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RHYTHMIC RESPONSES OF SMOOTH MUSCLE

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THE rhythmic responses with which this paper is concerned are set up in the smooth muscle either by impulses in the motor nerve fibres or by the intravenous injection of adrenaline. Though in some experiments Lambert & Rosenblueth [1935] observed that rhythmic potential waves (their III*a* waves) were set up by a single maximal nerve volley, they only suggested "that each wave might represent a new set of elements brought into play", no mention being made of a possible rhythmic response of any units of the smooth muscle. Bacq & Monnier [1935] describe a depolarizing action of adrenaline on smooth muscle which runs a time course similar to the mechanical response, but repetitive responses of smooth muscle to adrenaline have never previously been recorded [cf. Rosenblueth *et al.* 1933; Bacq & Monnier, 1935], a failure which presumably may be attributed to the relatively low amplifications hitherto employed in electrical investigations. The method of experiment is similar to that described in the preceding paper, with the exception that smaller coupling condensers (0·5 μF.) were used in the later stages of the amplifier in recording some of the adrenaline responses.

The rhythmic responses set up by a single motor nerve volley

Figs. 1 and 5 of the present paper and Figs. 2 and 3 of the preceding paper show that, following the initial complex potential wave which is set up in the smooth muscle by a single motor nerve volley, there is a rhythmic series of irregular potential waves, each of which precedes a step in the contraction. As the motor nerve volley is made smaller, the rhythmic potential waves also become smaller in amplitude (cf. the series of observations 5, 2, 6, 1, 3, 4 of Fig. 2, preceding paper), and the associated contraction steps are seen to decrease correspondingly in the series of observations 4, 3, 2 of Fig. 3 (preceding paper). This decrease is

still more obvious in observations 3, 2, 1 of Fig. 1, the contractions associated with the rhythmic waves in observation 1 being so small that the contraction progressively declines from the summit of the response associated with the initial complex wave. Usually (cf. Fig. 2, preceding paper) the rhythmic waves disappear when the motor nerve volley is still sufficiently large to give rise to a large initial complex wave and associated contraction, but there is a great variability from one experiment to another, a maximal volley in some experiments even failing to

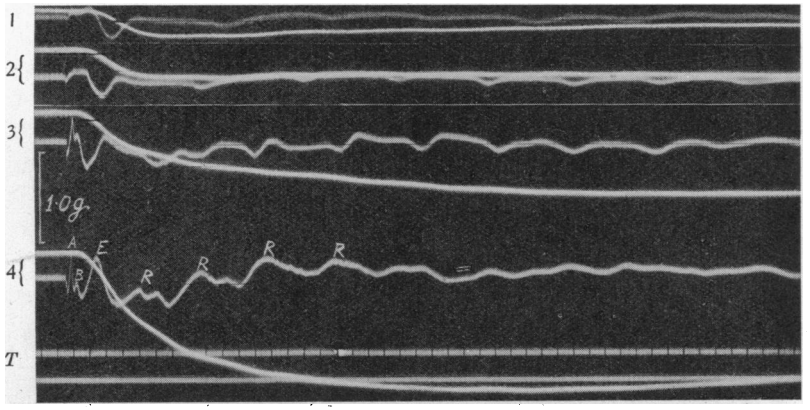

Fig. 1. Observations 1, 2 and 3 show a series of electrical and mechanical responses (myograph pulling downwards) of the nictitating membrane to a progressively larger single nerve volley, which is maximal with observation 3. In the response to two maximal nerve volleys about 20 msec. apart (observation 4) the first rhythmic wave (*E*) is seen to be very early and also much sharper than the later rhythmic waves (*R*). The *A* and *B* waves of the initial complex are also labelled. The line marked *T* is broken every 0·2 sec.

produce any detectable rhythmic waves, and in others these waves are produced by the smallest nerve volleys with which an initial complex wave is detectable. The most frequent type of response in our experience is illustrated by Fig. 3 of the preceding paper and by Fig. 1 of this paper, where, as the stimulus is weakened, the contraction associated with the rhythmic waves is seen to diminish more rapidly than the initial contraction.

The frequency of the rhythmic waves has never been observed to exceed 2·5 per sec., but such a high rate is only produced by summation of the exciting effects of two or more maximal volleys (see later). With single volleys the rate has approached 2 per sec., but is more usually

about 1 per sec. and may even be as slow as 1 beat in every 1·5 sec. As shown in Figs. 2 and 3 of the preceding paper and in Fig. 1, decrease in the size of a volley is always associated with a slowing in the frequency, but large changes are not usually observed.

The irregular shape of the rhythmic waves (cf. Figs. 2, 3, 5 of the preceding paper; Figs. 1, 2, 4, 5, 8, 10), which may even appear to be composed of short diphasic potentials, and their invariable association with a contraction of the smooth muscle suggest that each of these waves is produced by propagated impulses in the units of the smooth muscle, such impulses being identical with those producing the *A* and *B* waves of

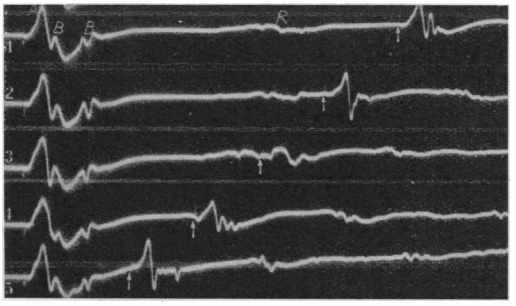

Fig. 2. A single, almost maximal, nerve volley is set up (as shown by the arrows) at various times relative to the first rhythmic wave (labelled *R* in observation 1) of the response to a previous maximal volley. In observation 1 the *A* and the double *B* wave are also labelled. In observation 3 this second volley acts towards the end of a rhythmic wave and is seen to produce a greatly diminished response. Time—1 d.v. = 10 msec. in this and all other figures in which it is shown.

the initial complex potential. Such a suggestion has been investigated, as shown in Fig. 2, by firing a testing motor nerve volley into the muscle at varying times before, during and after a rhythmic wave. The position and irregular shape of the first rhythmic wave are seen in observation 1, Fig. 2, and in observation 3 a testing volley arriving during the latter part of this wave produces a greatly diminished initial complex (the response to the testing volley alone is only slightly less than that for the initial volley in Fig. 2). In observation 2 the testing volley produces a large response markedly diphasic in character and with a greatly diminished *B* wave, thus resembling its response in observation 5 and in other observations late in the refractory period after the initial complex set up by the first volley (cf. observations 5, 6 and 7, Fig. 8, preceding paper). Finally in observation 1, Fig. 2, a further stage of recovery in the

A and *B* waves is apparent. *Thus the rhythmic wave in Fig.* 2 *is associated with a refractoriness of many units of the muscle similar to that following the initial A and B waves, identical units being involved, and not fresh parts of the muscle as suggested by Lambert & Rosenblueth* [1935]. Such a refractoriness is always demonstrable after each rhythmic wave, and it may even be almost total, a maximal testing volley producing in some experiments little or no initial complex, exactly as was observed in observations 3 and 4 of Fig. 8 in the preceding paper. Such rhythmic waves would appear to be produced by the setting up of an impulse in almost every unit of the smooth muscle. More usually the testing volley produces a small response, which may arise either in those units not responding to the rhythmic wave, or, on account of the large temporal dispersion of the responses of the various individual units (cf. Fig. 2), in those earlier units of the wave which have recovered their excitability before the responses of the most delayed units.

Thus, except for the greater asynchronism of the constituent unit responses, the rhythmic waves resemble the initial *A* and *B* waves in all respects. This asynchronism often increases during any one rhythmic series, and, in addition, the mechanical and electrical records indicate that units drop out progressively during the series. *Hence a single motor nerve volley sets up, not a true twitch response of the smooth muscle units, but an irregular tetanus in which, after an initial response giving the A and B waves, the various units respond a variable number of times often with a progressively increasing asynchronism.*

This repetitive response of the smooth muscle units would seem, in some experiments at least, to account for all the contraction response of the muscle. This is particularly well illustrated in Fig. 5, where the contractions bear a very close resemblance to the tetanus of a skeletal muscle. Thus the rapidly decreasing frequency of the rhythmic responses to the double volley (observations 1 and 2) is associated with an early onset of the decline in the contraction, while the initially increasing and then steady frequency of the responses to a single volley (observation 3) is associated with a steady rise of the contraction to a plateau from which decline occurs as the rhythm eventually slows. The figure indicates that the contraction tensions evoked by the single and double volleys would be equal when the frequencies of the rhythm become equal in the later stages of the responses, i.e. the greater maximum tension produced by a double volley is due solely to the higher initial frequency of the rhythmic response which it produces. Similar conclusions are indicated in all those experiments in which such comparisons are possible, i.e.

when approximately the same number of units are responding rhythmically to both single and double volleys. Thus there is no evidence that one or two maximal nerve volleys produce any contraction in smooth muscle except that which is secondary to the impulses which they set up in the muscle units.

