In fifteen experiments the inhibitory curve has been determined for both the right and left vagi, and in each case the curves have been closely alike. In twelve experiments both curves were of the double-wave type (Text-fig. 9, and Text-fig. 2 of next paper), and in the remaining three experiments both were single waves only (Text-figs. 1 and 5). The inhibition of the right vagus always has been greater than the left, the inhibitory curve of the left vagus being indistinguishable from that given by a submaximal volley in the right vagus. For example, in experiments with inhibitory curves of the double-wave type the primary wave of the right vagus curve is, in comparison with the left, relatively higher than the secondary wave and the trough is less well marked and later (cf. Text-figs. 9 *a* and *b*); but such differences also appear when the curve of a maximal vagal volley is compared with that of a submaximal, and so are to be attributed to differences in the intensity of the inhibition rather than to differences in the type of inhibitory action.

(6) *Effect of a tetanic background stimulation of the accelerantes or other vagus.*

The effect with accelerantes has been investigated in five experiments. In two the inhibitory curve was of the single-wave type, and it was unaltered by the accelerantes stimulation, *e.g.* Text-fig. 10*a* was obtained before and Text-fig. 10*b* during an accelerantes tetanus. In the other three experiments the inhibitory curve was of the double-wave type and in all the accelerantes tetanus altered it towards the single-wave type, the slow secondary wave being less prominent, and even completely disappearing with a marked acceleration. Thus, during tetanic stimulation of the right nervi accelerantes, the double inhibitory curve of Text-fig. 7 is transformed to the simple curve shown in Text-fig. 11, the original double curve being shown by the broken line. When owing to fatigue the acceleration is less marked, the secondary wave does not completely disappear. It remains as a hump on the descending part of the primary wave (Text-fig. 12). A slight hump is also present on the descending part of Text-fig. 11.

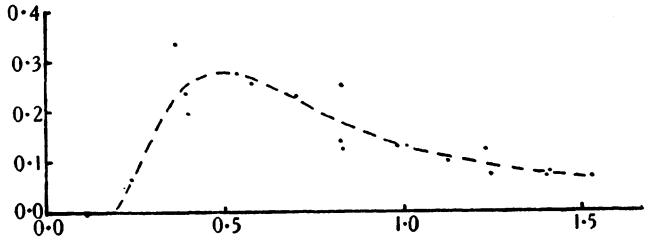


Fig. 10 *a*.

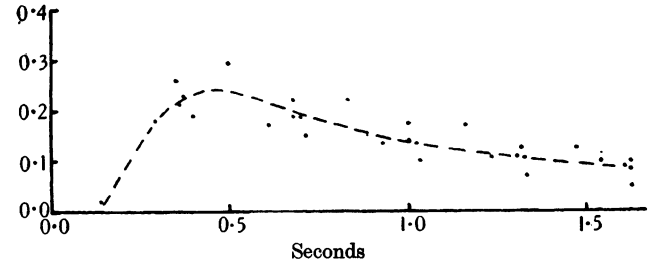
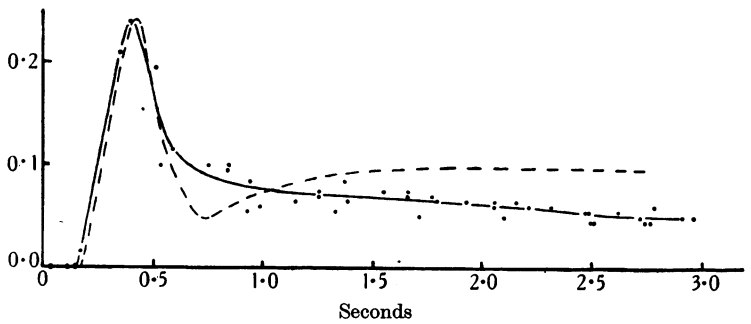


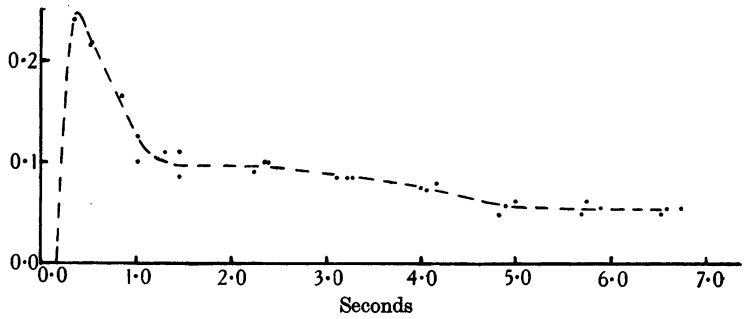
Fig. 10 *b*.

Text-fig. 10. Two inhibitory curves similar to Text-fig. 1, but in another experiment, the right vagus being stimulated. *a* was obtained with the normal rhythm (cycle = 380 σ), and *b* during tetanic stimulation of the right nervi accelerantes (cycle = 275 σ). The rising phases of the curves are approximate only, but their general shapes were indicated by other series of observations from the same experiment.



Text-fig. 11. A series of observations taken concurrently with those of Text-fig. 7 (same experiment), but with, in addition, tetanic stimulation of the right nervi accelerantes, which shortened the cycle from 520 to 380 σ. The broken line shows the curve of Text-fig. 7.

The normal tonic action of accelerantes is similar in effect to tetanic stimulation. If the tonic action is considerable, the inhibitory curve has a single wave or at most a slight second hump. Removal of accelerantes tone by cutting both nervi accelerantes causes the slow secondary wave to appear. The secondary inhibitory wave is therefore closely related to the absence of an accelerantes action on the heart, but it may not be present even when both nervi accelerantes and both vagi are cut (see Text-fig. 10*a*). Tetanic stimulation of one vagus nerve has also been observed to remove the secondary inhibitory wave produced by the other vagus.



