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THE ELECTRICAL ACTIVITY OF ISOLATED MAMMALIAN INTESTINES

By N. AMBACHE

From the Department of Pathology, Guy's Hospital Medical School

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This paper describes experiments on the origin and regulation of the pendulum movements of the intestine. Previous work (Ambache, 1946) has suggested that these movements may depend upon a nervous mechanism, acting eventually upon the musculature of the intestine by the liberation of acetylcholine, and there is already some evidence (cf. Keith, 1915) for the existence of a 'nodal' tissue in the intestine, which may control the rhythmic contractions.

The action potentials associated with pendulum movements have been recorded with a device allowing simultaneous presentation of electrical and mechanical changes on the same cathode-ray tube. The time relations of the electrical changes and the effects of suppressing the contraction upon the various phases of the action potential, suggest that the intestinal nerve net acts as a pacemaker for pendulum movements.

METHODS

The Preparation. The majority of experiments were carried out on rabbits, but a few preparations were also made from mice. All the animals were killed by concussion and bled. A coloured ligature was tied round the duodenum to identify its oral end. The small intestine was exsected and washed thoroughly with Tyrode solution, both inside and out, and a short piece of duodenum (1–2 cm.) was prepared as follows:

The gut was supported during manipulation by passing a glass rod through its lumen. Two coloured cotton threads (0·3 mm. in diameter), destined later to serve as leading-off electrodes, were stitched into the surface of the gut with a small curved cutting needle (Lane's No. 2). The stitches were placed in line on the antimesenteric border of the gut at its oral end, and the distance between them was measured. The aim was to place the stitches in the longitudinal muscle of the gut, and for this reason they were made as small and as superficial as possible. As a check on the depth of the stitches, the gut was evaginated and its interior surface was examined under a binocular dissecting microscope; in most cases the stitches were either not visible or just visible through the mucous membrane, which was itself in every case intact. Apart from this, it was difficult to assess the exact depth of the stitches, and it is probable that in some of the experiments the leads reached the plane of the circular muscle.

When this examination was completed, the intestine was re-invaginated, and after the fluid in its lumen had been gently squeezed out, it was ligated at both ends. The preparation was then connected to the apparatus which is shown in Fig. 1. It was suspended in a glass supporting

frame (*F* in Fig. 1) with the oral end upwards; the lower end was tied to a light whale-bone lever, *L*, which was hinged on to the lower end of *F*. The loose ends of the suture threads, E_1 and E_2, were then cut short (1 cm. or under) and the cut ends were slipped into the hooks, *H*, on the silver leads to the input of the pre-amplifier. A firm connexion was made by closing the hooks tightly over the threads with a pair of artery forceps. The firmness of this join (which was tested in each case) and the 'slack' in the suture threads effectively eliminated friction artefacts of the kind to which attention was drawn by Evans (1926).

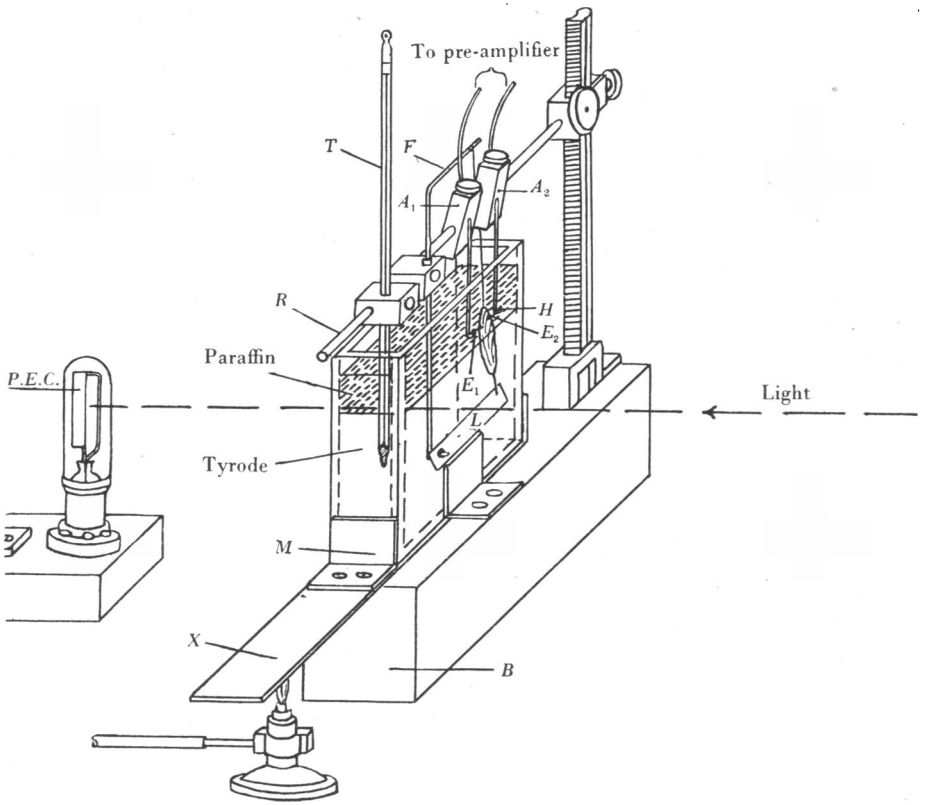

Fig. 1. Arrangement of muscle bath, electrodes and photoelectric cell. A_1, A_2, ebonite blocks; *B*, wooden block; E_1, E_2, electrode threads held in; *H*, hooked ends of silver input leads to pre-amplifier; *F*, glass frame for suspension of muscle and hinging of *L*; *L*, light whale-bone lever; *M*, metal holder for the glass bath, warmed at *X*; *P.E.C.*, photoelectric cell (Osram CMG25); *R*, glass rod; *T*, thermometer.