The interaction of two nerve volleys

Volleys individually too small to set up rhythmic waves. Observations 1 and 6, Fig. 3, show the action potentials set up by each of two submaximal nerve volleys which have been diminished in size until almost no

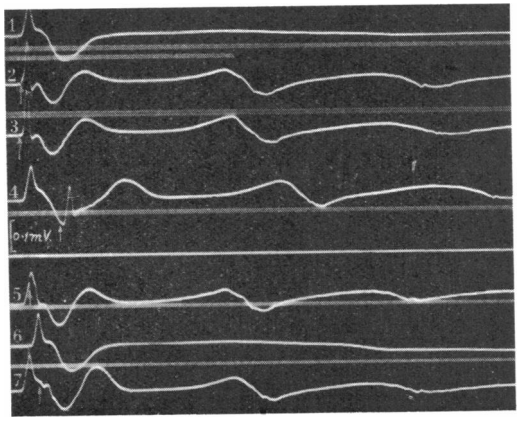

Fig. 3. Action potentials of the nictitating membrane showing the rhythmic waves produced by two similar submaximal volleys at various intervals, though as shown by observations 1 and 6 there is almost no sign of rhythmic waves with either volley alone. The stimulus artefact of each second stimulus is shown by an arrow. Stimulus intervals in milliseconds are for observation 2, 31; for 3, 7·2; for 4, 317; for 5, 80; for 7, 170.

rhythmic response is produced by the smooth muscle. However, as shown in the remaining observations, the characteristic rhythmic waves are produced by summation of the exciting actions of these two volleys when set up at intervals varying from 7·2 msec. (observation 3) to 317 msec. (observation 4) apart. The rhythmic response resulting from the summation is seen to be almost independent of the stimulus interval, the only appreciable change being a slight slowing with the longest interval. Further, this summation to give rhythmic waves is not affected when the second volley sets up no initial *A* and *B* waves (on account of the refractoriness of the units resulting from their response to the first volley, cf. observations 5, Fig. 3; and 5, Fig. 4), and hence it appears to be

independent of the effect of the second volley in setting up these *A* and *B* waves.

When still longer stimulus intervals are employed, as in Fig. 4, the summation can be observed to decrease progressively with lengthening of the stimulus interval, there being at the longest interval (10 sec.) almost no effect of the first volley on the response to the second. The time course of the excitatory condition thus shown to underlie the production of the rhythmic waves is so much slower than that responsible for the facilitatory effect on the initial *A* and *B* waves, which almost disappears in 300–400 msec. (see preceding paper), that such conditions are clearly

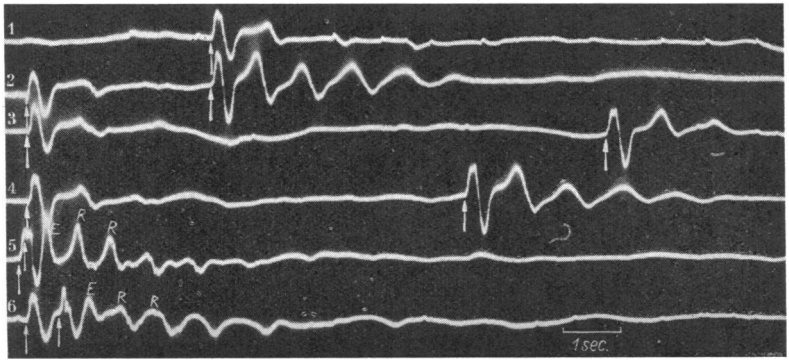

Fig. 4. As in Fig. 3, but at longer intervals to show that there is very little summation of rhythmic wave responses at an interval of nearly 10 sec. (observation 3). Observation 1 shows the response to the second volley alone. In observations 5 and 6 the early rhythmic wave is labelled *E* and the later rhythmic waves *R*. The times of all stimuli are indicated by arrows.

distinguished from one another. Moreover, since summation of two volleys sets up rhythmic waves though neither alone does so, it may be concluded that such waves are not produced by a special mechanism excited by impulses in high threshold nerve fibres. The nerve fibres concerned are not distinguishable from those setting up the *A* and *B* waves either by threshold or refractory period, and presumably are identical with them.

The production of rhythmic waves by a single large volley and not by a small volley must be explicable by the more intense excitatory effects exerted by the increased number of impulses in the larger volley, summation occurring in observations such as those of Fig. 2 in the previous paper, just as with successive volleys down the same nerve fibres in Figs. 3

and 4 of this paper. *Such a summation proves that, in setting up the rhythmic response, many nerve fibres exert their excitatory action on each individual unit, there being a convergence of action comparable with that observed in setting up the initial A and B waves in the preceding paper.*

Volleys which individually set up rhythmic waves. As shown in observations 1 and 2, Fig. 5, the rhythmic waves produced by two nerve volleys at a short interval apart always have a faster rhythm than with either volley alone (observation 3). As with the summation of volleys subliminal for the rhythmic response (Fig. 3), the summed response is not

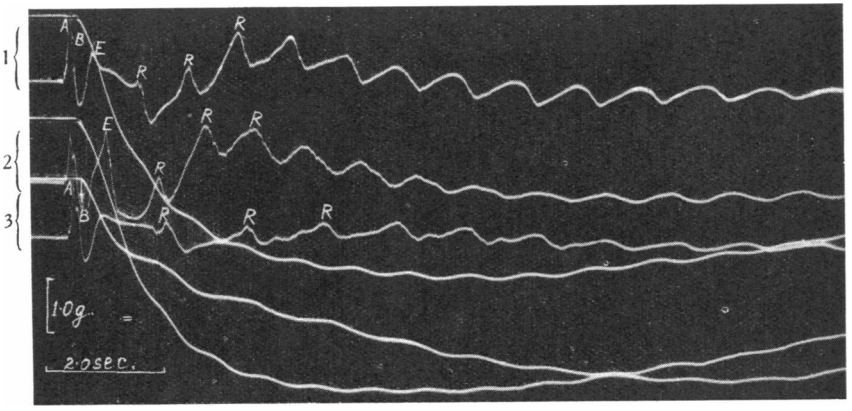

Fig. 5. Records as in Fig. 1 of the electrical and mechanical responses of the nictitating membrane to a single maximal nerve volley in observation 3, and to two maximal nerve volleys at intervals of 15 and 310 msec. in observations 1 and 2 respectively (second stimulus artefact at the latter interval is shown by an arrow). The rhythmic waves in the electrical and mechanical observations are particularly well shown, the early rhythmic waves being labelled *E*, and the later rhythmic waves *R*. The *A* and *B* waves of the initial complex are also labelled in observations 1 and 3.

appreciably affected by variations in the interval from values as short as 14 msec. to as long as 300 msec. Presumably, therefore, the second volley is adding its full excitatory effect at all such intervals, and, since the excitatory effect of the first volley in producing these rhythmic waves shows a relatively slow decline, two similar volleys within such a range of stimulus intervals must together be producing an excitatory effect almost double that produced by a single volley. Hence the ratio of the frequencies of the resulting rhythms is of interest.

However, in any one observation, the rhythmic cycles of the double response do not bear any constant relation to the corresponding cycles of the single response. Thus the measurements of Fig. 5, which are plotted

in Fig. 6, reveal that, after an initial very short cycle, the rhythmic cycle of the double response becomes steady for a few cycles and then progressively lengthens to approach in duration the longer cycles of the single response. In Fig. 6 there is actually a progressive shortening of the early cycles of the single response (cf. Fig. 3, preceding paper). More usually the successive cycles of the single response are either fairly constant in duration (observations 1, Fig. 8; and 3, Fig. 10), or else show a lengthening which is relatively less than that of the double response. In

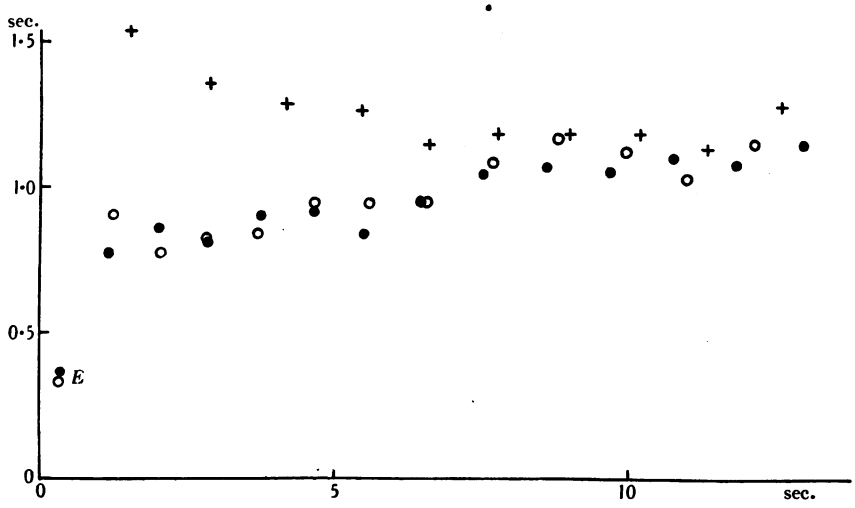

Fig. 6. For each observation of Fig. 5 the durations of the successive rhythmic waves (measured between their summits) are plotted as ordinates against, as abscissæ, the intervals between these summits and the crest of the *A* wave. In observation 2, Fig. 5, the second volley sets up a large *A* wave and measurements are made from its crest. The crosses show the points for the response to a single volley, the solid circles and the hollow circles for two volleys at intervals of 15 and 310 msec. respectively. The two early rhythmic waves are labelled *E*.

all cases, therefore, the rhythm of the double response continuously approaches that of the single response, there being no constant ratio between the two frequencies. Presumably the ratio of the durations of the first few rhythmic cycles gives the most reliable index of the change in frequency produced by doubling the excitatory effect.

When the cycle of the rhythmic response to a single volley is long, it may even be halved with the double response, e.g. a cycle of 1·5 sec. has been shortened to one of 0·78 sec. On the other hand, when the cycle of the single response is short, it is much less affected, e.g. a cycle of 0·58 sec. is only shortened to 0·44 sec. with the double response. There appear to be

definite limits to the shortening which can thus be produced with the double response, for rhythmic cycles are frequently shortened to a little more than 0·4 sec. in duration, while cycles shorter than this have never been observed. Apart from such limitations there is a large degree of variation in the relative shortening from one experiment to another, and, as both the duration of the cycle and the wave form often show a considerable degree of irregularity from one beat to the next, no precise determination is possible for the changes produced in rhythms of various frequencies by doubling the excitatory effect.