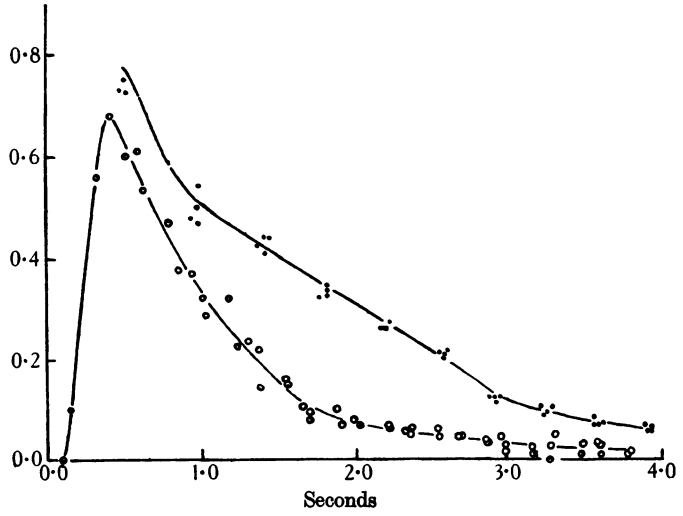
Text-fig. 12. A series of observations later in the same experiment (concurrently with Text-fig. 8). Tetanic stimulation of the right nervi accelerantes now only shortened the cycle from 500 to 420 σ. The second inhibitory wave is still present.

Acceleration or slowing of the rhythm is often accompanied by shift of the pacemaker to another rhythmic centre, and it might be thought that this would account for the change in the shape of the inhibitory curve, but with such an explanation it would be unlikely that the double-wave type should always change towards the single-wave type, and that the single-wave type should remain unaltered. Moreover, in some experiments the absence of an appreciable change of action potential during acceleration has indicated that the change in the curve was not due to a displacement of the pacemaker.

(7) *The action of eserine and atropine.*

(*a*) Eserine has been given intravenously in doses as small as 25γ. When the inhibitory curve belongs to the single-wave type, the summit of the curve is made higher by eserine and its descent is slowed as shown in Text-fig. 13. This effect is due to the eserine inhibiting to some extent the esterase which destroys the A.C. substance [Loewi and Navratil,

1926; Engelhart and Loewi, 1930; Matthes, 1930]; hence it may be concluded that the decline of the inhibitory curve is due to the esterase destroying the A.C. substance. Since the latter part of the rising phase of the inhibitory curve is made steeper by the action of eserine (Text-figs. 14 and 15), this enzymatic destruction of the A.C. substance must be proceeding even during the short time that the concentration of A.C. substance is increasing. It has been suggested that, in addition to inhibiting the esterase, eserine might also increase the production of A.C. substance, but there are no experimental observations which cannot be explained by its known inhibitory action on the esterase.

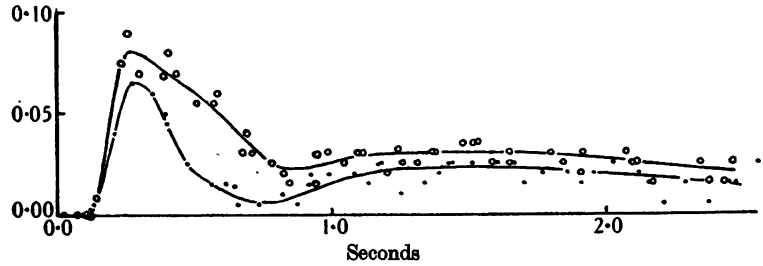


Text-fig. 13. The curve drawn through the circles is the normal inhibitory curve produced by a single volley in the left vagus, the curve through the dots being obtained after injection of 25γ eserine. Weight of cat, 1·75 kg.

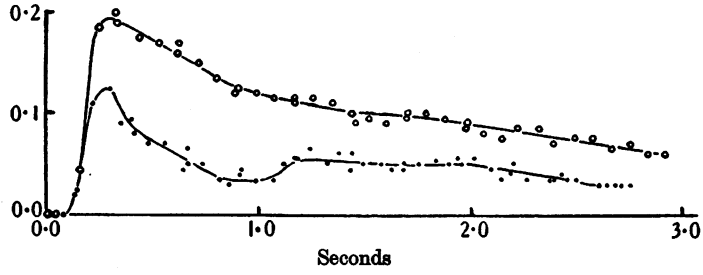
If the A.C. substance only suffered enzymatic destruction after it had diffused into the blood, changes in the rate of this enzymatic destruction would have very little effect on the curves expressing the time course of the concentration of A.C. substance acting on the pacemaker, for the flow of blood would in any case rapidly remove such A.C. containing blood from the region of the sino-auricular node. But, since partial paralysis of the esterase by eserine causes a corresponding slowing in the removal of A.C. substance, it may be concluded that most of the esterase destroying the A.C. substance is not in the blood but in the tissues. Moreover, the rapidity of the destruction of the A.C. substance acting on

the pacemaker indicates that the esterase must be in very close proximity to it.

When the inhibitory curve is of the double-wave type, eserine increases the height of both waves and slows their decline, but the secondary wave is affected to a less extent than the primary. This is observable in Text-fig. 14 after a dose of only 25γ. With larger doses of eserine the primary wave may be so prolonged that it overlaps the secondary wave



Text-fig. 14. The curve drawn through the dots is the normal inhibitory curve produced by a single volley in the left vagus, and the curve through the small circles is the inhibitory curve obtained 3–10 min. after injection of 25γ eserine. Weight of cat, 2·17 kg.