The preparation was then lowered into a parallel-sided glass bath of 120 c.c. capacity filled, but for a layer of medicinal liquid paraffin on top, with unaerated magnesium-free Tyrode solution. This bath was encased in a metal holder, *M*, which was mounted on a wooden block, *B*, and protruded over one edge of this block at *X*, where it was heated by a small gas flame till the temperature of the bath was between 32 and 40° C. (35–39° C. when records were taken). When electrical records were being made, the preparation was raised so that the electrodes lay in the layer of paraffin, but care was taken to avoid too long immersion of the gut in paraffin as this led to inactivity of the tissue; at the same time, the lever, *L*, was brought into the edge of a light beam previously directed on to the photoelectric cell.

The separation of the electrodes. During the actual experiment, the distance between the electrode sutures was liable to depend upon the tone of the preparation and the load on the muscle, both of which were variable. It has, therefore, been more convenient, in order to standardize the conditions of this measurement, to quote, in the legends and in the results, the distances measured at room temperature when the gut was fully relaxed.

Recording system. The recording system owes much to the kindness of Mr E. S. McCallister of the Mullard Wireless Service Co., and is described by him (1947) elsewhere in greater detail. The apparatus consisted of a Mullard cathode-ray oscillograph (type E800) modified to provide two separate channels of amplification, one for the photoelectric impulses produced by the mechanical contractions, and the other for the action potentials.

Switching device. The outputs from the two amplifiers were fed into a mechanical vibrator (Wright and Weaire, type ACD/6), which connected the vertical deflector plates of the cathode-ray tubes alternately to each amplifier at a frequency of 100 cyc./sec. Owing to this 'chopping' frequency being high compared with the frequency of the two phenomena studied, a faithful reproduction of them both appeared simultaneously on the screen of the cathode-ray tube. To provide a separate base-line for each phenomenon a variable bias voltage was applied to the output of one amplifier relative to the other. A continuous record of the two phenomena was taken with a moving-film camera.

Characteristics of the pre-amplifier. Records were taken between a caudally situated earthed electrode and another, proximal electrode connected to the grid of the first valve of the pre-amplifier. In all records shown, an upward deflexion indicates negativity of the grid electrode. The high input impedance ($2\,\mu$F. + 3 megohms) permitted the use of 'polarizable' metallic electrodes (cf. Hodgkin, 1937). The inter-valve coupling time constants ($1{\cdot}5\,\mu$F. + 3 megohms) were adequate for the requirements of this research, being sufficiently large compared with the duration of the action potentials studied.

The overall sensitivity of this channel was approximately $70\,\mu$V. (d.c.)/cm. deflection on the cathode-ray tube. The noise level was 15–20 μV. Small fluctuations of the base-line were effectively prevented by the second valve in the pre-amplifier which acts as a stabilizing device.

Interpretation of the photoelectric record. The movements of the muscle lever in the beam of light produce a voltage variation which is amplified in the second channel. Because of the condenser coupling between the valves, it is evident that long-lasting changes of tone will not be faithfully reproduced by this amplifier. Thus the records provide no indication of the tone level of the preparation. This is a drawback which did not matter in the present experiments, the aim being to show the time relation of the action potentials to the onset of each rhythmic contraction.

Despite the large coupling time constants, a certain amount of distortion of the contraction curve is apparent in some of the photoelectric records. Thus, when the interval of relaxation between successive pendulum movements is prolonged (Fig. 2*c*), there is a slow upward deflexion, starting some 2–3 sec. before the true contraction of the muscle. This is probably due to the condenser decay of the steady p.d. from the photoelectric cell during the 3–4 sec. of the relaxation phase. The true onset of contraction is indicated in the records by the sharp upward inflexion in this curve, which is coincident with the beginning of each pendulum movement.

The tracings were timed at the end of each experiment by interrupting the light beam at fixed intervals by means of a clock.

RESULTS

The electrical activity attending pendulum movements

The experiments were carried out without oxygenation of the bath and with the lumen of the gut completely empty. This procedure was calculated to abolish active peristalsis without affecting the pendulum movements (Ambache, 1946); in fact, peristaltic contractions were never observed.

The records obtained under these conditions reveal the existence of

rhythmically recurring electrical changes associated with each pendulum movement. With the preparation as short as possible (1 cm.) and the distance between the electrodes small, the potential changes observed are of two kinds, each with its own temporal characteristics. There is first (Fig. 2) a slow diphasic wave, 'A', which *precedes* the contraction; in Fig. 2*b* the time interval

