RELATION OF THE EXTRINSIC NERVES OF THE INTESTINE TO THE ACTION OF ATROPINE ON THE INTESTINAL MOTILITY

by

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INTRODUCTION

1. Historical:

Extracts of the belladonna plants have been known and employed in various ways for many centuries, and atropine, the principle alkaloid responsible for their actions, was isolated in 1831. (1) However, little is said of its pharmacology prior to 1866, other than to describe its effects on the central nervous system and its toxicology. Therapeutically, it was used both internally and locally for a great variety of ailments. Among other uses Wood in 1860 (2) mentions mydriasis in ophthalmic surgery, relief of emuresis nocturna in children, and the relief of intestinal spasms. According to Wood, the first suggestion of an understanding of its fundamental action was made by Müller. Johannes Müller knew that constriction of the pupil was produced by electrical stimulation of the short ciliary nerves (1844)(3) from his own experiments, as well as those of Mayo in 1923 and the earlier work of von Fowler, Reinhold, and Nysten. Wood (2) states that Muller suggested that atropine produced mydriasis by relaxing the circular muscle of the iris by some influence on the ciliary nerves. Bernstein and Dogiel in 1866 (4), confirming Müller's suggestion, showed that atropine blocked the pupillary constriction produced by oculomotor stimulation.

The demonstration of the inhibitory action of the vagus nerve on the heart by the Weber brothers in 1845 (5) and the secretory effect of chorda tympani stimulation by Ludwig (6) and Claude Bernard in 1851 (6) accelerated the investigations leading to the generally accepted present day concept of the action of atropine on the peripheral autonomic

nerves. Though the fast heart produced by Atropa Belladonna had been observed previously, Bezold and Bloebaum in 1867 (4) were first to demonstrate the cause by showing that atropine blocked the cardio-inhibitory effect of vagal stimulation in the frog and dog. They noted the preliminary slowing of the heart after administration of atropine and attributed it to central stimulation of the cardio-inhibitory centers in the medulla, an effect later blocked by the paralysis of the vagal endings. Schmiedeberg in 1870 (7) further localized the action on the heart of the frog. By electrical stimulation separately of the vagal trunk and the sinus crescent with its parasympathetic ganglion cells and postganglionic fibers, both before and after the application of atropine or nicotine, he showed that both drugs blocked the effects of preganglionic stimulation, but only atropine blocked the ganglionic or postganglionic stimulation. In 1872 Heidenhain (8) showed that atropine blocked the secretory effects of chorda tympani stimulation. Again, Langley in 1890 (9) showed the action, in contrast to that of nicotine, to be postganglionic. Following this, similar observations were made on most of the other glands of the body, all bearing out in general the blocking effect of atropine on postganglionic parasympathetic nerves.

Both the effects of vagal stimulation and of atropine on the small intestine were disputed for many years. Bayliss and Starling in 1899 (10) pointed out that prolonged vagal stimulation was necessary to produce a consistent effect on the intestinal motility. In general their results show a stimulatory effect, but vary somewhat with the previous state of intestinal activity. If the loop activity were low, vagal stimulation tended to increase activity during stimulation, while

if the loop activity were high, inhibition often lasted throughout the period of stimulation, but was immediately followed by marked increase in tone and amplitude of contractions. Doses of atropine up to 30 milligrams in their dogs produced no effect on vagal stimulation. This result was confirmed by Henderson in 1923 (11). Furthermore, Bayliss and Starling stated that they noted no effects of atropine on the power and rhythm of contractions, nor the local reflexes of the intestine, though it blocked the excitatory effect of muscarine. Many other workers, as reviewed by Schubel in 1924 (12), added contradictory results to the effect of atropine on the intestines by a variety of techniques. While clinicians generally were showing that atropine in doses of 1 to 2 milligrams, (Wood, 1905)(15) which produce blood concentrations of 1/5,000,000 or less, produced therapeutic effects in man, Magnus in 1905 (14), introducing the isolated gut technique, reported that baths containing a 1/4,000 concentration of atropine were stimulatory while lesser concentration had no effect. In much higher concentrations, the drug paralyzed the bowel wall. Unger in 1907 (15) showed that concentrations of 1/2,000,000 or less produce a decrease in tone and rhythm of the isolated intestine of rabbits. His results have been amply verified, though at first denied by Magnus in 1908 (16) on repetition of his experiments. It was evident that experimental conditions and dosage were responsible for many of the contradictory results reported in the literature of those days.