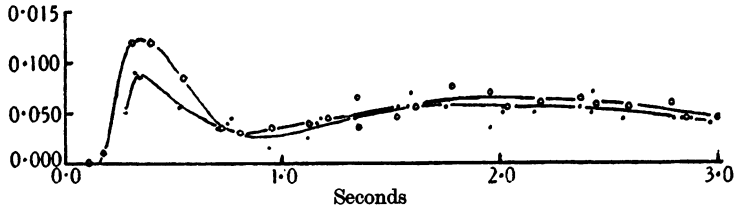


Text-fig. 15. As in Text-fig. 14, but in another experiment. The upper curve was obtained 2½–7½ min. after injection of 50γ eserine. Weight of cat, 2·75 kg.

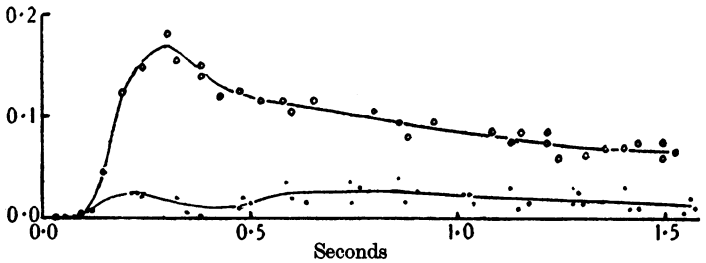
as is seen to occur in Text-fig. 15 after an injection of 50γ eserine. The relatively smaller effect of eserine on the secondary wave is to be expected, because, when the height of the primary wave is changed in other ways, the secondary wave suffers a relatively smaller change, *e.g.* it is decreased to a relatively less extent when submaximal stimuli are applied to the vagus, or when the left vagus is stimulated instead of the right, and it is increased to a relatively smaller extent when there is summation of the inhibitory effects of two vagal volleys [Brown and Eccles, 1934]. Eserine therefore seems to have an identical effect on

both waves; hence it may be concluded that both inhibitory waves are due to the action of the same A.C. substance and that the decline of both curves is due, at least in part, to the esterase destroying this A.C. substance.

The maximum effect of eserine is attained in 3 min. after its injection into the saphenous vein. This action is much more rapid than that occurring *in vitro*, for under such conditions eserine was observed by Matthes [1930] to take longer than 15 min. to exert its maximum inhibitory effect on the esterase. The effect of small doses of eserine had completely passed off in 2–3 hours after injection.



Text-fig. 16. The upper curve drawn through the circles is the normal inhibitory curve produced by a single volley in the left vagus, the lower curve through the dots being obtained after injection of 5γ atropine. Weight of cat, 2·35 kg.



Text-fig. 17. As in Text-fig. 16 in another experiment, but right vagus stimulated. The curve through the dots was obtained after injection of 25γ atropine. Weight of cat, 2·75 kg.

A dose of 25γ eserine given to a 2·5 kg. cat approximately halved the rate of decline of the first wave (Text-figs. 13 and 14). This corresponds to a dilution of 1 in 10^8 for the whole cat. Matthes [1930] observed that the action of the esterase *in vitro* was usually halved by a dilution of eserine in the proportion of 1 in 3×10^7, a value in agreement with our results if allowance be made for the fact that very little eserine would be distributed to such parts of the cat as the bones, and so the concentration in the pacemaker would be considerably higher than 1 in 10^8.

(*b*) Very small injections of atropine diminish both waves, but the primary relatively more than the secondary, *i.e.* the inhibitory curve resembles a normal submaximal curve. Text-fig. 16 shows the effect of 5γ atropine. The results with atropine therefore also indicate that the same A.C. substance is responsible for both inhibitory waves. When larger doses of atropine are given in experiments having an apparent single-wave type of inhibitory curve, a small secondary wave may be unmasked as shown in Text-fig. 17. Such small secondary waves may also be submerged in other examples of apparently simple waves, *e.g.* in Text-fig. 1 the flattening towards the end may be due to the small secondary wave [cf. Brown and Eccles, 1934].

III. Discussion.

A. *The double-wave type of inhibitory curve.*

(1) A possible simple explanation of double inhibitory waves would seem to be afforded by assuming a transient change of the pacemaker to another rhythmic centre, which was only subjected to an inhibition of the slow type indicated by the secondary inhibitory wave. Such an explanation would, however, not be consistent with the fact that in Text-fig. 7 there was no inhibition of the cycles preceding those which fell at the bottom of the depression between the two waves. If the second rhythmic centre became the pacemaker at that instant, *i.e.* if it were able to assume a dominant rhythm so little slower than the initial normal rhythm, then still more so should it have assumed the dominant rhythm in those observations in which there was more lengthening of the first inhibited cycle, *i.e.* in all those observations of the primary inhibitory wave. The hypothetical second rhythmic centre must, therefore, also have suffered the initial inhibition of the first wave, and hence the explanation breaks down.

The above argument applies still more to Text-fig. 9*a*, where it will be seen that with the left vagus there is no detectable lengthening of those cycles which end in the trough between the two waves. There is therefore no possibility of a change of pacemaker at this point. In all other experiments with primary and secondary inhibitory waves the above argument also excludes the likelihood that the secondary inhibitory wave could be due to a change of pacemaker, though its application is less obvious than it is in Text-fig. 9*a*. Moreover, in most experiments the action potential of the beats showed no appreciable change, *i.e.* there was probably no shift of pacemaker, so it would seem that the

two inhibitory waves occur in the one rhythmic mechanism. Thus in Pl. I, fig. 4, no change in the shape of the initial part of the action potential is detectable in any of the observations. These form part of the series from which Text-fig. 9 was constructed.

(2) Another plausible explanation is that the double inhibitory waves are produced by a transient acceleration introduced within a single inhibitory effect. For example, in Text-fig. 8 the true inhibitory curve might follow the broken line. Now a background tetanic stimulation of accelerantes changes the double-wave to the single-wave type, and this would seem to conform with this explanation of the double-wave type of inhibitory curve, for such an interpolated acceleration might be occluded by the background acceleration. There are two possible sources of such an interpolated acceleration.