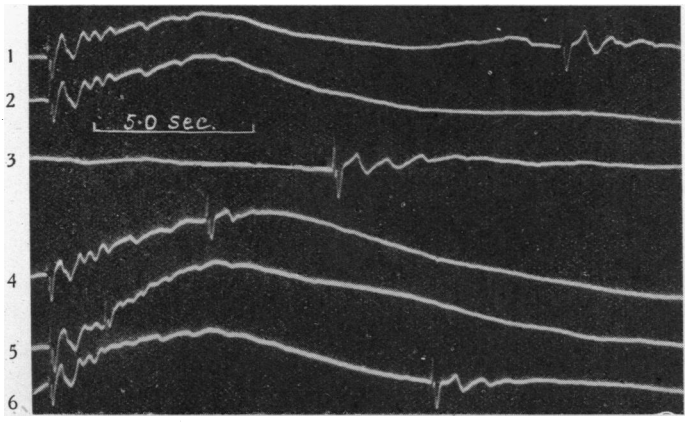

Fig. 7. A series of observations as in Fig. 4, but with single volleys which are large enough to set up several rhythmic waves. In observation 3 is shown the response to the second volley alone. This volley is considerably smaller than the first volley (shown in observation 2), and consequently it sets up a slower and less complex rhythmic response.

When a single volley sets up either no rhythmic waves or only a few feebly developed ones, we have seen that it is followed by a period of several seconds during which there is an increased production of rhythmic waves by a second volley (Fig. 4). Further we have seen that two volleys at a short interval give rise to rhythmic waves which are initially much quicker than those set up by either volley alone. However, as shown in Fig. 7, after a series of rhythmic waves set up by a single volley, there is a period of depression during which a testing volley sets up rhythmic waves which are smaller and which die away more quickly than in control responses to this volley alone. This condition of depressed rhythmicity of the units reaches a maximum towards the end of the rhythmic response to the first volley, progressive recovery occurring after this point (cf.

Fig. 7). Since this depressed rhythmicity is absent when the first volley sets up very little or no rhythmic response (cf. Fig. 4), it appears to arise as a result of this response. It may thus be regarded as a type of "adaptation" produced by the rhythmic response, and it seems likely that this "adaptation" would also be effective during the actual response, tending to slow and eventually to stop it. "Adaptation" may thus provide an explanation of the slowing of rhythm which was more rapid for the double than for the single responses in Figs. 5, 6 and 10, for, presumably, the higher the frequency of the rhythm, the more rapid the "adaptation". "Adaptation" may also be responsible for the progressive decline in rhythmicity which occurs when series of observations are taken in rapid succession, e.g. at more than 1 per min.

The initial beat of the rhythmic series

Usually the first beat of the rhythmic response set up by a single nerve volley follows the initial negative complex (the *A* and *B* waves) by an interval approximately equal to (cf. observations 1, Fig. 8; and 3, Fig. 10) or a little longer than (cf. Fig. 3, preceding paper; observation 3, Fig. 5 of this paper) the subsequent cycles of the rhythmic series, i.e. the rhythm appears to continue on directly from the initial *A* and *B* waves. Such a condition also obtains for observations 3 and 4 of Fig. 2 in the preceding paper, but the larger nerve volleys of observations 6, 2 and 5 are seen to set up a very early wave of the rhythmic series, this wave being less than half a cycle after the initial *A* and *B* complex. In this series it is obvious that this wave has not been produced by the forward shift of the earliest rhythmic wave in observations 3 and 4. It has arisen independently in this early position. It is perhaps first evident with the volley employed for observation 1, and progressively increases with increase in the size of the volley (observations 6 to 2 to 5), until its size rivals that of the next beat. At the same time it becomes slightly earlier. Meanwhile the phase of the subsequent beats has become realigned so that the rhythmic series now appears to commence from this early beat and not from the initial *A* and *B* waves as in observations 3 and 4. Only in four experiments have *single* volleys been observed to set up such early rhythmic beats, but in three other experiments the first rhythmic beat has followed the initial *A* and *B* waves by a cycle much shorter than the subsequent rhythmic cycles, and, as the volley has been made smaller, all transitions have been observed to the usual condition where the cycles have been approximately equal. Such early rhythmic waves thus appear to have no affinity to those described above.

The rhythmic response set up by two maximal nerve volleys at a short interval apart almost always commences with a beat (labelled *E* in all our figures) which is very early after the initial *A* and *B* waves—usually beginning as soon as 200 msec. after the initial *A* and *B* complex, and often being larger and much sharper than the subsequent beats of the rhythmic series (cf. observations 4, Fig. 1; 5 and 6, Fig. 4; 1 and 2, Fig. 5; 3, Fig. 8; 1, 2, 4, 5, 6, Fig. 10). Early rhythmic waves are also usually produced by the interaction of two large submaximal volleys (cf. Fig. 3). In many experiments these early waves are clearly seen to be associated with an increase in the steepness of the contraction curve, which in its short earlier course is produced only by the impulses giving the initial *A* and *B* complex (cf. observations 4, Fig. 1; 1 and 2, Fig. 5). Moreover,

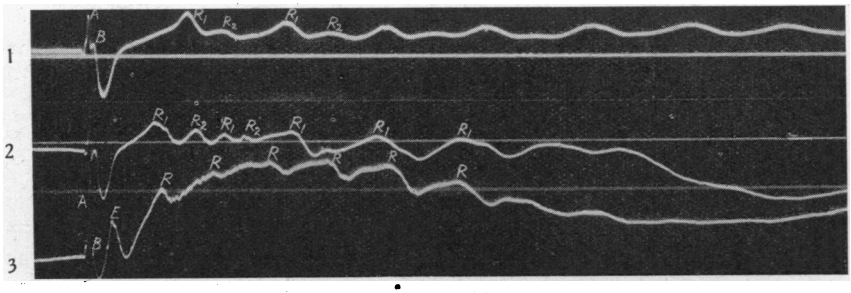

Fig. 8. Observations 1 and 2 show the primary, R_1, and secondary, R_2, rhythmic waves set up by a submaximal and a maximal nerve volley respectively. Observation 3 shows the response to two maximal nerve volleys at 15 msec. interval, the *A* and *B* waves of the initial complex and the early rhythmic wave, *E*, also being labelled.

in the preceding paper this additional contraction has been shown to follow the early rhythmic wave at an interval corresponding to that between the *A* and *B* complex and its associated contraction. Finally, a testing motor nerve volley demonstrates that this early rhythmic wave is followed by a period of refractoriness in every way comparable to that following any of the subsequent waves or the initial *A* and *B* complex (cf. observations 1, 5 and 7, Fig. 9). *Thus there can be no doubt that the early rhythmic wave is due to impulses in the units of the smooth muscle, and these impulses are identical with those giving the initial A and B waves and the later rhythmic waves.*

It might be suggested that, with such observations as 1 and 2 of Fig. 8, an early rhythmic wave is set up by the single volley as well as by the double volley, but it is obscured by the potential change at the end of the positive wave. However, a testing nerve volley reveals that there is no

sign of any developing refractoriness at a point corresponding to the refractoriness produced by the early rhythmic wave set up by the double nerve volley (cf. observations, 4, 5, 6 and 7, Fig. 9), hence no rhythmic wave could be present. A similar conclusion may be drawn from an investigation of the effects of lengthening the interval between the two nerve volleys. The early rhythmic wave then becomes progressively later (cf. the series of observations, 1, 2, 4, 5, 6, Fig. 10; 5, 6, 2, 4, 3, Fig. 4), until, with long intervals, the first rhythmic beat after the second volley is

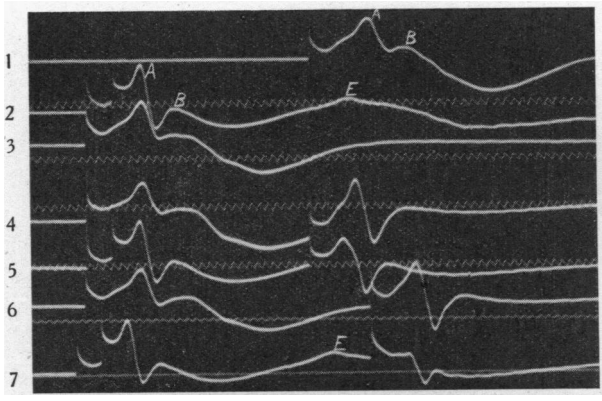

Fig. 9. A maximal nerve volley (response shown in observation 1) tests the excitability of the smooth muscle at varying times after the response to single (observations 4 and 6) or to double maximal nerve volleys. The control responses are shown in observations 3 and 2 respectively. The early rhythmic wave set up by the double volley is labelled *E* in observations 2 and 7, and the *A* and *B* waves of this initial complex are also labelled. Further description in text.

no earlier than that following a single volley, i.e. all transitions are observable between the early rhythmic wave and the first rhythmic wave with a single volley.

This effect of lengthening the stimulus interval is not usually evident as long as this interval remains less than 100 msec. (cf. observations 2, 3, 4, 5, Fig. 8 of preceding paper), but, as the interval is still further lengthened, the early rhythmic wave rapidly becomes more delayed (cf. observations 1, 2, 4, 5, 6, Fig. 10). This suggests that the rate of decay of the excitatory process underlying the production of these early rhythmic waves runs almost as rapid a time course as that responsible for facilitation of the initial *A* and *B* waves, which also decays rapidly after 100 msec. (see preceding paper). Thus this facilitatory process may be in part responsible for setting up the first rhythmic beat so soon after the

initial complex. Possibly it acts by inducing a quicker recovery from the refractory period following this complex, and hence conditions the muscle units to respond so prematurely to the enduring excitatory mechanism responsible for setting up the rhythmic waves.