While in general, though not without arguments to the contrary, the actions of atropine at that time might be described as parasympathetic blocking, the exact mechanism was disputed. About 1906 a series of

observations began which culminated in the theory of chemical transmission of nerve impulses with acetylcholine as the neurohormone produced at most of the neuroeffector junctions of the parasympathetic system. The possibility of chemical transmission of nerve impulses was probably first suggested by Dubois-Reymond in 1877 (17). In 1904 T. R. Elliot (18) pointed out the similarity between the actions of extracts of the adrenal medulla and sympathetic nerve stimulation, and noted that adrenal extracts stimulated the effectors even after the degeneration of the sectioned sympathetic nerves. He postulated that adrenalin was the chemical transmitter at the neuroeffector junctions of the sympathetic nervous system. In 1906 Dixon (19) pointed out the faithful similarity between parasympathetic stimulation and the effect of muscarine. He postulated that the parasympathetic nerve endings liberated a muscarine-like chemical which acted to stimulate the effector. Also in 1906, Reid Hunt (20) reported his studies on choline and choline esters, making the discovery of the extraordinary physiological effects of acetylcholine. He suggested that acetylcholine was the depressor substance of the adrenal extracts of Abel (21). In 1914 Dale (22) studied the physiological action of acetylcholine in detail and described its "muscarinic" action as "parasympathomimetic", and noted its "nicotinic", ganglion stimulatory, action as well. He observed the effect on the isolated intestines, noted the evanescent effect when injected intravenously, and postulated a tissue esterase for its breakdown. Though no one had as yet shown acetylcholine to be present in the body, Dale recalled the work of Hunt, which suggested the possibility of acetylcholine liberation in the adrenal gland, and raised

the question of whether acetylcholine might not have a normal physiological function in the animal body.

In 1912 Wieland (23) noted that a piece of isolated rabbit intestine contracting in saline, imparted to the saline the property of stimulating a second normal isolated intestinal strip; the action on the second strip was blocked by atropine. Le Heux in 1918 (24) traced the active principle in the bath through many extractions and precipitations and hit upon the fact that Reid Hunt's method of acetylation increased the physiological activity of the extract many thousand times with regard to depressing of the pressure, slowing of the isolated heart, and inhibiting of the isolated intestine. The increase in effectiveness for each indicator was in close agreement with the corresponding increases reported by Reid Hunt for the acetylation of choline. Le Heux (24) isolated the active principle as crystalline choline and identified it chemically. He showed that at least 75 per cent of the original activity of the extract could be accounted for as extracted choline.

Le Heux in 1920 (25) then offered an explanation for the variable results obtained for the action of atropine on the intestine of the experimental animal. He showed that an actively contracting intestine, yielding high content of choline into the solution in which it was suspended, was always inhibited by very small concentrations of atropine, 1/1,000,000 or less. If the activity and choline production were low, an excitatory action of atropine sometimes overruled the choline antagonism. Moderate increases in the concentration of atropine, above anything that would ever be attained therapeutically, usually resulted in a stimulating effect, while higher concentrations paralyzed the intestine.

After reviewing the experimental methods and results, he offered an explanation for all the contradictory results previously reported concerning the action of atropine on the intestine.

Le Heux in 1921 (26) reinvestigated the effect of organic compounds on the intestine first reported by Rosa and Neukirch in 1912 (27), and found that d-glucose, d-mannose, and the sodium salts of acetate and pyruvate and a few other similar aliphatic acids stimulate in significantly small doses. He suggested that the acids might be acting by virtue of the formation of choline esters. The relative effects of the various acids, as compared with their choline esters, bore out his hypothesis. He further suggested that the stimulating action of the sugars used might be due to the organic acids made available for esterification with choline during the metabolic breakdown of the sugars. Had he had the information concerning the "active" acetate acetylation of choline following pyruvic acid oxidation, he would no doubt have added that to his argument. (Lipmann, 1941)(28)

The most widely known work in the demonstration of the fundamental correctness of the theories of Elliot and Dixon was begun by Otto Loewi in 1921 (29). He stimulated the vagus nerve to an isolated perfused frog's heart and showed that a substance was liberated into the perfusion fluid which, when perfused through a second frog's heart, produced slowing of the recipient heart. Since atropine blocked both the effects of vagal stimulation and the effects of the perfusate on the donor heart, he believed that a chemical was being liberated at the cardiac vagal endings which was acting as the transmitter from nerve ending to effector.