(*a*) In order to interfere as little as possible with the blood supply to the vagus, it was usually dissected out for stimulation without being separated from the cervical sympathetic. It was possible, therefore, that any cardiac accelerator fibres in the cervical sympathetic would be stimulated at the same time as the vagal inhibitory fibres. However, the double inhibitory wave was still present in experiments in which the cervical sympathetic was excluded.

(*b*) Many investigators have demonstrated a cardio-accelerator action of the mammalian vagus. The observations of the earlier workers, *e.g.* Rutherford, Boehm, Schiff and Arloing appear to be due to stimulation of sympathetic fibres which arise from the middle and inferior cervical ganglia and join the vagus low down in the neck [cf. Dale, Laidlaw and Symons, 1910; Hering, 1924]. Anatomically there is no evidence that sympathetic fibres to the heart arise from the superior cervical ganglion [Perman, 1924], and physiological evidence is also against the existence of such fibres [Dale, Laidlaw and Symons, 1910; Hering, 1924]. Recently Morgan and Goland [1932] have found that cardiac acceleration is sometimes produced by prolonged tetanic stimulation of the vago-sympathetic trunk of the dog after the vagus has been cut proximal to the nodose ganglion and allowed to degenerate. It is probable that this acceleration is produced reflexly by afferent fibres in the sympathetic trunk.

With the exception of the cardiac acceleration described by Dale, Laidlaw and Symons [1910] and by Dale [1921] prolonged tetanic stimulation of the vagus has produced only a relatively small acceleration after a latent period of several seconds. It is clear that the accelerating effect of a single volley would be so slight and so long delayed that it could not produce the trough between the two inhibitory waves. The cardiac acceleration described by Dale and his co-workers, however, requires more careful consideration, for it had a short latent period and quickly reached its maximum intensity. The effect is developed during a series of tetanic stimulations of the vagus after its normal inhibitory action has been abolished by certain drugs, *e.g.* nicotine, tropine or quinidine. The action of the true sympathetic fibres has been excluded, so there are two possible explanations. One is that the vagus contains fibres which produce acceleration of the heart beat, and this effect is unmasked by drugs which paralyse its normally preponderant inhibitory action. The other is that, on account of the effect of the drugs, a series of tetanic stimulations of the vagus produces a curious state in the postganglionic neurones as a result of which they fire off repetitively when no impulses are reaching them from

the preganglionic terminals, the presence of such impulses immediately inhibiting their discharge.

Dale and his co-workers incline to the former more direct explanation, but as Hering [1924] points out their evidence is strongly in favour of the latter explanation. Thus the acceleration was usually absent or at most very slight until a marked and prolonged cardiac slowing developed after each tetanic stimulation, and acceleration during the stimulation usually just sufficed to restore the rhythm to the rate obtaining before the appearance of this slowing. Again the response of the heart to acetylcholine and other agents acting directly was observed to be normal, and the slowing between the periods of stimulation was quickly abolished by atropine, so it was almost certainly due to a prolonged discharge from the postganglionic neurones. Finally, it was impossible to obtain the acceleration apart from the inhibitory periods between the successive stimulations.

It therefore seems very unlikely that these experiments do demonstrate an accelerator action of the vagus sufficient to give rise to the trough between the primary and secondary inhibitory waves set up by a single vagal volley. The improbability of such an explanation of double inhibitory waves is more apparent when it is realized that a single volley would have to produce an acceleration of at least 10 p.c. in Text-fig. 8, and 15 p.c. in Text-fig. 7, the latent period of the effect being about 0·4 sec. and the maximum being reached in 0·8 sec. after the setting up of the vagal volley.

(3) Another explanation of the double inhibitory wave is that the secondary wave might be due to after discharge from the postganglionic neurones. But the secondary wave could only be produced in this way if the after discharge commenced about 300σ after the first discharge and continued for a second or more. Bishop and Heinbecker [1932], Brown [1934] and Eccles [1934] find no after discharge from the superior cervical ganglion. Even in the simple reflex pathways after discharge is a much simpler phenomenon, commencing immediately after the first discharge and continuing for a much shorter time. Finally, if the secondary wave were due to after discharge, it should not be reduced or abolished by a background tetanic stimulation of accelerantes.

It would seem therefore that the shape of the inhibitory curve is inherent in the rhythmic centre and is not conditioned by combinations of inhibiting and accelerating impulses, *i.e.* a single volley of inhibitory impulses in the postganglionic fibres of the vagus gives rise to the double inhibitory effect. The remaining two explanations conform with this conclusion.

(4) It has been seen that both waves of the double inhibitory curve are due to the action of A.C. substance on the pacemaker, so it seems possible that a single volley of impulses in the postganglionic fibres of the vagus might produce in the pacemaker a sudden accumulation of

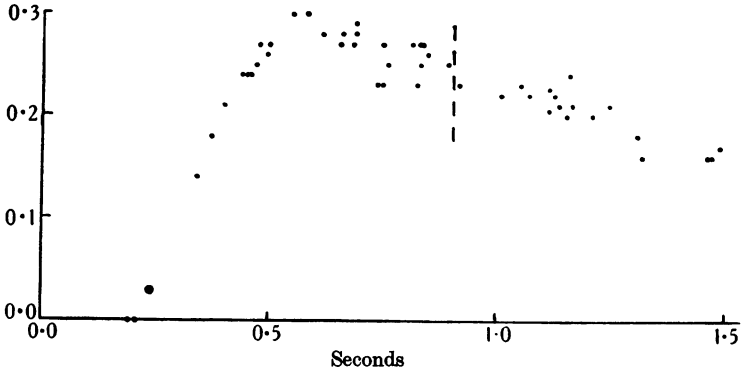
A.C. substance which is rapidly destroyed by the esterase, and is followed by a slower accumulation which is more slowly destroyed. This second accumulation of A.C. substance could perhaps be due to diffusion from surrounding tissues. Thus, for example, the muscle fibres of the sino-auricular node interlace through abundant elastic tissue. A.C. substance liberated from the nerve fibres passing through this elastic tissue would perhaps not be subjected to such strong esterase action as that liberated near the muscle fibres of the pacemaker, and so would survive long enough to diffuse into the pacemaker and produce the secondary wave. However, it does not seem that this plausible explanation can be reconciled either with the diminution or removal of the secondary wave by tetanic stimulation of accelerantes or with the observation that changes in the height of the primary wave are accompanied by a relatively smaller change in the height of the secondary wave.