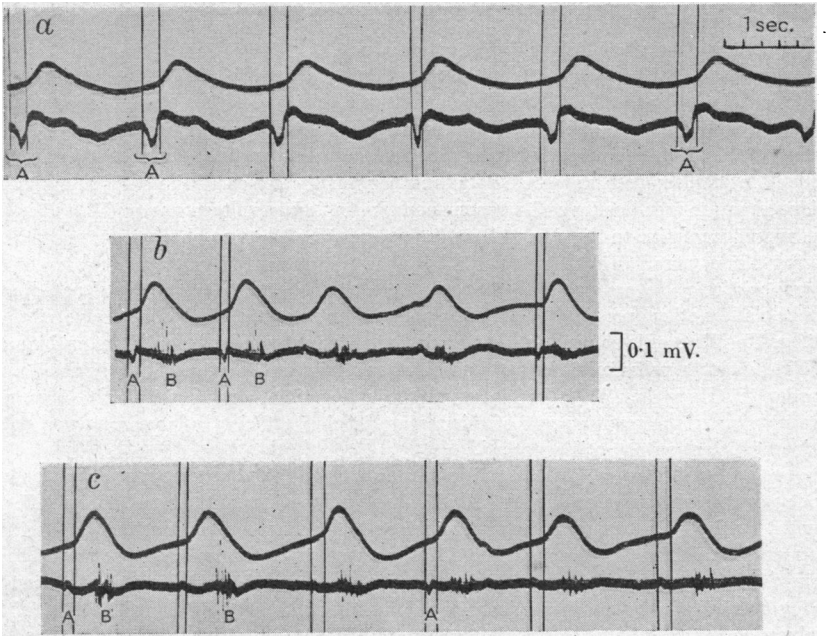

Fig. 2. Simultaneous records of the rhythmic contractions (top line: photoelectric record) and the action potentials (bottom line) of a preparation (1 cm. long in the relaxed state) of fresh rabbit's duodenum. This and all subsequent records should be read from left to right; time, 1 sec. Calibration: 0·1mV. (d.c.). (*a*) Leads $\alpha\gamma$ (1 cm. apart). Diphasic 'A' waves are seen preceding each contraction. An upward deflexion indicates negativity of the grid electrode (oral end of the muscle) in this and all subsequent records. Paired vertical lines have been drawn through (*a*) the onset of the 'A' waves, and (*b*) the beginning of each contraction, to show the time lag between the two phenomena. (*b*) and (*c*) Leads $\alpha\beta$ (1 mm. apart); showing the polyphasic response 'B' which follows 'A'. 'B' occurs during the contraction.

between these two phenomena is 0·5–0·75 sec. This time interval is also found when the contractions are recorded by a direct optical method. For this purpose, a ground-glass screen was mounted above the face of the cathode-ray tube, and a beam of light was reflected from an optical lever (in air) on to this screen. The alignment of the two 'spots' was carefully checked with a plumb-line at the time of the experiment and, later, on the photographs. This experiment also showed that the damping effect of the bath fluid on the lever (in the photoelectric method) was negligible.

The 'A' wave is followed by a faster, polyphasic response, 'B', which occurs *during* the contraction. In Figs. 2*b*, *c*, the distinction between 'A' and 'B' is quite clear, but in other records it is sometimes obscured by the merging of 'B' into 'A'. By various means, which are described below, it has been possible to obtain the first wave, 'A', without the other (e.g. in Fig. 2*a*), and for this and a number of other reasons, given subsequently, it is possible to interpret these two types of potential recorded as manifesting the activity of two different tissue components within the gut.

Berkson, Baldes & Alvarez (1932) have reported the occasional occurrence of contractions without any electrical change. In the present experiments this has been noticed in fresh preparations only twice; it appears to happen when that part of the muscle which is immediately under the electrodes, i.e. the top end of the gut, is left for too long in the paraffin layer. To avoid this local failure, the records were only taken immediately after the muscle was raised into the paraffin.

Effect of electrode separation

The following experiment was designed to show the effect of varying the distance between the electrodes on the type of activity recorded. A third silver electrode was included in the bath, and the gut was prepared with three leading-off sutures: one (α) at the top end of the muscle; the second (β) 1 mm. below α, and the third (γ) at the bottom end of the gut, 1 cm. below α. The muscle was permanently connected to the grid of the input through α, but it could be earthed at will through β (arrangement $\alpha\beta$; electrodes 1 mm. apart) or through γ (arrangement $\alpha\gamma$; electrodes 1 cm. apart).

Leads $\alpha\beta$ (1 *mm.*). An inspection of the records shows that the waves 'A' and 'B' are invariably present (Fig. 2, *b*, *c*); their timing with respect to the mechanical contraction needs no further description. The amplitude of 'A' is usually 50 μV. from peak to peak, but the amplitude of the individual component waves in 'B' varies from one wave to the next, and often from contraction to contraction, ranging between 50 and 200μV. There appears to be a general tendency for the whole 'B' complex to decrease as the contractions get smaller, when, for instance, the muscle is left too long in the paraffin layer. In certain cases, where the component waves in 'B' are sufficiently spread out, one can make out their diphasic nature (Fig. 2*c*).

Leads $\alpha\gamma$ (1 *cm.*). In these records the 'A' wave is still regularly present and always precedes the onset of the mechanical contraction, often by as much as 1 sec. (Fig. 2*a*), but there has been a noticeable change in the shape of this wave, which shows considerable broadening. Thus the distance between the peaks of this diphasic wave (wherever these can be made out distinctly) shows a four- or fivefold increase. There has also been an increase in its amplitude (now 100–110μV.), but it is difficult to know whether this is merely due to a difference

in the depth of the electrode sutures at β and γ or to other causes. On the other hand, the broadening of 'A' indicates clearly an increase in the interelectrode conduction time of this response, as the electrodes are separated.

The other noticeable change in the $\alpha\gamma$ records is the frequent absence or attenuation of the polyphasic response 'B'. When present (in only sixteen out of ninety recorded contractions, in the experiments of Figs. 2 and 4), the average amplitude of this response is reduced, but its temporal characteristics are unaltered (see also Fig. 3). In another experiment the amplitude of the 'B' response was quite considerable in the $\alpha\beta$ records (Fig. 5*b*, *c*). Despite a certain amount of attenuation, these spikes are still clearly visible in the $\alpha\gamma$ records from the same preparation (Fig. 3).