This explanation would seem to be particularly significant in experiments such as that illustrated by Fig. 2 of the preceding paper. It was noticed that, as the single volley was increased in size, the early rhythmic

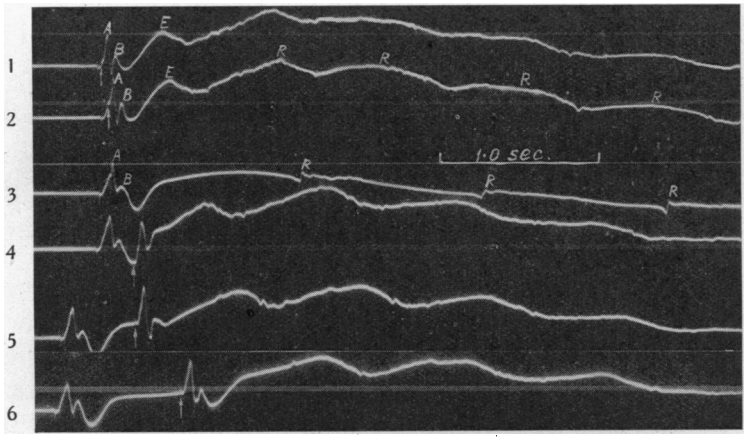

Fig. 10. In observations 1, 2, 4, 5 and 6 two maximal nerve volleys are set up at intervals of 26, 46, 228, 470 and 790 msec. respectively, the second stimulus artefact being indicated by the arrows. Observation 3 shows the response to the first volley alone, and in the first three observations the *A* and *B* waves of the initial complex, the early rhythmic waves, *E*, and the later rhythmic waves, *R*, are labelled.

wave first appeared in the characteristically early position which it maintains during the growth that is brought about by further increase of the volley. This is just the position where the early rhythmic wave would be expected if it owed its production partly to the above facilitatory process, for testing with a second maximal volley shows that the decay of this facilitatory process is then still in a relatively early stage and the recovery from refractoriness is well advanced.

A comparison of Fig. 3 of this paper with Fig. 2 of the preceding paper convincingly shows that, in the experiment from which these two figures were taken, the early rhythmic wave produced by summation of two submaximal volleys is identical with that produced by a single larger volley, the explanation in terms of the facilitatory process thus also being indicated for some at least of the early rhythmic waves produced by

summation of two volleys. Further evidence is, however, necessary before such an explanation can be developed in detail or extended to cover all examples of early rhythmic waves. This caution must particularly be applied to those experiments in which, by diminishing the size of a single volley, all transitions are produced between a short initial cycle (early rhythmic beat) and an initial cycle equal to the later cycles. Under such conditions the early rhythmic beat may occur almost as long as 1 sec. after the initial *A* and *B* waves, an interval which seems much too long for survival of any appreciable facilitatory effect of the type suggested above.

The co-ordination of the rhythmic responses of the units

In some experiments the various units which respond with each rhythmic wave rapidly get out of phase, and it is impossible to follow the rhythm for more than a cycle or two. For example, in observation 8, Fig. 5 of the preceding paper, an irregular continuous series of potential waves begins some time after the early rhythmic beat. More usually the regularity of the rhythmic waves is maintained for several cycles, the changes being in amplitude rather than in temporal dispersion (cf. Fig. 2 of preceding paper). Finally there are the remarkable experiments in which the rhythmic beating goes on regularly for a large number of cycles (cf. Fig. 5 of this paper), and even may become re-established after a temporary period of irregularity (observations 2 and 3, Fig. 8). *Such observations show conclusively that the various units of the muscle are not responding independently. There must be some co-ordinating process by which they are kept in phase or restored to phase after a period of asynchronism.*

Rhythms less regular and prolonged, e.g. those illustrated in Fig. 2 of the previous paper or in Fig. 4 of this paper, conceivably could be produced by summation of the rhythmic responses of units initially brought into phase by their almost synchronous *A* or *B* responses, and thereafter beating independently of each other, but at frequencies which are distributed about a certain dominant value dependent on the general intensity of the excitation. However, now that a process co-ordinating the responses of the units of some smooth muscles has been demonstrated, it seems likely that this co-ordination is also playing a part in maintaining these less regular rhythms.

In the preceding paper it was shown that a small motor nerve volley sets up a *B* wave after a long latent period. As the volley is increased in size, the latent period shortens, an increased number of units respond to

give the *B* wave, and their responses are less asynchronous. Thus, as soon as this increase in size of the volley becomes adequate to recruit any particular unit into the *B* group, that unit responds with a latent period much shorter than that obtaining for the low threshold units in their response to the very small volley initially employed. The latent period of the units just excited to the threshold intensity for eliciting a *B* response thus decreases progressively as the size of this response increases. Therefore there would seem to be a co-ordinating process tending to synchronize the responses of the units giving the *B* wave, an effect presumably similar to that acting during the rhythmic waves.

At times, e.g. Fig. 5, the wave form is simple and relatively constant throughout a long rhythmic series, thus indicating that there is an aggregation of the responses of the various units around an optimum position, and that this disposition is approximately maintained for each successive beat. Frequently, however, the wave form is complex and shows systematic variations. Thus in observation 1, Fig. 8, each of the first four rhythmic waves, R_1, is followed by a secondary wave, R_2, which eventually, in the sixth and seventh beats, appears to become merged into the primary wave. With the larger single volley of observation 2, Fig. 8, this secondary wave is large with the first beat, and the rhythm then becomes temporarily confused, the next primary and secondary waves being small, but the third primary wave is large and the primary wave clearly dominates the next four cycles, though the secondary wave is still evident. In other observations dominance may eventually be achieved by the secondary wave.

A further development of this antagonism between the primary and secondary waves would seem to be the explanation of the temporary almost complete asynchronism of the responses of the individual units in observation 3 after the first two rhythmic beats. However, the third beat of the primary rhythm is still detectable, and thereafter a progressive dominance develops with the fourth, fifth and sixth beats. Similar primary and secondary waves also appear in observations 3 and 4, Fig. 1, in the response to the first volley in the observations of Fig. 7, and in observations 3 and 4 of Fig. 3 in the preceding paper.

The development of secondary waves and the consequent disturbance of the rhythm is always accentuated by increasing the intensity of the excitation, larger nerve volleys or summation of the effects of two nerve volleys being shown to be thus effective in Figs. 1, 7 and 8. In experiments in which two maximal volleys set up a regular rhythmic response, the still more intense excitation produced by three volleys often results in

an asynchronism of the various units of the muscle. When the excitatory process setting up the rhythmic responses is intense, it would thus seem to excite the units to respond independently of the co-ordinating process. It would, therefore, be expected that a more regular rhythm would develop with the later waves of a rhythmic series, for then the intensity of the excitatory process would have decreased. Such an expectation is seen to be realized in Figs. 5 and 8.

Discussion. Thus the co-ordinating process does not constitute a rigid linking together of the various units of the smooth muscle, e.g. the condition of the smooth muscle does not become comparable to that of cardiac muscle, where the uninterrupted passage of a single impulse links all the muscle fibres in a single all-or-nothing unit. The irregularity in the shapes and sizes of the successive potential waves is sufficient to exclude such a condition for smooth muscle. Further, any participation of the nervous system in the co-ordinating process may be excluded, for all the fine meshwork of nerve fibres in the muscle arise from neurones in the superior cervical ganglion, and no anastomosis is demonstrable between the fibres from different neurones. Thus all the nerve fibres degenerate if the ganglion is removed, and part only after partial removal of the ganglion [Lawrentjew & Borowskaja, 1936]. Again, the graded effects produced by graded stimulation of the postganglionic trunk show that there is no anastomosis of the different nerve fibres with one another.

Hence there seem to be only two possible explanations of the co-ordinating process in smooth muscle.

(1) The units of the muscle are anatomically independent, approximate synchronization of beats being conditioned by a tendency for a beat in any one unit to set up a beat in adjacent units. Thus the unit in which a beat first arises (the temporary pacemaker) conditions a response in other units and these in turn in others, and so on for all the units of that wave. The contraction response of a unit is too sluggish to form the basis of such a spreading excitation, for it is never detectable within 100 msec. of the action potential, and in each rhythmic wave the action potentials of the various units must follow each other within intervals of a very few milliseconds. There is no experimental evidence to support an explanation based on transmission due to rapid diffusion of exciting chemical substances liberated by impulses in each unit. Thus there remains the possibility that the eddy currents associated with the propagation of an impulse in any one unit (cf. preceding paper) exert a weak exciting action on adjacent units, which consequently set up impulses

earlier than would be the case in the natural course of their own rhythmic cycles. Besides offering an adequate explanation of the rapidity of action of the co-ordinating mechanism, such an explanation is in accordance with experimental evidence, for a spread of the eddy currents does of course occur into the adjacent fibres, and smooth muscle fibres have been shown to be electrically excitable [Monnier & Bacq, 1935; Eccles & Magladery, 1937] though this has been denied by Rosenblueth *et al.* [1936]. Such an explanation is similar to that offered by Adrian [1930] and Adrian & Gelfan [1933] for the synchronous rhythmic activity of nerve and striated muscle fibres. It could, of course, only be securely established if it would be shown that the excitatory effect exerted by the eddy currents is adequate to condition the observed synchronization.

(2) An alternative explanation may be offered in terms of the protoplasmic bridges which have been described between the smooth muscle fibres [Boeke, 1932]. On account of the possible functional significance of such bridges the units of smooth muscle were, in the preceding paper, not definitely identified with single smooth muscle fibres, the possibility being envisaged that impulses may be transmitted from one fibre to another across the bridges. Still more tenuous bridges might link fibres of different units, and, though failing to transmit impulses, could serve as a pathway for spread of excitatory conditions, which have already been shown to spread from the region of any one nerve ending to the region of many other nerve endings. Such an explanation seems much less likely than that discussed above, depending as it does on purely hypothetical conditions, for we have no knowledge of the functional behaviour of protoplasmic bridges, whose very existence is by no means well established.