He later reported work to indicate that his "Vagustoff" was identical

with acetylcholine. He showed that his "Vagustoff", like acetylcholine, was rapidly inactivated by tissue extracts or blood. In the process choline was formed in amounts compatible with the theory that "Vagustoff" was acetylcholine, and was inactivated by hydrolysis (30). The inactivation could be blocked by enzyme blocking methods, such as cold, heat, or ultraviolet light; therefore he postulated that a tissue enzyme, an esterase, was responsible. Since eserine potentiated the effects of acetylcholine and "Vagustoff", but not pilocarpine and choline, he concluded that eserine was acting as a specific anti-esterase (31).

Stedman, Stedman and Easson (32), showed that his conclusions were essentially correct, that the tissue and blood contain an esterase specific for acetylcholine and closely related choline esters. Using a chemical method of following the hydrolysis of acetylcholine, they were able to show the anti-cholinesterasic action of eserine in vitro.

Following these early discoveries evidence rapidly accumulated suggesting that acetylcholine was the mediator of most of the parasympathetic nerve endings, as well as those sympathetic endings which Dale in 1914 (22) had recognized as "muscarinic" in character. Dale in 1933 (33) introduced the term "cholinergic" to describe the nerve ending type. The effectors may be divided into "cholinergic inhibitory" and "cholinergic excitatory", depending on whether they react to nerve stimulation or acetylcholine by being excited or inhibited.

There was general agreement that atropine, which blocked the effect of nerve stimulation at most of the parasympathetic endings, without the prevention of acetylcholine liberation at these endings, and blocked the effect of injected acetylcholine, was acting in both cases by preventing

the action of acetylcholine on the effector, regardless of whether that action was excitatory or inhibitory.(34).

2. Literature on the effect of atropine on the intestines of the normal dog and man.

The inhibitory action of atropine on the intestinal motility of the intact unanesthetized dog and man has been studied by a number of workers. Ganter in 1924 (35) in normal men used a balloon-tipped rubber catheter passed by mouth into the lower duodenum and recorded the results on a smoked drum by means of a bromoform manometer. He found that 1 milligram of atropine subcutaneously or intravenously gave a marked drop in tone and amplitude of rhythmical contractions. Ganter and Stattmüller in 1924 (36) used a similar method to record the movements and tone of the colon in a patient with a caecostomy. One milligram of atropine subcutaneously reduced the tone of the colon and decreased the strength of the rhythmical contractions. Jackman and Barger in 1938 (37) by similar technique took colonic activity records using patients with colostomies and found atropine in doses of 1/100 grain subcutaneously an effective spasmolytic. These methods do not determine propulsive activity.

Herrin in 1936 (38) carefully studied the emptying time of the stomach following doses of 1 to 2 milligrams of atropine in healthy medical students. He demonstrated an initial and final delay in the emptying time which he attributed to the vagal blocking action through antagonism of liberated acetylcholine.

Elsom in 1939 (39) in man followed a radiopaque progress meal introduced by Rehfuss tube into the duodenum to eliminate the factor of the stomach emptying time, and found that small doses of atropine subcutaneously, totalling 0.6 milligram for the entire experiment, more

than doubled the time required for the meal to reach the mid-colon

Employing Thiry-Vella fistulae in dogs, Gruber, Greene, Drayer, and Crawford in 1930 (40) showed that atropine in doses of 0.5 milligram in dogs of ten kilograms body weight produces a decrease in tone and rhythmical contractions of the fistulae. Quigley, Highstone and Ivy in 1934 (41) in the dog demonstrated a marked decrease in the speed of bolus propulsion in the Thiry-Vella fistula following the subcutaneous injection of atropine sulfate in doses of 0.025 milligram per kilogram of body weight. There was an initial latent period of about 8 minutes. The maximum action was reached at about 7 hours and action had terminated at 24 hours.