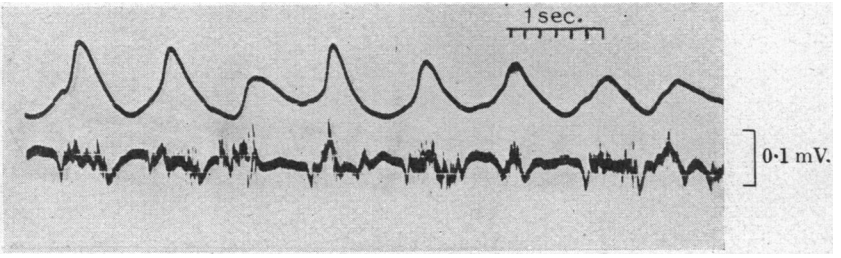

Fig. 3. Effect of electrode separation on the 'B' response. $\alpha\gamma$ records (electrodes 1 cm. apart) from a rabbit's duodenum, showing attenuation but no significant broadening of the individual waves in 'B'; for comparison, see the $\alpha\beta$ records (electrodes 1 mm. apart) in Fig. 5*b* and *c*, which were taken from the same preparation. Nicotine was present in the bath in a concentration of 1 in 10^4.

Spontaneous reversal of conduction of the 'A' wave

In their original experiments on pendulum movements, Bayliss & Starling (1899) noticed that the rhythmic contraction waves in the intestine 'pass as often in one direction as in the other', although there is 'a preponderance of descending contractions'. The electrical records sometimes show a corresponding change-over in the direction of conduction of the 'A' wave, occurring spontaneously in the middle of an experiment. The two phenomena appear to be related.

If we consider negativity as a sign of excitation, we see that throughout the experiment of Figs. 2 and 4*a*, the contractions are initiated by an excitation wave which passes the earth electrode first and then the grid electrode, and is in fact travelling, in this case, from the caudal end of the preparation upwards. Towards the end of this experiment a few contractions were recorded which were initiated by an excitation wave travelling in the opposite direction. This wave, the mirror image of 'A', has been called 'C'. This is shown in Fig. 4*b*, where the 'C' wave has for two beats superseded and replaced the 'A' wave, the excitation

travelling downwards (from grid to earth); but at the third beat excitation from below preponderates again and 'A' is reinstated. This phenomenon has been recorded in a number of experiments. The time interval between the conducted electrical variations ('A' or 'C') and the onset of contraction (or its peak, in other experiments) is independent of the direction of the conduction. The 'C' wave is also seen, sometimes, just after 'A', as, for example, in Fig. 4*a*, where it would seem that the preparation is discharging at both ends, excitation from below occurring first. The possible effects of such a double excitation is shown in another experiment in which the length (4 cm.) of the preparation

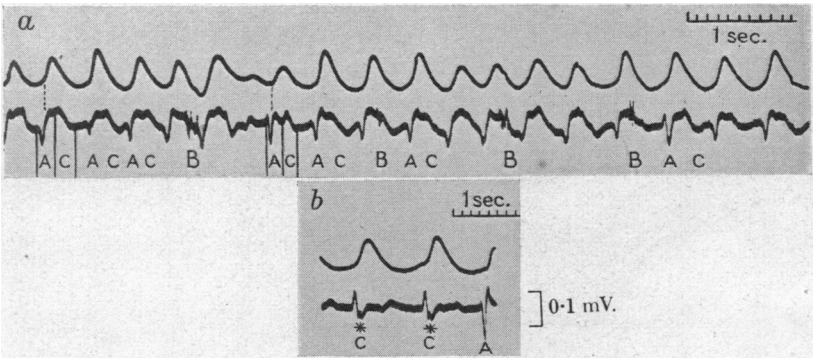

Fig. 4. (*a*) Continuation of the same experiment as Fig. 2. Leads $\alpha\gamma$ (1 cm. apart) showing 'A' and 'C' waves, and occasional 'B' responses. The continuous vertical lines delimit the 'A' and 'C' waves; the dotted vertical line shows the onset of contraction. For explanation see text. (*b*) Later. Spontaneous reversal, for two 'beats' (marked with asterisks) of the electrical disturbance which precedes contraction, producing a 'C' wave (see text); conduction is now from grid to earth, i.e. from the oral end downwards. The third 'beat' is preceded by an 'A' wave; conduction is again from the earth to the grid electrode, i.e. from the caudal end upwards. The time interval between the electrical change and the onset of contraction is independent of the direction of conduction.

was greater than usual. The photoelectric records from this preparation (Fig. 6*e*) showed a 'step' in each contraction, the muscle appearing to contract in two halves. The electrical change which was recorded at the top end of the muscle just preceded the second half of the contraction.

Persistence of both types of potential after nicotine

A few experiments were carried out in the presence of nicotine. A concentration of 1 in 10^4 was used, as previous experiments had shown that this was more than sufficient to paralyse the ganglion cells in the rabbit's gut. The results of two experiments are shown in Fig. 5. In the first (Fig. 5*a*), the 'A' waves were well marked and continued after the introduction of nicotine, but the 'B' waves were diminished or absent, possibly through depression of the muscles fibres in the interpolar field; in fact, such a concentration of nicotine

has in previous experiments sometimes produced a noticeable depression in the size of pendulum movements. In the second experiment polyphasic 'B' responses were seen both before (Fig. 5*b*) and after (Fig. 5*c*) nicotine. This experiment was remarkable for the irregularity of the contraction record, and for the merging of the two components in the electrical records. The two experiments taken together show that both the 'A' and 'B' waves can persist in the presence of high concentrations of nicotine (see also Fig. 3).