The action of adrenaline

Observation 1, Fig. 11, shows the typical electrical response produced in the nictitating membrane by intravenous injection of a small quantity of adrenaline (15γ). Simultaneous mechanical recording (cf. observations 1 and 2, Fig. 12) reveals that these action potentials are always associated with a contraction of the smooth muscle, and they continue, but with diminishing intensity, during and even beyond the rising phase of the contraction. Moreover, since adrenaline never produces a contraction of normal smooth muscle in the absence of these action potentials, their relation to the contraction is comparable to that obtaining for the responses produced by motor nerve impulses.

The faster records of observations 3 and 4, Fig. 11, and the higher amplification of observation 6 allow a more detailed examination of the adrenaline action potentials, which are seen to be compounded of spike-like waves, the earthed lead being usually initially negative to the grid lead. Many of these waves are seen to have a diphasic shape and are very brief, e.g. the initial wave in observation 6 has a total duration of about 20 msec., and so is as fast as the fastest action potentials set up in response to a nerve volley. This similarity between the spike-like waves

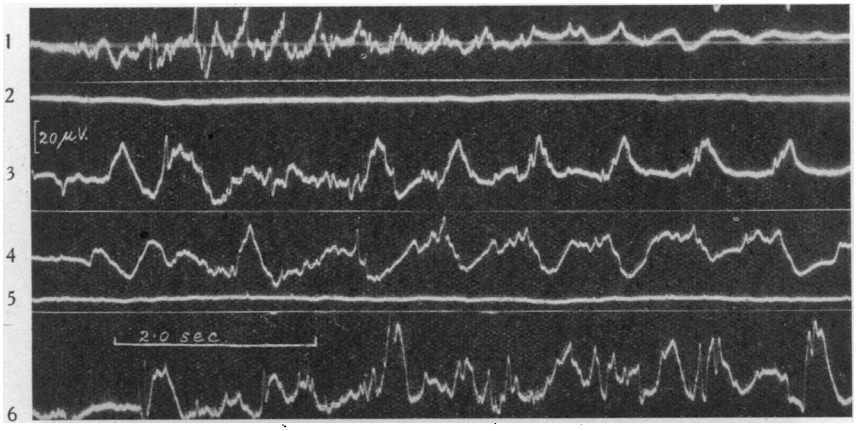

Fig. 11. Observations 1, 3, 4 and 6 show the action potentials which intravenous injections of adrenaline set up in the smooth muscle of the nictitating membrane, the respective doses being 15, 10, 10 and 5γ. Observation 1 is taken at a much slower rate than the other observations, and with observation 6 the amplification is increased 1·7 times. Observations 2 and 5 show the electrical base line in the absence of an adrenaline injection.

of the adrenaline action potentials and those of the action potentials set up by motor nerve impulses has been observed in all experiments. Since, as we have seen, both these potentials are similarly related to the contraction of the muscle, there would seem to be no doubt of their identity. *Thus the adrenaline responses presumably are also produced by propagated all-or-nothing impulses in the units of the smooth muscle. The polarity of the action potentials shows that most of these impulses are propagated in the direction of earthed lead to grid lead, i.e. there is a general tendency for them to be set up at the same region as the impulses produced by motor nerve impulses.* This recalls the observation of Langley [1907] that nicotine only sets up the rhythmic discharge of

impulses in frog's muscles when it is applied to the region of the motor end plates, and, similarly, the impulses set up in mammalian muscles by

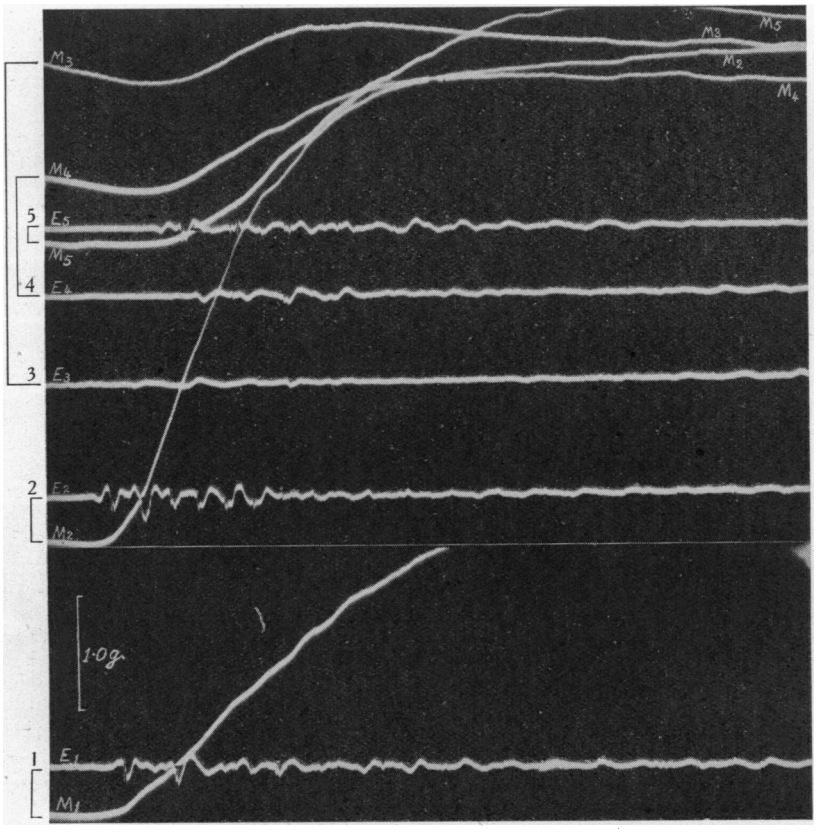

Fig. 12. Observations 1 and 2 show the electrical (E) and mechanical (M) responses of the nictitating membrane to the intravenous injection of 10 and 50γ of adrenaline respectively. Early in the relaxation of the response to 50γ a second injection of 10γ was given (observation 3), the respective electrical and mechanical responses being labelled E_3 and M_3. The initial contraction tension is indicated by the height of M_3 above E_3 at the outset, for the completely relaxed M is below E as shown in observations 1 and 2. Observations 4 (E_4 and M_4) and 5 (E_5 and M_5) similarly show the responses to 10γ adrenaline at later stages of relaxation from the response to 50γ adrenaline. The quasi-artefact produced by arterial pulsation in the membrane may be detected in some mechanical records, e.g. M_3. All injections given at approximately the same time before taking the records.

acetylcholine and eserine probably arise at the motor end plates [Brown *et al.* 1936; Brown, 1937; Eccles, 1936, pp. 404, 407].

Observations 1, 3 and 4, Fig. 11, further show that, after an initial period of asynchronism, the short spike-like waves exhibit a tendency to come into phase, forming complex waves whose frequency is at first about 2 per sec., but thereafter it gradually slows and the waves become smaller,

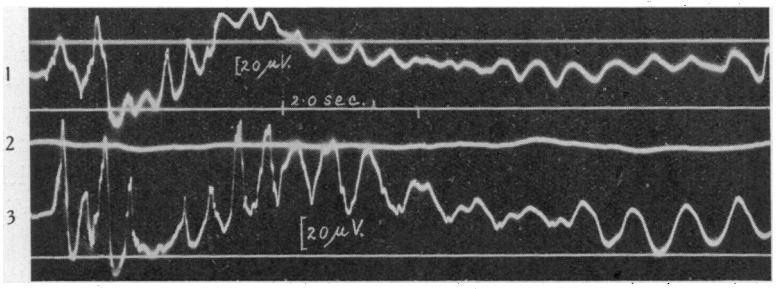

Fig. 13. As in Fig. 11, 10γ of adrenaline being given intravenously with observations 1 and 3. Observation 2 shows the control base line. The time scales for observations 1 and 2 are shown respectively above and below the central line.

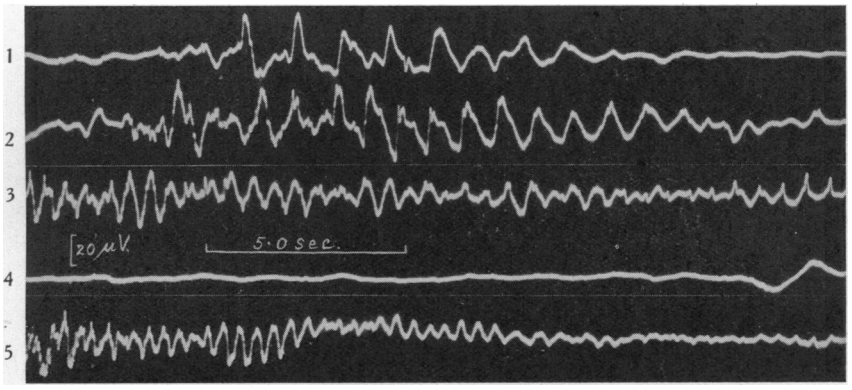

Fig. 14. As in Fig. 11, observations 1, 2, 3 and 5 showing respectively the responses to 5, 10, 25 and 150γ of adrenaline injected intravenously. Observation 4 shows the control electrical base line.

the final rhythm being about 1 per sec. in observation 1. This tendency to synchronism is particularly well shown in Figs. 13 and 14. In Fig. 13 the grouping into complex waves is seen to be established right at the outset of the response, the intervals between the successive waves being there as short as 0·35 sec. The similarity of successive waves in some parts of Figs. 11, 13 and 14 shows that these waves are compounded of

the responses of units beating rhythmically with one beat for each wave, i.e. the frequency of the waves gives the frequency of the individual units, exactly as with the rhythmic waves set up by motor nerve impulses.