(5) The following two observations suggest an alternative explanation. When there is a well-developed trough between the two inhibitory waves, it has already been noticed that the decline of the primary wave is always much more rapid than in the single-wave type, the lengthening being halved in as short a time as 0·1 sec. In one experiment the trough actually descended just below the base line, *i.e.* there was actually a period during which there was a slight shortening of the cardiac cycle. This does not necessarily mean that there was a stimulation of accelerantes fibres, for the very early subsequent beats observed by Eccles and Hoff [1934*c*] provide a comparable phenomenon. In order to explain their production it was suggested that a rhythmic centre contained "inhibitory components," and that these might be dissociated from the remainder of the centre consequently freeing it from their inhibitory action. If this dissociation were temporarily to occur during the inhibition from a single vagal volley, the trough between the two inhibitory waves would be formed and this trough could descend below the base line. In addition the abnormally steep descent of the primary wave would be explained.

B. *The single-wave type of inhibitory curve.*

By its negative chronotropic action the A.C. substance liberated by the vagus in some way delays the setting up of the next beat. It must do this by acting on some continuous process of excitement [cf. Eccles and Hoff, 1934*b*] underlying the production of the beat, for gradations in the extent of the vagal slowing may be produced by altering the size of the vagal volley. Such gradations are easily observed during

tetanic stimulation of the vagus even when the action potentials show that there has been no appreciable shift of the pacemaker. Gradations of this type indicate that, if a beat is delayed (inhibited), the continuous process of excitement which the A.C. substance inhibits goes on increasing after the normal time of this beat, otherwise a beat once delayed by a continued inhibition would be delayed indefinitely, *i.e.* there would be no such thing as a steady graded slowing of a rhythm. Inhibition would be all or nothing. But, if the excitement goes on increasing, it will eventually overcome the inhibitory effect of the A.C. substance, and so the beat will occur after a cycle longer



Text-fig. 18. An inhibitory curve as in Text-fig. 1, but in another experiment, the right vagus being stimulated. Normal cycle = 500 σ. The perpendicular broken line separates points with a previous inhibited cycle from those in which the previous cycle was not inhibited.

than normal. The lengthening of the cycle will bear some relation to the concentration of the A.C. substance; hence the occurrence of gradations in the inhibitory lengthenings.

In all inhibitory curves there is a transition between those points in which the previous cycle was not inhibited and those in which it was. Thus in Text-fig. 1 the points from each observation are marked in a characteristic way. The perpendicular broken line divides these points according as the previous cycle was or was not inhibited. It will be seen that there is no sign of a discontinuity of the curve in this region. Text-fig. 18 also gives a particularly good illustration of this absence of a discontinuity, for in this case even a small change would be detectable in the very straight curve which runs through the transitional region. In fact in no experiment has a discontinuity been apparent. Now the experiments with eserine show that the decline of the inhibitory curve

must be largely due to the destruction of the A.C. substance by the esterase, *i.e.* the A.C. substance present at the summit is gradually removed and so its inhibitory action gradually diminishes. If the setting up of a beat affected the inhibitory action of the A.C. substance, this change should be apparent as an alteration in the inhibition of the next cycle. A discontinuity would therefore be expected at the transitional region of the curves in Text-figs. 1 and 18. The invariable absence of any such discontinuity shows that the setting up of a beat by the rhythmic centre does not affect the inhibitory action of the A.C. substance existing in that centre. A comparable conclusion may be drawn from the results of Gilson [1932] who observed that the time course of the negative inotropic effect of a single vagal volley was unaltered by driving the auricle electrically at rates much faster than normal.

There are two ways in which the A.C. substance could exert its inhibitory effect.

(*a*) It could check the building up of excitement throughout the cycle.

(*b*) It could antagonize the action of excitement without affecting or being affected by its production. In the first case the inhibitory lengthening of a cycle would be dependent on the integration of the inhibitory effect of the A.C. substance throughout the whole cycle, in the second case it would only be related to the inhibitory effect of the A.C. substance present at the end of the cycle.

On account of a supposed hyperbolic relationship between the frequency of tetanic stimulation and the response of various autonomic systems (including vagal inhibition of the heart) Rosenblueth [1932] has postulated that a mediator is liberated from autonomic nerve fibres and enters into reversible chemical combination with a substance in the effector, the response of the effector being proportional to the amount of the compound so formed [cf. Cannon, 1933]. A careful reading of Rosenblueth's paper fails to convince one that his hypothesis has reliable experimental support.

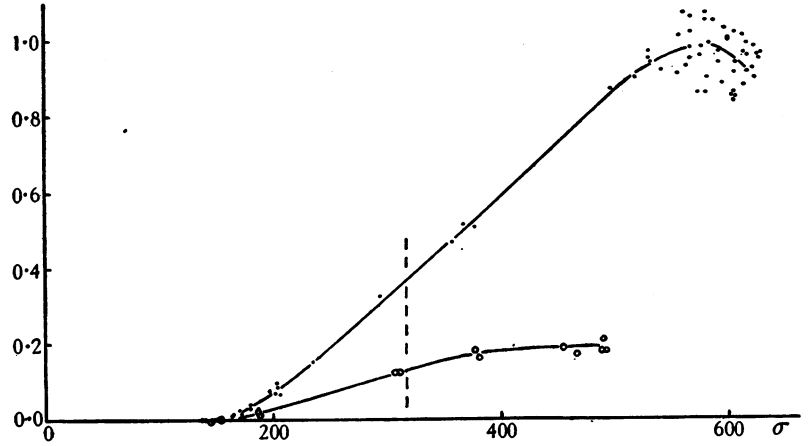
It has been seen that the optimal inhibitory effect is often exerted by a vagal stimulus when it is much later than the beginning of the cycle, the inhibition being often much less with earlier stimuli, *i.e.* such observations lie on the descending part of the inhibitory curve. Still more so is this the case if those observations be included in which the vagal stimulus was applied too late in the previous cycle to produce any lengthening of it. During the first inhibited cycle in both these cases the rhythmic centre has been subjected to a time integration of inhibitory effect much greater than with the later stimuli, and yet there