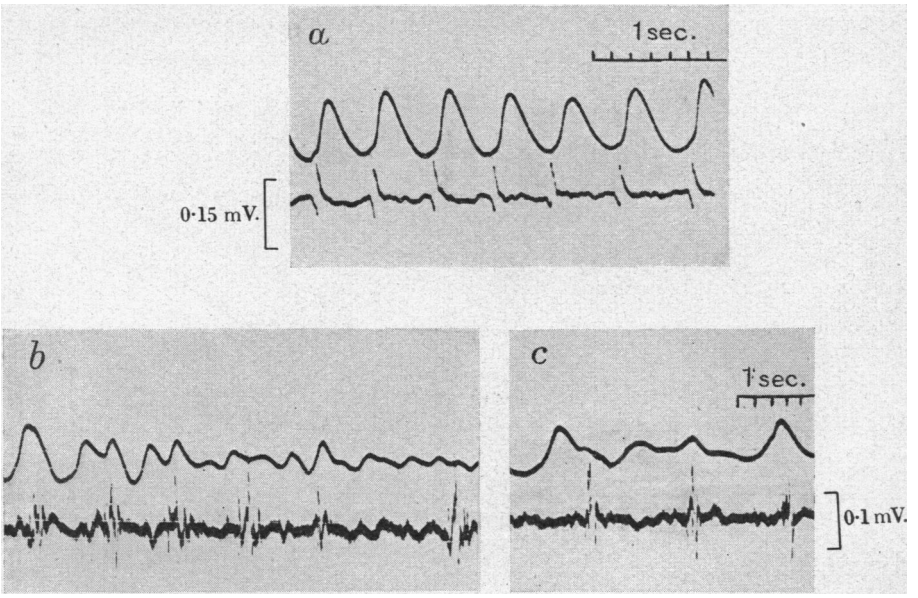

Fig. 5. Effect of nicotine on the duodenum of two rabbits. (*a*) Persistence of 'A' waves in the presence of one part in 10,000 of nicotine (electrodes 1 mm. apart). (*b*) and (*c*) From another preparation (electrodes 1 mm. apart). (*b*) Initial activity consisting mainly of polyphasic 'B' responses merging into the 'A' waves. (*c*) Persistence of the 'B' response in the presence of nicotine (10^{-4}).

Electrical activity in the absence of contraction

Several different ways have been employed of arresting the movements of the gut. With the exception of chloroform they all produce the same modification in the recorded response.

(*a*) *Calcium.* The suppression of pendulum movements by an excess of calcium ions has been shown, previously, to be attributable to an inhibition of acetylcholine release at the nerve endings (Ambache, 1946). It was of interest, therefore, to see what changes were produced by this substance in the electrical response of the gut. The more sensitive method of recording the contractions photoelectrically shows that, even with concentrations of $CaCl_2$ as high as

0·4 % (20 × normal), pendulum movements may persist, although they are now slightly irregular and very minute (Fig. 6*d*). With the reduction in size of

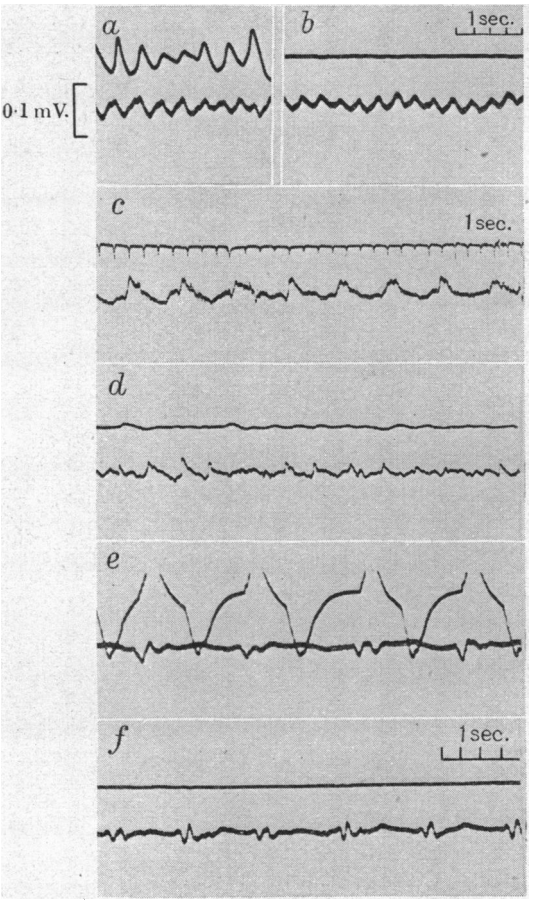

Fig. 6. Effect of inhibitory substances. Top line: photoelectric contraction-record, except in (*c*), where it indicates time. Bottom line: as before. (*a*) and (*b*) Mouse gut. Grid electrode, sewn on to the oral end, in paraffin; earth electrode not attached to the muscle, but immersed in Tyrode solution; top $\frac{3}{4}$ of the preparation (2 cm. long) in the paraffin layer, bottom $\frac{1}{4}$ in the Tyrode layer. (*a*) Initial activity. (*b*) Effect of adrenaline (10^{-5}). Time in 1 sec. (*c*) and (*d*) Rabbit duodenum; usual fixation of electrodes (distance 1 mm.). (*c*) Initial activity (electrical change only; top line indicates time in 1 sec.). (*d*) Double recording; effect of 0·4 % $CaCl_2$; camera speed as in (*c*). (*e*) and (*f*) Another preparation. Rabbit duodenum; total length of the preparation 4 cm.; electrodes 4 mm. apart. Time in 1 sec. (*e*) Initial activity, showing contractions occurring in two halves; the electrical change precedes the second half-contraction. (*f*) Effect of 0·5 % $CaCl_2$. Inhibition is complete but the 'A' waves continue.