The frequency, thus determined, of the impulses in individual units is fastest at the beginning of the response, and is never more than 3 per sec. It then progressively slows to rather less than 1 beat per sec., rhythms slower than 1 beat in 1·5 sec. never being observed. This close correspondence between the frequencies of the rhythmic responses produced by adrenaline and by motor nerve impulses confirms the above conclusion of the identity of these two types of response. Fig. 14 shows that within limits the rhythm is faster the larger the quantity of injected adrenaline. 5, 10 and 25γ set up rhythms which at their outset are about 0·95, 1·26 and 2·1 per sec. respectively, while the much larger injection of 150γ in observation 5 only increased the rhythm to 3 per sec., i.e. there appears to be an upper limiting value of about 3 per sec. to the frequency of the rhythmic discharge of impulses by the individual units.

In Fig. 13 the rhythm is interrupted by temporary irregularities, though in both observations 1 and 3 a regular beat at a frequency of about 1 per sec. is eventually established. In the occurrence of these temporary breaks in the rhythm (cf. observations 3 and 5, Fig. 14), which are often accompanied by the appearance of secondary waves, the response to adrenaline again closely resembles the rhythmic waves set up by motor nerve impulses (cf. Fig. 8). Thus the co-ordinating process which is responsible for the tendency to synchronism both in these adrenaline responses and in the responses to motor nerve impulses can exercise no rigid control over the individual units. This is further shown by the variations in wave form in Figs. 13 and 14 which indicate that the different units must vary considerably, from one beat to the next, in their phase relations to one another. Generally the adrenaline responses exhibit more asynchronism than the rhythmic responses set up by motor nerve impulses, a difference presumably arising because in the latter case the responses are approximately synchronized at the outset by the response giving the initial complex (the *A* and *B* waves).

Discussion. In only one respect has a difference been observed between the rhythmic responses to adrenaline and to motor nerve impulses. The contraction produced by motor nerve impulses always seems to be produced entirely by impulses in the smooth muscle units, the tension always declining when a cessation of action potentials indicated that no more impulses were being set up. Though this correspondence may also occur

for the responses evoked by small quantities of adrenaline, e.g. 5γ intravenously, the contraction produced by larger quantities declines so slowly that it is prolonged far beyond the duration of detectable action potentials and hence presumably of the impulses (cf. observation 2, Fig. 12). It may be that the individual units have become completely asynchronous, their action potentials then being too small to be detectable, but the closely parallel observations of Brown [1936] on striated muscle make it more likely that a contracture has supervened on the contraction associated with the impulses. Thus Brown finds that a small quantity of acetylcholine given by intra-arterial injection into normal frog's or denervated mammalian muscles sets up a contraction associated with the irregular series of diphasic action potentials which are produced by impulses in the muscle fibres. Larger quantities of acetylcholine produce a still longer mechanical response of the muscle, but the action potentials are now restricted to a short initial burst. Since the potential produced by a single impulse in a single fibre could be detected, the prolonged mechanical response must occur independently of propagated impulses in the muscle fibres, i.e. it must be a contracture.

A further parallel with Brown's experiments is illustrated in Fig. 12. Testing doses of 10γ of adrenaline were injected at various times during the declining phase of the prolonged mechanical response set up by the injection of 50γ of adrenaline (observations 1 and 2 show respectively the initial responses evoked by 10 and 50γ of adrenaline). Observation 3 shows that early in the declining phase the testing dose produces almost no action potential and a diminished mechanical response, and observations 4 and 5 show that the testing dose evokes progressively larger electrical and mechanical responses as it is applied later in the declining phase. Thus the prolonged mechanical response evoked by 50γ adrenaline is associated with a striking depression of the rhythmic discharge of impulses which is normally evoked by adrenaline. A similar depression of the rhythmic discharge of impulses which is evoked by ACh. in striated muscle has been shown by Brown to occur during the contracture produced by a large injection of ACh. Thus there seems to be no doubt that a contracture is also produced in smooth muscle by a large injection of adrenaline.

It may be that, if the excitation produced by motor nerve impulses is made sufficiently intense, e.g. by several maximal volleys in quick succession, the smooth muscle would respond by a contracture response resembling that produced by large quantities of adrenaline. It is not even possible to exclude the presence of small contractures in the responses to

single or double volleys. *However that may be, the rhythmic waves produced by motor nerve impulses so closely resemble the adrenaline responses that it may be regarded as established that these rhythmic responses are produced as a consequence of an adrenaline-like substance (Ad.-substance) liberated by the motor nerve impulses.* Further evidence in support of this is provided in the next paper by the actions of cocaine and 933F.

The hypothesis has been put forward that this Ad.-substance combines with a receptive substance in the smooth muscle cells to form a substance called sympathin, which alone is capable of evoking a contraction response [cf. Cannon, 1933, 1934; Rosenblueth, 1932, 1935, 1936]. A critical examination of this hypothesis [Eccles, 1936, pp. 414, 430] has shown that it lacks reliable support, and that the simpler view that the Ad.-substance acts directly on the smooth muscle fibres is adequate to explain all the available experimental evidence. There is some evidence [cf. Bacq, 1935] that Ad.-substance differs slightly from adrenaline, but Loewi [1936] has recently provided good evidence that in the heart Ad.-substance is identical with adrenaline. For the present, however, it seems advisable to retain the term Ad.-substance.

The effect of adrenaline on the response to a motor nerve volley

Rosenblueth and Cannon [1936] and Eccles & Magladery [1936] have independently described the diminution which adrenaline effects in all phases of the initial complex potential set up by a maximal nerve volley. As shown in Figs. 15 and 19, which are typical of our thirteen experiments, the maximum diminution of the *N*, *A*, *B* and the positive waves is reached at about 30 sec. after the intravenous injection of adrenaline, or about 15–20 sec. after the beginning of the adrenaline action potentials. Recovery is almost complete in 2–3 min. In the eight experiments in which records were fast enough for accurate time measurement, the latency of the beginning of the initial complex has not been altered, but the *A*, *B* and positive waves have been made quicker in their time course (cf. Figs. 15, 17 and 18), an effect

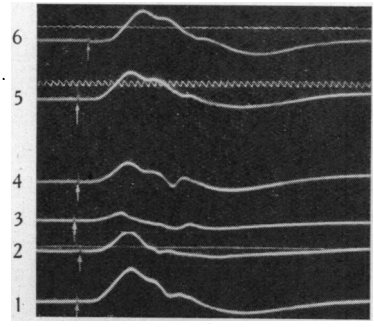

Fig. 15. Observation 1 shows the smooth muscle action potential set up by a single maximal nerve volley, and observations 2, 3, 4, 5 and 6 show respectively the action potentials which such a volley sets up 15, 30, 90, 150 and 210 sec. after the intravenous injection of 25γ of adrenaline.

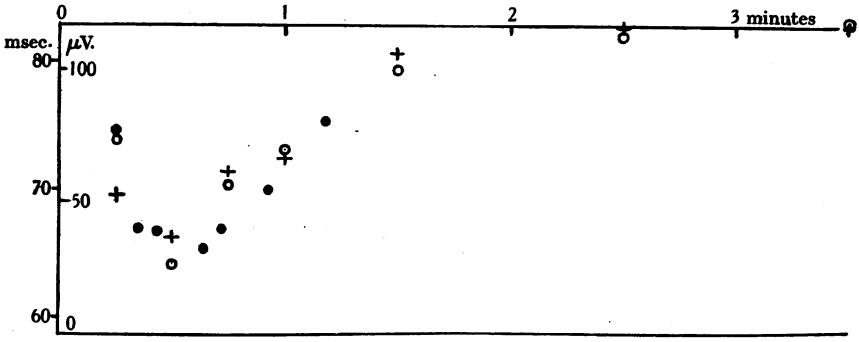

Fig. 16. Each circle, open or solid, represents the potential of the summit of the *A* wave set up by a maximal nerve volley plotted against the time (abscissæ) after the intravenous injection of 25γ of adrenaline, while each cross shows the time from the nerve stimulus to the summit of the *A* wave similarly plotted against time after the adrenaline injection. The open circles are derived from the same observations as the crosses. The top horizontal line shows the potential and latent period of the control *A* wave before the adrenaline injection.

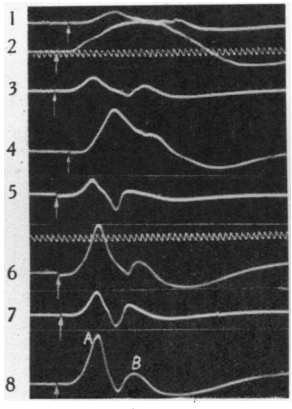

Fig. 17.

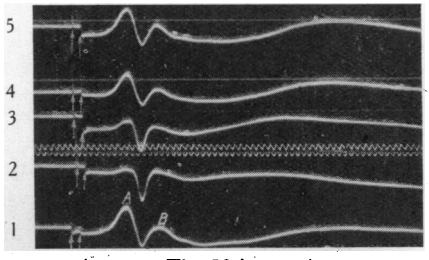

Fig. 18A.

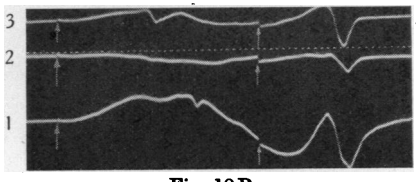

Fig. 18B.

Fig. 17. Observations 2, 4, 6 and 8 show the smooth muscle action potentials evoked by nerve volleys set up by stimuli whose strengths in arbitrary units are respectively 6, 9, 17 and 33. Corresponding stimulus strengths were employed for the series of observations 1, 3, 5 and 7, but 25γ of adrenaline had been given intravenously about 40 sec. earlier than each of these observations.

Fig. 18A. As in Fig. 15, but the action potentials are set up by two maximal nerve volleys at 15 msec. interval (stimuli shown by arrows), the control response being shown in observation 1. Observations 2, 3, 4 and 5 are taken respectively 30, 60, 90 and 150 sec. after the intravenous injection of 25γ of adrenaline.