was actually a smaller lengthening with the earlier stimuli. The inhibitory action of A.C. substance during the early part of the cycle does not seem to have any lasting effect on the rhythmic centre. A.C. substance, therefore, does not appear to exert its effect by checking the building up of excitement throughout the cycle. It probably acts according to the second alternative mentioned above, *i.e.* A.C. substance antagonizes the action of excitement without affecting its production. The lengthening of the cycle would then only be related to the inhibitory action of the A.C. substance present at the actual end of the cycle. The optimum inhibition by vagal stimuli late in the cycle would signify that with earlier stimuli the inhibitory effect of the A.C. substance was already declining by the end of that cycle. The above conclusion provides the justification for plotting as abscissæ the intervals between the vagal stimuli and the ends of the cycles when constructing inhibitory curves.

The evaluation of the intensity of the inhibition produced by a given concentration of A.C. substance is only possible in terms of an equivalent intensity of excitement. When a cycle is lengthened by inhibition we have seen that the excitement goes on increasing after the normal end of the cycle, and this increase will continue until at some particular instant it surpasses its normal threshold intensity by an amount equivalent to the inhibitory effect of the A.C. substance at that instant. A beat will then be set up, for the uninhibited excitement has reached threshold. The time course of the increase of excitement after the normal time of the beat will, therefore, condition the relation between the inhibitory effect of the A.C. substance and the lengthening of the cycle. In the inhibitory curves the lengthening of the cycle has been plotted as ordinates and so such curves do not give a correct representation of the time course of the intensity of the inhibition produced by the A.C. substance. They can only be adjusted to do this if the time course of the increase of excitement is known.

An approximate solution of this problem may be attempted in the following way. An inhibitory curve is determined for maximal stimulation of the vagus, and then the vagus is partly cut through so that the inhibition is diminished to a relatively small fraction of the maximum, and a second inhibitory curve is determined. Text-fig. 19 shows the rising phases of two such inhibitory curves. Let i and I be the respective submaximal and maximal concentrations of A.C. substance, and let l and L be the respective lengthenings of the cardiac cycle produced thereby. The assumption may be made that for any interval after a

vagal stimulus i is directly proportional to I, *i.e.* that the submaximal concentration of A.C. substance is a fair sample of the maximal. This assumption is probably only justifiable for the early parts of the rising phases of the inhibitory curves, for, in accordance with the general behaviour of enzyme-substrate systems the rate of enzymatic destruction of A.C. substance probably would not be increased proportionally with its concentration, *i.e.* a larger concentration would suffer a relatively slower decrease than a smaller. This factor is probably of significance even during the short rising phase of inhibitory curves, for the action of



Text-fig. 19. The rising phases of two inhibitory curves obtained by applying a single stimulus to the right vagus. Normal cycle = 335 σ. The upper curve shows the maximal inhibitory effect, the lower curve the submaximal, which was obtained after partly cutting through the vagus.

eserine shows that enzymatic destruction of A.C. substance is occurring during the latter part of the rising phase of the inhibitory curve.

If the early rising phases of the two curves of Text-fig. 19 be considered, *i.e.* to the left of the broken line, it will be seen that l is directly proportional to L. Moreover, it may be assumed that any particular inhibitory lengthening of a cycle is always due to the same concentration of A.C. substance acting on the pacemaker at the end of its cycle[1]; hence

[1] The rhythmic mechanism may become "adapted" to the inhibitory effect of A.C. substance, and on analogy with the behaviour of receptor organs this "adaptation" would be expected to be more rapid the greater the concentration of A.C. substance [cf. Matthews, 1931]. There is, however, no evidence that this "adaptation" actually occurs, so for the present it will be assumed that any particular concentration of A.C. substance always has the same inhibitory effect, *i.e.* that A.C. substance and excitement are equivalent in their action on the rhythmic mechanism.

it may be concluded that, during the early part of the rising phase of inhibitory curves, the inhibitory lengthening of a cycle is directly proportional to the concentration of A.C. substance acting on the pacemaker at the end of that cycle, *i.e.* that L bears the same proportional ratio to I as l does to i. In the case of Text-fig. 19 $L=2{\cdot}9l$, therefore $I=2{\cdot}9i$, *i.e.* the submaximal concentration of A.C. substance is slightly more than one-third of the maximal.

However, if the curves be considered beyond the perpendicular broken line, l bears a progressively smaller ratio to L. This could be due to the relatively slower enzymatic destruction of A.C. substance mentioned above, or it might indicate that, as I increased beyond a certain value, it produced a lengthening of progressively more than proportional amount.