the contractions there is a great reduction in, and in places a disappearance of, the fast polyphasic response 'B', but 'A' continues unabated. In another

experiment (Fig. 6*f*), the inhibition produced by calcium was, for a time, complete, with a higher concentration than usual (0·5 % $CaCl_2$ = 25 × normal). Yet the 'A' waves (which showed reduplication in this experiment) continued; in fact, their frequency was slightly raised, from 11·5 to 15/min.

(*b*) *Adrenaline.* With adrenaline, although the mechanism of inhibition is different, the result is the same, both in rabbits (thus confirming an earlier observation of Berkson *et al.* 1932) and in mice (Fig. 6*b*).

(*c*) *Short periods of cooling.* On one occasion, the gut was left in the refrigerator for 3 hr. before the experiment. The preparation was then transferred to warm Tyrode solution in the bath and examined at intervals. After 25 min. muscular contractions were still in abeyance; nevertheless, slow rhythmic 'A' potentials of the usual type were observed at this stage, but fast impulses were absent. Half an hour later, i.e. 55 min. from the start of the experiment, vigorous pendulum movements had returned. Each contraction was now preceded by its 'A' wave, and fast impulses of type 'B' were also present during the contractions.

(*d*) *Chloroform.* When the Tyrode solution was saturated with chloroform, both the 'A' and 'B' types of activity were abolished.

The observations under headings (*a*), (*b*) and (*c*) all show that the slow potentials of type 'A' not only precede the pendulum movements but are also independent of the process of contraction, whereas the faster impulses 'B' appear to be more intimately associated with that process.

Effects of eserine

The effect of eserine on pendulum movements is difficult to analyse in fresh preparations because it also brings a reappearance of peristaltic reflexes. It was, therefore, convenient to examine the electrical changes produced by this drug in preparations cooled for 1 or 2 days, when the second action of eserine, which is undesirable in these experiments, is eliminated. Owing to the gradual decrease in the excursion of the pendulum movements produced by cooling, the records were taken with higher lever magnifications and a brighter light intensity of the beam falling on the photoelectric cell.

In the experiment of Fig. 7 *a–c*, the preparation was the same throughout, and the experiment was conducted in such a way as to avoid any alteration in the position of the electrodes from beginning to end. The initial activity of the gut was of the usual type (Fig. 7*a*), although the mechanical contractions tended to be slightly irregular. After the records were taken on the first day, the muscle was left in the bath and the whole bath was kept overnight in the refrigerator at 0–2° C. After cooling for 20 hr., the bath fluid was renewed and warmed to 37° C., and the preparation was re-examined. There was no appreciable change, at this stage, in the frequency of the rhythmic contractions, but they were smaller. At the same time, the electrical activity of the gut consisted