EXPERIMENTAL

1. Methods:

The following experiments are concerned with the effect of atropine on the tonus and motility of the intestinal loop "in situ", both before and after various extrinsic denervations. For this study two Thiry fistulae were made from adjacent segments of jejunum in each animal. The operations performed on the nerves are listed as follows: 1) The vagi were cut in the thorax at the level of the lower esophagus, to thus produce vagal denervation of both loops. 2) All the mesentery to one loop was cut, leaving only the vessels intact; these were carefully stripped of nerve fibers and painted with phenol followed by alcohol. This produces complete extrinsic denervation only for the loop concerned. 3) The large splanchnic nerve was cut at the diaphragm and traced from the abdomen to its point of origin from the sympathetic chain in the thorax; the sympathetic chain was removed from above that point on down to the lower lumbar region. This procedure done bilaterally produced a preganglionic denervation of both loops, decentralizing the pre-aortic ganglia. 4) Various combinations and sequences of the above operations were used. In most cases the preparation was such that the two loops in the same animal differed only with regard to one set of nerves so that the relation of this set to the sensitivity of the intestine to the inhibitory action of atropine could be determined.

The motility of the jejunal segments was recorded by the balloon-mercury-manometer method described by Krueger (42). This method stimulates rhythmic contractions which continue at four to five second intervals for hours. The kymographic records show pressure changes which

are recorded from the mercury manometer. The method records contractions which are virtually isometric since a large variation in pressure is recorded with only a small reduction in volume of balloon content. Under these conditions all loops, regardless of the presence or absence of extrinsic innervation, show strong rhythmic contractions.

The procedure for performing an experiment was as follows. The trained dog was allowed to lie unrestrained on the table while pressure changes were recorded from each intestinal loop. No drug other than that being tested was administered; no anesthetic was used. After a period long enough for the heart rate to become constant and to allow for adjustment of the intestinal loops to the introduction of the balloons, the heart rate and intestinal motility were recorded for ten or fifteen minutes. Then 0.1 milligram per kilogram of atropine sulphate dissolved in one cc. of distilled water was injected subcutaneously, and the record continued until the full effect on intestinal motility and heart rate was demonstrated. In some cases the record was continued into the recovery period. Heart rate was determined from time to time by counting the apex beat.

2. Results:

The present studies include an analysis of 30 records from six dogs.

Our results indicate that the inhibitory action of atropine is exerted independently of extrinsic nerves, that the intestine is sensitized to the action of atropine by certain denervations, that the inhibition is not produced by adrenalin or sympathin, and that the dose of atropine sufficient to inhibit the denervated loop is sufficient to exert an inhibitory effect on the action of minimal intestine-stimulating

doses of acetylcholine or carbaminoylbetamethylcholine. Evidence is presented which suggests that atropine may be acting in the "classical" role of blocking intestinal stimulation by acetylcholine formed continuously in the intestines independent of extrinsic innervation.

A. Normally innervated loops:

The subcutaneous injection of atropine sulphate in doses of 0.1 milligram per kilogram of body weight produced a gradual onset of inhibition of the rhythmical contractions with a slight decrease in the pressure within the lumen. (Fig. 1.) It is to be noted that the two loops with their identical innervation are influenced exactly alike, a fact born out consistently in all of the records.

Examination of the records reveals that the heart rate does not begin to increase until after the onset of inhibition of the intestine, indicating that the threshold for intestinal effects is below that for any blocking action on the cardiac vagus.

B. Influence of vagotomy:

It is impossible to prepare an animal so that one loop is normal while the other loop is vagally denervated only, or so that one loop is completely denervated extrinsically while the other has only its vagal innervation intact. In the absence of this ideal situation for determining the exact influence of vagotomy, comparison was made of the effect of a standard dose of atropine before and several days after vagotomy at the level of the diaphragm. Such a comparison shows that the action of atropine is undiminished by vagotomy. It cannot be definitely stated

whether or not this procedure produces sensitization to atropine. (Compare figure 1, upper record, with figure 3A and 3B) Under these conditions atropine could not be producing intestinal inhibition by blocking a tonic excitatory action of the vagus.

C. Comparison of the influence of preganglionic and postganglionic sympathectomy following vagotomy:

Figure 3 compares the effect of atropine upon a completely extrinsically denervated loop and one having only its sympathetic innervation intact. It clearly shows that considerable sensitization is produced by postganglionic sympathectomy.