Fig. 18B. As in Fig. 18A, but the stimulus interval is 320 msec. Observations 2 and 3 are respectively 30 and 90 sec. after the injection of 25γ of adrenaline, observation 1 showing the control response before the adrenaline.

which apparently was not noticed by Rosenblueth & Cannon [1936]. Fig. 16 shows that this quickening of the *A* wave (measured by the latency of the crest of its spike) runs a course which closely corresponds to that of its simultaneous diminution in size by adrenaline.

The slower action potentials produced by submaximal volleys suffer even greater changes of the same kind, e.g. the smooth rounded negative wave of observation 2, Fig. 17, is altered to a small sharp early spike in observation 1, and a comparison of observations 4 and 3, and even 6 and 5 also shows a greater change than in observations 8 and 7. On the other hand adrenaline has relatively little effect on the *time course* of the *A* wave when it is formed by almost synchronized responses of the muscle units, such as are produced by two maximal volleys in quick succession (Fig. 18A), or by a second volley in the relatively refractory period of the response to a previous volley (Fig. 18B). The alteration which adrenaline effects in the time course of the components of the initial potential wave would, therefore, appear to be due to a decreased asynchronism of the responses of the individual units rather than to a change in the duration of these responses.

Fig. 18A illustrates a change in shape which adrenaline produces in the action potential. The series of observations at 30, 60, 90 and 150 sec. after the adrenaline injection clearly shows that adrenaline has produced a great diminution in the negative component of the diphasic *A* wave, the positive component even being increased in the observation at 30 sec. A similar relative increase of the positive component is also seen in diphasic potentials produced by the second volley at a long interval in observations 2 and 3, Fig. 18B, and indeed it has been detectable to some extent in all our observations (cf. Figs. 15 and 17). A similar effect appears to have been noticed by Rosenblueth *et al.* [1936] in one experiment on pilomotor muscles, but, as they failed to recognize the normal diphasic character of the *A* wave, they interpreted a sharp positive wave resembling that of observation 2, Fig. 18A, as due to the suppression of the *A* wave making apparent the early onset of the slow positive wave (their II wave). In some of our experiments the positive component of the diphasic *A* wave was very large even in the absence of an adrenaline action.

Presumably the rhythmic responses of the muscle units to adrenaline are followed by refractory periods, just as are the initial and rhythmic responses evoked by motor nerve impulses. Such a refractoriness must provide at least a partial explanation of the diminished and less asynchronous action potentials which a motor nerve volley evokes during an adrenaline response. However, these effects of adrenaline considerably

outlast the irregular action potentials produced by the rhythmic responses of the units. For example, in observation 5, Fig. 19, the testing

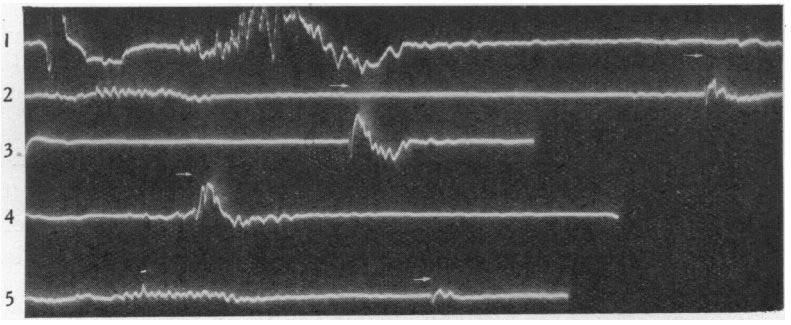

Fig. 19. Smooth muscle action potentials (nictitating membrane) set up by intravenous injection of adrenaline and a testing maximal motor nerve volley. Observation 3 shows the response to this testing volley alone, and in observations 4, 5 and 2 are shown the responses to this volley at 6, 29 and 54 sec. respectively after the beginning of the action potentials set up by the intravenous injection of 25γ adrenaline. Observation 1 shows the adrenaline response at 4·5 times the amplification of the other observations. The horizontal arrows indicate the height of the summit of the *A* wave set up by each nerve volley.

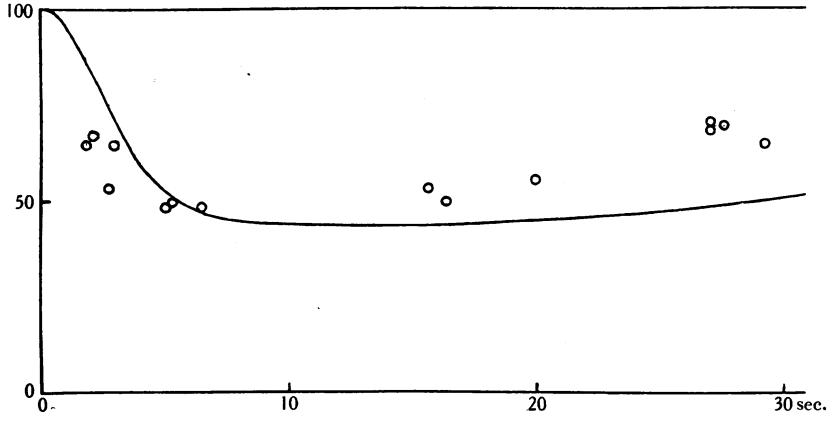

Fig. 20. An injection of 20γ adrenaline intravenously sets up the contraction (myograph pulling downwards) shown by the continuous line, and also diminishes the *A* waves (plotted as ordinates as a percentage of the control *A* wave) produced by testing maximal nerve volleys at various times during this response (shown by the circles).

motor nerve volley is set up after the end of the smallest adrenaline responses that can be detected in the highly amplified record of observation 1, yet its *A* wave is only about one-quarter of the control response

shown in observation 3, while in observation 2 a testing volley a further 25 sec. later still has its *A* wave greatly diminished. Thus the effect of adrenaline on the initial action potential which is evoked by a testing nerve volley considerably outlasts the period during which a rhythmic discharge of the muscle units is revealed by the action potentials, but on the other hand, as shown in Fig. 20, it always runs a shorter time course than the contraction evoked by the adrenaline.

Fig. 19 further shows typically that the rhythmic waves produced by a testing nerve volley are greatly diminished for a long time after the adrenaline action potentials have ceased, there being, for example, only two small rhythmic waves in observation 5, while even at the very long interval of observation 2 recovery is by no means complete. On the other hand in observation 4 the testing volley in the early stage of the adrenaline response seems to produce an effect comparable with the control response in observation 3, the adrenaline-like substance liberated by the nerve impulses presumably having added its effect to that of the injected adrenaline. Records of the contractions confirm these electrical observations. Thus, after the period of the adrenaline action potentials, the depression of the rhythmic wave response evoked by a testing volley runs a time course similar to the depression of the initial potential wave. Moreover, during this period of depression, we have seen that both the electrical and mechanical responses to a second injection of adrenaline are also diminished. Presumably, therefore, this slowly increasing depression produced by an injection of adrenaline is at least partly responsible for the diminution and eventual stopping of the rhythmic action potentials which, as we have seen, are only set up during the early stages of the response to a large quantity of adrenaline.

Discussion

The depressed excitability of the smooth muscle units during that part of the adrenaline response which is beyond the period of the rhythmic action potentials provides a further parallel with the conditions obtaining during acetylcholine contracture of some striated muscles, for Brown [1936] has shown that during this contracture it is difficult or impossible to set up impulses in the muscle fibres either by the application of additional acetylcholine or by direct electrical stimulation. *Thus there can be very little doubt that, after the initial contraction produced by rhythmic impulses in the muscle units, a large quantity of adrenaline sets up a later prolonged contracture of the muscle, during which tension may*

be maintained, though the rhythmic production of impulses has ceased and the excitability of the muscle units is greatly depressed.

Evidence has been presented which indicated that, with the mechanical responses evoked by one or two maximal nerve volleys, no contractile mechanism need be postulated other than that associated with impulses set up in the muscle units either as the initial *A* and *B* waves or as the later rhythmic waves. Apparently the adrenaline-like substance is liberated in such small quantities or in such a relation to the muscle fibres that little or no contracture is produced. However, it would be difficult to exclude a small contracture in such cases and the depressed excitability of the muscle units ("adaptation") may perhaps be identical with the depression which is associated with the adrenaline contracture, though of course no invariable relationship has been established between depressed excitability and contracture. Thus a small contracture possibly may be evoked even by single or double nerve volleys, and, with a tetanic stimulation, the accumulating Ad.-substance liberated by the successive volleys might give rise to a contracture comparable to that produced by large quantities of adrenaline. Hence the progressive depression of the *A* waves during tetanic stimulation [Rosenblueth *et al.* 1936; Rosenblueth & Cannon, 1936] has two possible explanations.

(1) The accumulating Ad.-substance sets up a rapid spontaneous rhythm of the muscle units, on account of which the successive volleys would find more and more units refractory.

(2) The accumulating Ad.-substance sets up a contracture of the muscle, during which there is a depression of the ability of the units to set up impulses. The first explanation would certainly obtain during the early phase of a tetanus. The second may be applicable to the later phases, particularly of a rapid tetanus. It is to be noted that on neither of these explanations is there a breaking down of the analogy between the *A* wave and the spike potential of striated muscles; hence this diminution of *A* waves during a tetanus cannot be used, as Rosenblueth *et al.* [1936] have done, as an argument against the *A* waves being due to all-or-nothing impulses (see the preceding paper).