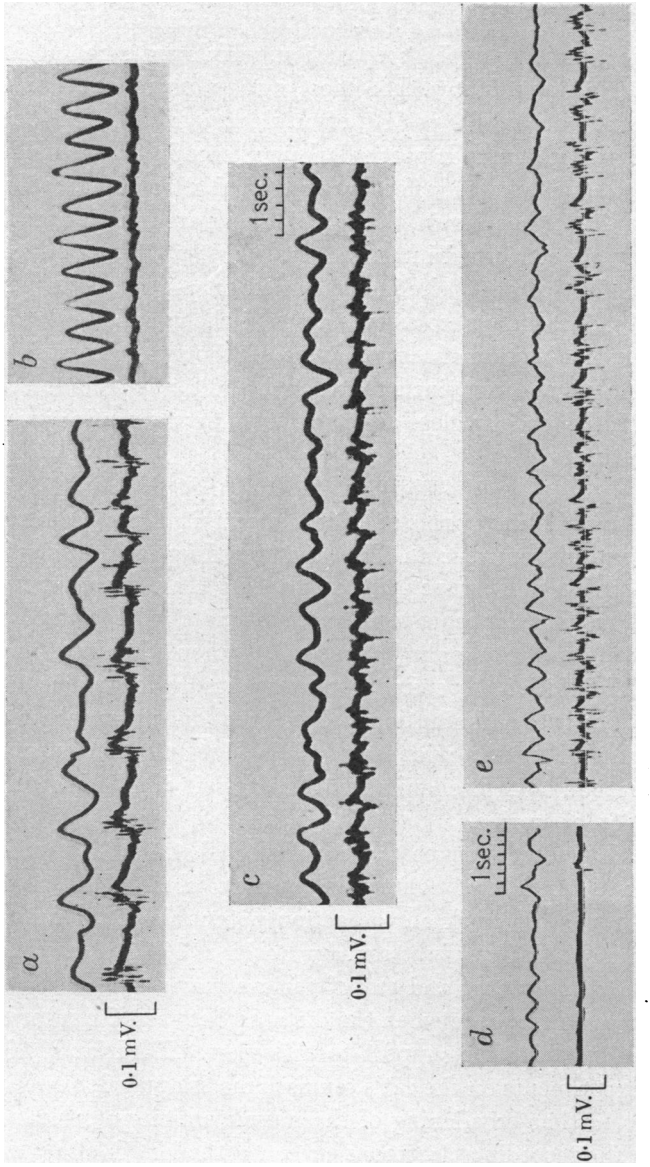

Fig. 7. Effect of cooling. (*a*), (*b*) and (*c*) From the same preparation (rabbit duodenum; electrodes 1·5–2 mm. apart). (*a*) Initial activity, before cooling. (*b*) Activity of the same preparation after cooling for 20 hr. at 0–2° C. Note disappearance of 'B' potentials. (*c*) Effect of eserine sulphate (4×10^{-7}) showing return of spike potentials of type 'B'. The excursion of individual pendulum movements is smaller because the muscle is in a contracted state. (*d*) and (*e*) From another preparation (rabbit), cooled for 27 hr. at 0–2° C. Electrodes 1·5 mm. apart. (*d*) Initial activity of the cooled preparation. (*e*) Effect of eserine sulphate (8×10^{-7}) introduced into the bath 6 min. previously. Grouping of the 'B' potentials is seen in the latter half of the tracing.

mostly of 'A' waves (Fig. 7*b*); but in some experiments these were so small as to be barely detectable with the amplification available. In another experiment (Fig. 7*d*) there was after 27 hr. very little electrical activity. In both these experiments the addition of eserine (Fig. 7*c*, *e*) brought about a progressive increase in the electrical activity of the preparation, which was small at first, but very marked after a few minutes. The most noticeable change was the reappearance of spike potentials of type 'B', often clearly grouped, with each rhythmic contraction; this grouping is particularly well seen in the second half of Fig. 7*e*. A similar, though smaller, effect was recorded after 50 hr. cooling; at such a time, as Vogt (1943) has shown, the ganglion cells are functionless and it is unlikely that they are concerned in this effect.

Acetylcholine. The effect of acetylcholine was tried on preparations which were cooled previously for 2–4 days, so as to inactivate the ganglion cells, and thus eliminate the 'nicotine' action of acetylcholine. Although the muscle was shortened by acetylcholine and remained contracted, there was no detectable electrical activity, with the leads as close as 1 mm., even in preparations which later responded to eserine.

DISCUSSION

In these experiments, two distinct forms of electrical activity have been found to accompany pendulum movements: an 'A' wave preceding each mechanical contraction and a polyphasic 'B' wave occurring during the contraction.

The polyphasic 'B' response appears to be most intimately associated with the contraction proper and can only be recorded when the intestine is actually contracting. Inhibition of pendulum movements by adrenaline or by excess of calcium ions, leads to a diminution and eventual disappearance of the 'B' electrical activity, and similar evidence of their close interdependence is provided by the increase in both mechanical and 'B' activity in the cooled gut after the administration of eserine.

It seems therefore difficult to escape the conclusion that the 'B' activity represents the action potentials of contracting muscle fibres. This view is supported by Fletcher's (1937) work on the action potentials of the anterior retractor of the byssus of *Mytilus edulis* and by Bozler's (1942) observations on the smooth muscle in the uterus and gut of various mammals.

The behaviour of the 'B' response when the distance between the recording electrodes is changed gives further support to this view. If Adrian's (1925) argument, based on experiments on the cat's tenuissimus, is applied, one would expect there to be no further broadening of the muscle action potential once the distance between the recording electrodes exceeded the length of a muscle fibre or active unit of the intestine. This, in fact, is what occurs; increased separation between the electrodes causes no obvious change in the shape of the 'B' potential, which would therefore appear to arise in units less than 1 mm. in length. In fact, the muscle fibres in the longitudinal layers of the rabbit's

intestine have been measured in conjuction with these experiments; in histological sections their average length is between 70 and 100μ.

The 'A' potentials present, on the other hand, an entirely different picture. Their onset precedes by a second or more the onset of contraction. They persist in intestine in which the pendulum movements have been inhibited by a variety of means. They show a clear dependence upon the distance between the recording electrodes, and it is difficult to avoid the conclusion that they are arising in some continuous conducting tissue with a fibre length much greater than that of the muscles concerned.

It is, of course, possible that the 'A' waves arise in muscles in which excitation is occurring without the appearance of internal work, such as occurs in the heart (Clark, 1938) or in the muscles of the crayfish (Marmont & Wiersma, 1938). Previous experiments (Ambache, 1946), however, have shown that a muscle in which movements have been stopped by calcium can still respond by contraction to acetylcholine and to electrical stimulation, and it appears unlikely that the 'A' wave could spread over tissue in this state of excitability without itself causing contraction.

If muscles are excluded as a source of the 'A' wave, the most likely tissue responsible for it in the gut would appear to be some nervous network. It seems improbable that Auerbach's plexus proper could be involved, since the wave persists after doses of nicotine sufficient to prevent transmission of excitation over synaptic junctions on ganglion cells.

The tissue which, in the author's opinion, is most likely to be involved is the nerve net which is present round Auerbach's plexus and in the interstices of the smooth muscle (Cajal, 1893, 1905). This structure seems to be identical with the 'nodal' tissue found by Keith (1915) in the rat's intestine. It is a truly syncytial structure consisting of the 'interstitial cells of Cajal' with their numerous anastomotic processes (Li, 1940).