Figure 4 illustrates that postganglionic sympathectomy causes greater sensitization to atropine than preganglionic sympathectomy. No situation could be anatomically created where one of the two loops retained its complete sympathetic innervation, while the other was only preganglionically sympathectomized. Therefore, it was impossible to state definitely, that preganglionic sympathectomy produced any sensitization, though such sensitization is suggested by comparison of some of the records obtained in using standard doses of atropine before and after preganglionic sympathectomy.

Another method of determining whether preganglionic sympathectomy produces sensitization is to compare figure 3 and figure 4. In these cases all the loops were vagally denervated, the differences being only in sympathetic innervation. If the records in figure 3 showed a greater difference in sensitivity to atropine than the records in figure 4, it would indicate that preganglionic sympathectomy produces some sensitization. However, in this case, it cannot be stated that preganglionic

sympathectomy produces definite sensitization.

D. Influence of complete extrinsic denervation:

The records comparing the effect of 0.1 milligram per kilogram of body weight on the normally innervated and on the mesenterically denervated loop (fig. 2) illustrate in every case that atropine depends on no extrinsic innervation for its characteristic inhibitory action; instead, the intestine is definitely sensitized to atropine by extrinsic denervation.

Several possibilities will be considered concerning the mechanism of the inhibitory action of atropine in this case.

Atropine might be causing the release of adrenalin or sympathin from other sources in the body. Youmans 1938 (43) studied the effect sympathin produced by rectal stimulation, as well as injected adrenalin. The sensitization produced by extrinsic denervation was more marked in the case of adrenalin and sympathin than is the case for atropine. Moreover, a relatively large amount of adrenalin is required to produce such prolonged inhibition of denervated intestine as is manifested by the doses of atropine used. Youmans, Haney, and Aumann in 1940 (44) showed that the minimal effective blood concentration of sympathomimetic substances for the speeding of the denervated heart and the transient inhibition of denervated intestine are approximately equal. Therefore, if a given dose of atropine causes the release into the blood stream of sympathomimetic substances in amounts sufficient to produce prolonged inhibition of the extrinsically denervated intestine, the same dose of atropine should cause a marked increase in the rate of the denervated

heart. The doses of atropine used never speeded the denervated heart in our own experiments. Brouha and Nowak in 1939 (45) likewise found that doses of 0.2 milligram per kilogram of body weight in dogs do not produce speeding of the denervated heart. Furthermore, there was no decrease in the inhibitory action of atropine on the denervated loop in those dogs with more extensive sympathectomies and with the adrenal medulla cauterized. These facts suggest that blood-borne adrenalin or sympathin is not the cause of the intestinal inhibition produced by atropine in the doses used.

Atropine might exert a blocking action against acetylcholine formed locally in the intestine and acting as a continuous stimulus for intestinal motility. To further clarify this point, the minimal effective dose of carbaminoylbetamethylcholine necessary to produce a definite increase in the motility of the bowel when injected continuously intravenously by motor driven syringe was determined. It was found that atropine in doses of 0.02 milligrams per kilogram of body weight, which only partly inhibited the denervated loop, definitely dimished the response to the minimal effective dose of carbaminoylbetamethylcholine. Also atropine in the doses usually employed, 0.1 milligram per kilogram of body weight, diminished the brief spasm produced by the intravenous injection of small doses of acetylcholine, 0.025 to 0.05 milligram per kilogram of body weight. The recovery of the sensitivity of the intestine to the excitatory action of acetylcholine following atropine, parallels the return of intestinal motility. These results indicate that the dose of atropine sufficient to produce inhibition of the denervated loop is

also effective in exerting an inhibitory action on stimulating effects of minimal amounts of choline derivatives. If atropine in these doses is effective against these choline derivatives arriving by blood stream, it could be effective against acetylcholine produced locally and diffusing locally in the intestine.

DISCUSSION

Evidence may be found in the literature to indicate that acetylcholine is continually produced in the intestine "in situ", whether it be innervated or denervated. Likewise the isolated intestinal loop and the sliced intestine have been shown to produce acetylcholine.

Feldberg and Rosenfeld in 1933 (46), following doses of eserine of from 3 to 10 milligrams, found a substance indistinguishable from acetylcholine in the portal blood of dogs. Blood from the jejunum showed higher concentration of the substance than blood from the colon and stomach, while blood from the splenic, jugular, and femoral veins and femoral artery showed no detectable amount. Feldberg suggested that the continuous production of acetylcholine was a function of the intrinsic nerve plexus of the intestine.