Bacq & Monnier [1935] find that with monophasic recording an electrical potential change accompanies the mechanical response produced by adrenaline or by a series of motor nerve volleys, and they regard this potential as being due to a depolarization of the smooth muscle cells. Lambert & Rosenblueth [1935] with diphasic leading also recorded potentials (their III*b*) having the same time course as the mechanical response, but such potentials are so variable that it is difficult to exclude

movement artefacts as a possible explanation, though they have attempted to do so. We, too, have often recorded such potentials (cf. Figs. 7, 8 and 10), but they have generally disappeared on movement of the electrodes to minimize movement artefacts. Thus it is doubtful if such potentials are recorded when leading diphasically. Experiments on many excitable tissues [Adrian, 1930, 1931; Adrian & Gelfan, 1933; Barron & Matthews, 1936; Eccles, 1935*a*, *b*; 1936; Eccles & Pritchard, 1937; Erlanger & Blair, 1936; Fessard, 1936] indicate that depolarization of cells is conducive to the discharge of impulses, hence it seems likely that the repetitive response of the smooth muscle units is set up both by adrenaline and Ad.-substance on account of their depolarizing action. A similar explanation has recently been offered by Cowan [1936] for the rhythmic discharge of impulses which acetylcholine sets up in striated muscle [Brown *et al.* 1936; Brown, 1936, 1937].

However, it seems likely that contracture is also associated with a depolarization, for Cowan finds that the injury potential of an isolated prostigmin-treated frog's sartorius is greatly diminished by application of acetylcholine to its pelvic end. Though Cowan regards such experiments as suggesting that a repetitive discharge of impulses in muscle fibres is set up by acetylcholine by virtue of its depolarizing action, Brown's results [1936] indicate that in Cowan's experiments the muscle would actually be in a state of contracture. Hence these experiments are more significant in indicating a relationship between depolarization and contracture in striated muscle fibres. The prolonged depolarizations suggested by Bacq & Monnier's experiments may, therefore, form the basis not only of the initial rhythmic discharge of impulses by the muscle units, but also of the subsequent contracture. Clearly, further work on the depolarization is necessary before its relationship either to the rhythmic discharge of impulses or to the contracture can be regarded as established. It certainly at first seems anomalous that depolarization, while initially setting up a rhythmic discharge, later results in a contracture with depression of this rhythmicity, but parallel examples are provided by other excitable tissues. Thus, for example, during the passage of a constant current, the excitability of a nerve first increases at the cathode and later decreases [Blair & Erlanger, 1936].

Presumably the basis of the rhythmic production of impulses by a smooth muscle unit is some continuous stimulus (probably provided, as we have seen, by a partial depolarization), and the gradual recovery of the unit from a depression which follows each impulse [cf. the discussion

on rhythm by Eccles & Hoff, 1932, 1934]. The longest observed rhythmic cycles, 1·5 sec., give an approximate measure of the time for complete recovery from this depression, while at intervals shorter than the shortest observed rhythmic cycle, 0·3 sec., very little recovery from this depression can have occurred. Evidence has been adduced [Eccles, 1936, p. 392] to show that, with nerve cells discharging rhythmically, the time of each discharge is conditioned by the gradual recovery from a depressed excitability which is set up by the previous discharge, and which is associated with a positivity of the soma relative to the axon of the nerve cell. In this connexion significance may attach to the positive wave which follows the discharge of the impulses of the *A* and *B* waves. Many observations, e.g. Figs. 3, 4 and 5, indicate that this positive wave also follows the discharge of impulses at each of the rhythmic waves, and, if the positive wave is associated with a depressed excitability, there would thus be an explanation of the rhythmic response. Such an explanation is related to that suggested above for the early rhythmic wave, recovery from depression being then accelerated by the increased excitability associated with the *N* wave. However, our knowledge of these slow potential changes in smooth muscle and the associated excitability changes is so inadequate that these explanations can only be tentatively suggested.

An asymmetrical depolarization of the muscle fibres is suggested by the relative increase which adrenaline brings about in the positive phase of the diphasic *A* wave (cf. Figs. 17 and 18). Normally during the *A* wave set up by a motor nerve volley, more impulses are travelling in the muscle fibres from the attachment of the membrane to its free edge than in the reverse direction. When an *A* wave is set up during an adrenaline response, there must still be this preponderance of impulses travelling peripherally, for the forward movement of the positive phase of the diphasic potential no more than corresponds to the forward movement of the preceding negative phase. The diminution of this negative phase relative to the positive appears, therefore, to depend on the production of an increasingly greater potential by impulses as they run from the proximal part of the muscle towards the free border of the membrane, an effect which possibly is due to a greater depolarization by the adrenaline of the muscle fibres in the more proximal regions of the muscle where the nerve endings predominate.

SUMMARY

In addition to the initial complex potential wave, a motor nerve volley usually sets up, in the smooth muscle of the nictitating membrane, later rhythmic potential waves, each of these waves preceding a corresponding step in the mechanical contraction. The irregular contour of these rhythmic waves indicates that they are compounded of short spike-like waves, and a testing motor nerve volley shows that each rhythmic wave is followed by a refractory state of the muscle units. Hence it is concluded that these rhythmic waves are produced by the rhythmic discharge of all-or-nothing impulses in the units of the smooth muscle, such impulses being identical with those forming the *A* and *B* waves of the initial complex. Thus a single motor nerve volley sets up an irregular tetanus of the muscle units, the limiting frequencies being on the one hand almost as high as 3 beats per sec. and on the other as low as 1 beat in every 1·5 sec.

A small nerve volley usually gives rise to no rhythmic responses, such responses being produced either by summing the effect of two such volleys, or by increasing the size of a single volley. With two maximal nerve volleys, and occasionally with a single volley, the first beat of the rhythmic series may occur very soon after the initial complex. It is suggested that this may be due to the facilitatory process associated with the *N* wave of the initial complex.

Injections of adrenaline produce rhythmic responses of the smooth muscle units, which in all respects are similar to those described above, hence it is concluded that rhythmic responses are produced by motor nerve volleys on account of the Ad.-substance which is liberated in the muscle. The initial part of the adrenaline contraction appears to be largely, if not entirely, produced secondarily to the rhythmic impulses in the smooth muscle units, but, following on this contraction phase, there is a contracture during which the mechanical response continues in the absence of impulses in the muscle units. During this phase it is difficult or even impossible to set up impulses either by a further injection of adrenaline or by a testing motor nerve volley, this inexcitability of the units thus being analogous to the condition described by Brown during the acetylcholine contracture of normal frog's or denervated mammalian muscles. Single or double motor nerve volleys set up very little if any of this contracture response, possibly because the concentration of the liberated Ad.-substance is too small. It is suggested that small concentrations of adrenaline or Ad.-substance partly depolarize the smooth muscle

fibres, and hence render them spontaneously rhythmic, while the further depolarization produced by larger concentrations rapidly results in the contracture and an inability of the muscle fibres to conduct all-or-nothing impulses.

There is a considerable tendency to synchronization of the rhythmic responses which either Ad.-substance or adrenaline set up in the individual smooth muscle units. It is suggested that this is due to the exciting action which the action current in any one unit produces on adjacent units.

REFERENCES

Adrian, E. D. (1930). *Proc. Roy. Soc.* B, **106**, 596.
Adrian, E. D. (1931). *J. Physiol.* **72**, 132.
Adrian, E. D. & Gelfan, S. (1933). *Ibid.* **78**, 271.
Bacq, Z. M. (1935). *Ergebn. Physiol.* **37**, 82.
Bacq, Z. M. & Monnier, A. M. (1935). *Arch. int. Physiol.* **40**, 467.
Barron, D. H. & Matthews, B. H. C. (1936). *J. Physiol.* **87**, 26 *P.*
Blair, E. A. & Erlanger, J. (1936). *Amer. J. Physiol.* **114**, 317.
Boeke, J. (1932). *J. comp. Neurol.* **56**, 27.
Brown, G. L. (1937). *J. Physiol.* **89**, 12 *P.*
Brown, G. L. (1937). *Ibid.* **89**, 220.
Brown, G. L., Dale, H. H. & Feldberg, W. (1936). *Ibid.* **87**, 394.
Cannon, W. B. (1933). *Science*, **78**, 43.
Cannon, W. B. (1934). *Amer. J. med. Sci.* **188**, 145.
Cowan, S. L. (1936). *J. Physiol.* **88**, 3 *P.*
Eccles, J. C. (1935*a*). *Ibid.* **85**, 207.
Eccles, J. C. (1935*b*). *Ibid.* **85**, 464.
Eccles, J. C. (1936). *Ergebn. Physiol.* **38**, 339.
Eccles, J. C. & Hoff, H. E. (1932). *Proc. Roy. Soc.* B, **110**, 483.
Eccles, J. C. & Hoff, H. E. (1934). *Ibid.* **115**, 327.
Eccles, J. C. & Magladery, J. W. (1936). *J. Physiol.* **87**, 87 *P.*
Eccles, J. C. & Magladery, J. W. (1937). *Ibid.* **89**, 45 *P.*
Eccles, J. C. & Pritchard, J. J. (1937). *Ibid.* **89**, 43 *P.*
Erlanger, J. & Blair, E. A. (1936). *Amer. J. Physiol.* **114**, 328.
Fessard, A. (1936). *Recherches sur l'activité rhythmique des nerves isolés.* Paris: Herman.
Lambert, E. F. & Rosenblueth, A. (1935). *Amer. J. Physiol.* **114**, 147.
Langley, J. N. (1907). *J. Physiol.* **36**, 347.
Lawrentjew, B. I. & Borowskaja, A. J. (1936). *Z. Zellforsch.* **23**, 761.
Loewi, O. (1936). *Pflügers Arch.* **237**, 504.
Monnier, A. M. & Bacq, Z. M. (1935). *Arch. int. Physiol.* **40**, 485.
Rosenblueth, A. (1932). *Amer. J. Physiol.* **102**, 12.
Rosenblueth, A. (1935). *Quart. Rev. Biol.* **10**, 334.
Rosenblueth, A. (1936). *Symp. Quant. Biol.* **4**, 132.
Rosenblueth, A. & Cannon, W. B. (1936). *Amer. J. Physiol.* **116**, 414.
Rosenblueth, A., Davis, H. & Rempel, B. (1936). *Ibid.* **116**, 387.
Rosenblueth, A., Leese, C. & Lambert, E. (1933). *Ibid.* **103**, 659.