Many of the phenomena observed in these experiments are compatible with the theory that such a nerve net acts as a pacemaker controlling pendulum movements and that its activity is manifested electrically in the 'A' wave. Such facts as the reversibility of conduction and the synchronization of the circular and longitudinal muscle layers of the gut (Bayliss & Starling, 1899; Trendelenburg's tracings, 1917) would be compatible with it. It would further explain the persistence of co-ordinated pendulum movements after nicotine, since this drug, in doses which paralyse synaptic ganglion cells, has no effect on other nervous tissues such as the dorsal root ganglia, the ganglion nodosum vagi and the bipolar nerve cells of the skate, all of which are non-synaptic (Langley & Dickinson, 1889; Langley, 1901).

Both Fischer (1944) and Rosenblueth & del Pozo (1942) have pointed out that smooth muscles form a heterogeneous class, and that comparisons between them may be misleading. This is very obvious when the electrical responses of

different smooth muscles are compared. For instance, a rhythmic component has been recorded electrically from the cat's nictitating membrane in response to single motor nerve volleys or to injections of adrenaline (Eccles & Magladery, 1937). These authors have suggested that 'adrenaline partly depolarizes the smooth muscle fibres, and hence renders them spontaneously rhythmic'. Yet, Rosenblueth & del Pozo (1942), who have criticized these deductions, failed to find any rhythmic component in the response of the pilomotors in the cat's tail, another muscle with an adrenergic innervation. These differences cannot be explained in our present state of knowledge. For instance, there is at present little information regarding the differences in the nerve-net innervation of the nictitating membrane and of the pilomotors, and any interpretation which does not take into account such possible differences between various smooth muscles is necessarily incomplete.

In comparing Eccles & Magladery's results with the above, it must be pointed out that their records were obtained with leads situated at either end of the nictitating membrane, i.e. many fibre lengths apart. In the present experiments it has been found that the muscle fibre potentials are considerably attenuated when the leads are so far apart. Also, with such an unrestricted lead it is impossible to obtain an action potential in skeletal muscle in response to injected acetylcholine (Brown, 1937), because the responses of the individual units are so out of phase as to cancel out; the failure to elicit, with acetylcholine, any electrical change from the gut in the present experiments could possibly be explained on similar lines. Yet, Eccles & Magladery obtained rhythmic responses to injected adrenaline, and they state that 'the various units are not responding independently. There must be some co-ordinating process by which they are kept in phase.' If it could be shown that a nerve net is also present in the nictitating membrane then Eccles & Magladery's findings would receive an explanation and their results could be brought into line with these.

SUMMARY

1. The electrical activity associated with pendulum movements has been recorded from isolated preparations of mammalian gut. An analysis of the records shows the existence of two distinct types of action potential.

2. There is, first, a slow diphasic wave, 'A', which *precedes* the onset of contraction by 0·5–1 sec. This electrical disturbance may start at either end of the preparation and is conducted for distances far greater than the length of one muscle fibre.

3. With electrodes close together there is also after the 'A' wave and *during* the contraction, a polyphasic response, 'B', consisting of faster diphasic spikes, the duration of which appears to be independent of the distance between the electrodes.

4. Both these responses persist after doses of nicotine which paralyse the ganglion cells.

5. When pendulum movements are inhibited by adrenaline or by an excess of calcium ions:

(*a*) The response 'B' disappears. It is thought that this polyphasic response consists of the action potential of asynchronous groups of muscle fibres.

(*b*) The 'A' waves continue. It is suggested that these represent the discharge of a pacemaker in the gut, and may arise in the nerve net which was described by Cajal (1905).

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REFERENCES

Adrian, E. D. (1925). *J. Physiol.* **60**, 301.
Ambache, N. (1946). *J. Physiol.* **104**, 266.
Bayliss, W. M. & Starling, E. H. (1899). *J. Physiol.* **24**, 99.
Berkson, J. Baldes, E. J. & Alvarez, W. C. (1932). *Amer. J. Physiol.* **102**, 683.
Bozler, E. (1942). *Amer. J. Physiol.* **136**, 553.
Brown, G. L. (1937). *J. Physiol.* **89**, 220.
Cajal, S. Ramon y (1893). *C.R. Soc. Biol., Paris*, **45**, 215.
Cajal, S. Ramon y (1905). *Manual de histologia normal.* Madrid.
Clark, A. J. (1938). *The Metabolism of the Frog's Heart.* Edinburgh.
Eccles, J. C. & Magladery, J. W. (1937). *J. Physiol.* **90**, 31, 68.
Evans, C. L. (1926). *Physiol. Rev.* **6**, 358.
Fischer, E. (1944). *Physiol. Rev.* **24**, 467.
Fletcher, C. M. (1937). *J. Physiol.* **90**, 233.
Hodgkin, A. L. (1937). *J. Physiol.* **90**, 183.
Keith, A. (1915). *J. Anat. Physiol.* **50**, 2 *P.*
Langley, J. N. (1901). *J. Physiol.* **27**, 224.
Langley, J. N. & Dickinson, W. L. (1889). *Proc. Roy. Soc.* B., **46**, 426.
Li, P. L. (1940). *J. Anat., Lond.*, **74**, 348.
McCallister, E. S. (1947). *J. Electronic Engineering.* In the Press.
Marmont, G. & Wiersma, C. A. G. (1938). *J. Physiol.* **93**, 173.
Rosenblueth, A. & del Pozo, E. C. (1942). *Amer. J. Physiol.* **137**, 263.
Trendelenburg, P. (1917). *Arch. exp. Path. Pharmak.* **81**, 55.
Vogt, M. (1943). *J. Physiol.* **102**, 170.