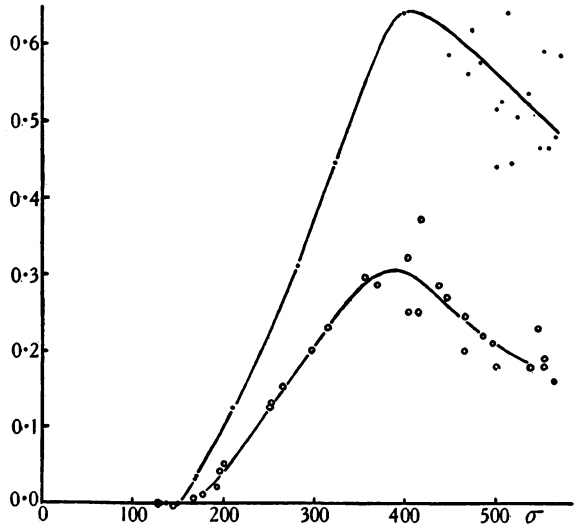
Similar series of curves were given by the two other experiments in which the number of points obtained was sufficient to determine the rising phase of the inhibitory curve with fair accuracy, but even in Text-fig. 19 the observations on this part of the curve were rather infrequent.

As already stated the inhibitory curve of the left vagus has always been similar to that of the right, but considerably lower. In fact it seems identical with a submaximal inhibitory curve of the right vagus. The inhibitory curves of the right and left vagi should therefore provide data similar to that of Text-fig. 19. Text-fig. 20 shows the inhibitory curves of the right and left vagi obtained from concurrent series of observations. If the slight difference in latent period be allowed for, the lengthening produced by the left vagus during the early part of the rising phase is a constant fraction (two-thirds) of that produced by the right vagus. As in Text-fig. 19 the smaller inhibitory lengthening bears a progressively smaller ratio to the larger beyond this phase of direct proportionality. Similar curves have been obtained in the two other experiments in which the inhibitory curves of the right and left vagi have been obtained with a sufficient accuracy. Those inhibitory curves having a prominent second inhibitory wave have not been used in this connection, because the factors responsible for this complication may also have interfered with the shape of the rising phase of the first inhibitory wave.

If the A.C. substance (I) inhibits the rhythmic centre by acting as a simple antagonist to its excitement E, then I at the end of an inhibited cycle is equivalent (in its action on the rhythmic centre) to the excess

of E which has been built up owing to the lengthening of that cycle. During the early part of the rising phase I is proportional to L, so the excess of E above the normal threshold value is also proportional to L, *i.e.* the excitement increases linearly for approximately as long as 0·3 of a cycle after the time at which the beat normally would be set up.

On account of the complicating factors already discussed the further course of E cannot be determined with certainty by analysis of curves



Text-fig. 20. Two inhibitory curves constructed as in Text-fig. 19, but the upper curve is for the right vagus, and the lower is simultaneously obtained for the left vagus. Normal cycle = 305 σ.

such as Text-figs. 19 and 20. However, the inhibitory effects of tetanic vagal stimulation would also be expected to throw light on the time course of the increase of E beyond the normal time of the beat, for it is this which accounts for the gradations of slowing produced by alterations in the strength of stimulation. For example, if the vagal stimulus be made strong, the heart often ceases to beat. This indicates that under such conditions E cannot be increased to an intensity which exceeds I by the threshold amount. Therefore, if the end of a cycle be delayed sufficiently long, E must attain a maximum value beyond which it cannot increase however long the cycle be continued. Moreover, tetanic stimulation of the vagus cannot as a rule be adjusted to produce a slowing of the sino-auricular rhythm to much less than half its normal rate [cf.

Gilson and Irvine-Jones, 1929]. Any stronger stimulation either completely stops the heart, or the rhythm arises in some region of the heart other than the sino-auricular node. Thus it appears that the increase of E does not continue for more than the length of a cycle beyond the normal time of the beat. This conclusion is in agreement with the following explanation which has already been suggested for the relation between the latter parts of the curves of Text-figs. 19 and 20—that, when I increased beyond a certain value, it produced a lengthening of progressively more than proportional amount—for this would indicate that E was increasing at a progressively slower rate.

Thus, only when the inhibitory lengthenings are small, may they be assumed to be directly proportional to the concentrations of A.C. substance acting on the rhythmic mechanism, *i.e.* under such conditions the curves expressing the time course of inhibitory lengthenings, *e.g.* Text-figs. 1, 10 and 18, also express the time course of concentrations of A.C. substance. When the inhibitory lengthenings are large, the concentrations of A.C. substance would follow a lower curve.

With maximal inhibitory curves the rate of decline is usually relatively slower than with submaximal. A similar result is recorded by Gilson [1932] for the negative inotropic effect on the tortoise auricle. Such observations appear to indicate that the rate of enzymatic destruction of A.C. substance is relatively slower the greater its concentration, and this should also modify the shape of the latter parts of the rising phases of the curves such as those of Text-figs. 19 and 20. Thus it seems that both of the suggested explanations are of significance in determining the relation between the maximal and submaximal curves.

IV. Summary.

Single vagal volleys have been set up by applying single induction shocks to the peripheral ends of the transected vagi. The heart beat is recorded electrically from the pacemaker, and it is found that a single volley in either vagus always produces a slowing of the heart rate which persists for many cycles, though the maximum effect is usually on the first inhibited cycle.

If the vagal volley is set up late in a cardiac cycle, that cardiac cycle is not inhibited, the latent period of the inhibition being usually 100–160 σ. Of this amount the conduction time to the region of the pacemaker probably only accounts for about 10 σ, *i.e.* the greater part of the latent

period appears to occur after the arrival of the inhibitory impulses at the nerve fibres of the pacemaker. It is probable that most of this time is occupied in the liberation of the A.C. substance and in its diffusion to the point of its action.

In order to show the time course of the inhibitory effect of a single vagal volley, curves have been constructed, the abscissæ representing the intervals between the vagal stimulus and the ends of the cycles, and the ordinates the lengthenings of the corresponding cycles. Such inhibitory curves have always a steep ascent to a summit, after which there may be either a descent at a progressively slower rate, or after a trough there may be a slow secondary inhibitory wave. A background tetanic stimulation of accelerantes always converts this double-wave type of curve towards the single-wave type. The double inhibitory wave does not seem to be due either to temporary displacement of the pacemaker or to an intercurrent acceleration produced by accelerantes fibres which might be present in the vagus. After discharge from the cardiac ganglia is also excluded. The shape of the inhibitory curve seems to be inherent in the rhythmic mechanism itself, for the right and left vagi always give similar curves, and the submaximal is similar to the maximal. It is suggested that a temporary dissociation of the diverse parts of the rhythmic mechanism may be the cause of the double inhibitory wave.