Dale and Feldberg in 1934 (47) found an acetylcholine like substance in the gastric venous blood following vagal section. Stimulation of the peripheral end of the cut vagi brought about a 2½ fold increase in the acetylcholine content of the gastric venous blood. Following perfusion with Locke's solution, acetylcholine was found in the perfusion fluid before vagal stimulation and was increased 2 to 4 fold by vagal stimulation. The fact, observed by Dale, that increases in motility were correlated with increases in acetylcholine output suggested that the muscularis rather than the mucosa was responsible for the acetylcholine output. Dale suggested that the continuous production of acetylcholine through activity of the intrinsic plexus of the stomach was responsible

for the motility of the vagally denervated stomach.

Bunting, Meek and Maaske in 1935 (48) perfused the small intestine of the dog with modified Locke's solution and eserine and obtained a concentration of acetylcholine-like substance in the effluent, equivalent in activity to a 1 to 100,000,000 concentration of acetylcholine. Following vagal stimulation, the concentration of acetylcholine-like substance in the effluent increased tenfold.

Bacq and Goffart in 1941 (49) reported on the "bound" acetylcholine content of the various portions of the gastro-intestinal tract of the dog. They found the content varied exactly as would be suggested by Alvarez's "metabolic gradient", being highest in the duodenum and upper jejunum, decreasing in the ileum, lowest in the colon and stomach. Denervation produced no decrease in the acetylcholine content during the first ten hours, that being the longest period observed; instead, Bacq's records show it to be increased. Analyzing the venous blood from various parts of the eserinized dog's intestine, he found, in general, in the innervated intestines a basal output of free acetylcholine corresponding to the content of "bound" acetylcholine previously determined. Intestines extrinsically denervated liberated even larger amounts of acetylcholine into the venous blood 24 hours later.

Dikshit in 1938 (50) reported on the acetylcholine production of the finely sliced dog intestine in warm Locke's solution, and found it equal to acetylcholine production in any part of the nervous system. (Table I and II). Allowing pieces of gut to remain in a refrigerator for 98 hours is believed to render the intrinsic plexus permanently

inactive. Dikshit found such pieces of gut produced no acetylcholine, though they could be made to show a typical rhythmical contractions, when suspended in warm Tyrode solution. He found the relatively plexus-free circular muscle layer of the dog intestine produced only about 1/10 as much as the longitudinal layer. (Fig. 5) The intestine of the guinea pig embryo, according to his results, began producing acetylcholine at the time that the intrinsic plexus is thought to become functional, and rapidly attained a level of production equal to that of the adult guinea pig intestine.

Feldberg and Solandt in 1942 (51) found that acetylcholine liberation into the eserinized perfusion fluid of isolated loops of rabbit intestine, contracting in the perfusion chamber, decreased as activity decreased. Pieces of refrigerated intestine which had lost their ability to synthesize and liberate acetylcholine, sometimes showed spontaneous activity, but had lost their normal sensitivity to atropine.

The evidence presented suggests that the intestine possesses the mechanism for the production of physiologically significant amounts of acetylcholine in the absence of activity of the extrinsic nerves. The exact site of production cannot be stated, but the evidence of Dikshit suggests that it is either in the cells and fibers of the intrinsic plexus, the synapses of the intrinsic plexus, or the neuro-myal junctions of the intrinsic plexus - or a combination of any or all of these. If the mechanism for the formation and liberation of acetylcholine exists in the intestine, it seems likely that a basal level of acetylcholine production and liberation may go on even in the absence of reflex activity of the nervous tissue present. Such acetylcholine might be a

factor in establishing the basal level of tonus, excitability, and activity of the muscle elements, as well as the excitability and activity of the neural elements of the intestine and peristaltic activity. If this were true acetylcholine production as a result of the actions of the extrinsic nerves would be superimposed on this basal level. It is also possible that acetylcholine may be formed and released from sites not directly related to the neural elements. That free acetylcholine can be formed in the absence of cellular function is known from the work of Nachmansohn and Machado, (52) who showed with incubation technique, that free acetylcholine can be formed by the use of extracts of nervous tissue and homogenized nervous tissue in which no intact cells are present.