A small intravenous injection of eserine increases the height of the primary wave and slows the rate of its decline. It also has a similar effect on the secondary wave. This action of eserine is explained by its known inhibitory action on the esterase hydrolysing A.C. substance, so it is concluded that the decline of the inhibitory wave is due to the enzymatic hydrolysis of A.C. substance, and that both inhibitory waves are due to the action of A.C. substance. This latter conclusion is supported by the finding that atropine also affects both inhibitory waves similarly.

Analysis of inhibitory curves shows that the A.C. substance antagonizes the action of the excitement on the rhythmic mechanism—there is no irreversible inactivation of the excitement. It is also shown that the setting up of a beat by a rhythmic centre does not affect the inhibition existing in that centre.

By comparing the rising parts of the maximal and submaximal inhibitory curves it has been possible to show that the concentrations of A.C. substance are directly proportional to the inhibitory lengthenings which they produce provided that such lengthenings are small. Larger concentrations of A.C. substance seem to produce a more than proportional inhibitory lengthening.

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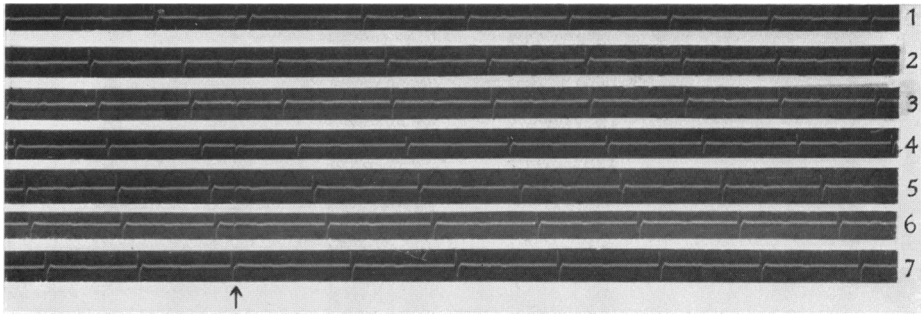

Fig. 1.

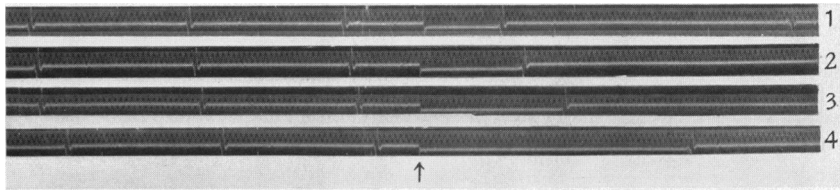

Fig. 2.

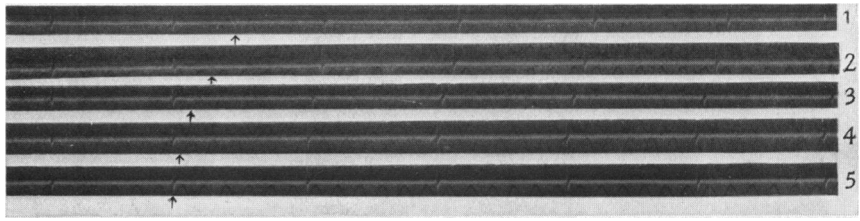

Fig. 3.

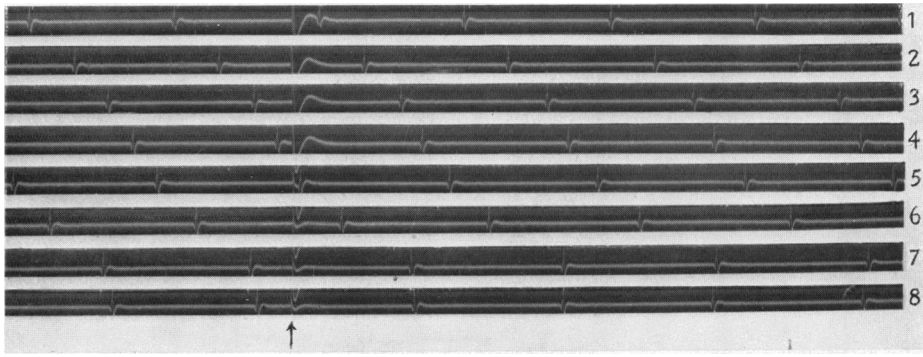

Fig. 4.

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EXPLANATION OF PLATE I.

Fig. 1. A series of observations of action potentials recorded with one lead on the pacemaker and the other on the auricle. A single induction shock is applied to the left vagus at the instant signalled by the small stimulus artefact (occurring in each observation at the time indicated by the arrow), and there is a subsequent slowing of the rhythm without any change in the action potentials of the beats. Observations are arranged from above downwards so that the vagal stimulus is progressively earlier in the cycle. Tuning fork, 1 d.v. $= 100\sigma$.

Fig. 2. A series of observations as in Fig. 1, the single shocks being applied to the right vagus at the time indicated by the arrow. Tuning fork, 1 d.v. $= 10\sigma$.

Fig. 3. A series of observations as in Fig. 1, the single shocks being applied to the right vagus. The observations are arranged so that the beats before the vagal stimuli are synchronized, the small artefacts of the vagal stimuli being indicated by an arrow below each observation. Tuning fork, 1 d.v. $= 100\sigma$.

Fig. 4. A series of observations as in Fig. 1, the single shocks being applied to the left vagus in the first four observations and to the right in the last four at the time indicated by the arrow. Tuning fork, 1 d.v. $= 10\sigma$.