The possibility that atropine may exert the most of its effect upon the intestine independent of the blocking of acetylcholine cannot be positively denied, though a series of coincidences would have to be postulated for such an explanation. Studies of Gasser in 1926 (53) and van Esveld in 1928 (54) on the relatively plexus-free circular muscle layer of the intestine showed inhibition of activity by atropine in some cases, but the results are unconvincing. Furthermore, there was nothing to show that the muscle strips did not owe what activity they showed to acetylcholine production. On the other hand, as pointed out by Alvarez (55) even striated muscle, which does not show rhythmical activity "in situ", developes rhythmical activity under appropriate environmental conditions. Therefore, results obtained under such abnormal environment may not be used to interpret responses of the intestine "in situ".

In every experimental situation where the action of atropine on the intestine in pharmacological amounts is clear cut, the output of

significant amounts of acetylcholine by the intestine has been demonstrated. The action of atropine alone, and its interaction with acetylcholine and eserine on the intestine can be explained as blocking the effects of intrinsically produced acetylcholine. It is considered that this is the most likely mechanism for the action of atropine on the denervated intestine.

SUMMARY

A brief history of the development of the present day concept of the action of atropine at postganglionic parasympathetic nerve endings is given. The experimental work concerning the action of atropine on the small intestine is reviewed.

The mechanism of the inhibitory action of atropine on the intestinal motility of the dog has been studied by recording from Thiry fistulae by the balloon-mercury-manometer method before and after certain operations on the extrinsic nerves supplying the loops. Trained, unanesthetized dogs were used.

Atropine exerts an inhibitory action on the tonus and motility of the intestine "in situ" independently of the extrinsic nerves. Vagotomy does not reduce the intestine-inhibiting action of atropine. Postganglionic sympathectomy produces an increase in the sensitivity of the intestine to the inhibitory action of atropine. It cannot be stated whether any such sensitization is produced by vagotomy or preganglionic sympathectomy.

Adrenalin or sympathin are not released into the blood stream from other sites in the body by the doses of atropine used, blood born adrenalin or sympathin is therefore not the cause of the intestinal inhibition.

Atropine in the doses used diminishes the brief intestinal spasm produced by the intravenous injection of small doses of acetylcholine. Likewise, even smaller doses of atropine diminish the response to minimal intestine-stimulating amounts of carbaminoylbetamethylcholine.

Evidence is cited from the literature which indicates that acetylcholine is continually produced in the intestine "in situ", whether it

be innervated or extrinsically denervated. The most likely interpretation of the mechanism of action of atropine is that it blocks the stimulatory action of acetylcholine produced at a basal level independently of extrinsic nerves.

BIBLIOGRAPHY

1. Henderson, V. E., and M. H. Roepke. Drugs affecting parasympathetic nerves. Physiolog. Rev. 17: 373, 1937.

2. Wood, George B. Therapeutics and Pharmacology, vol. 1, p. 800, J. B. Lippincott and Co., Philadelphia, 1860.

3. Müller, Johannes. Handbuch der Physiologie des Menschen, p. 672, Verlag von J. Holscher, Coblenz, 1844.

4. Wood, H. C. Therapeutics, Its Principles and Practice, 7th ed. J. B. Lippincott and Co., Philadelphia, 1890.

5. Hoff, H. E. The history of vagal stimulation. Bull. Hist. Med. 8: 461, 1940 (pt. 1).

6. Olmsted, J. M. D. Claude Bernard, Physiologist, p. 204, Harper and Brothers, Publishers, New York and London, 1938.

7. Heidenhain, R. Ueber arhytmische Herztatigkeit. Archiv f. d. ges. Physiol. 5: 150, 1872.

8. Heidenhain, R. Ueber die Wirkung einiger Gifte auf die Nerven der Glandula submaxillaris, Archiv f. d. ges. Physiol. 5: 309,1872.

9. Langley, J. N. On the physiology of the salivary secretion. J. Physiol. 11: 123, 1890.

10. Bayliss, W. M., and E. H. Starling. The movements and innervation of the small intestine. J. Physiol. 24: 99, 1899.

11. Henderson, V. E. On the sensitivity of different nerve endings to atropine. J. Pharmacol. & Exper. Therap. 21: 99, 1923.

12. Schubel, K. Ueber die wechselnde Wirkung des Atropins auf den Darm. Klin. Wchnschr. 3: 687, 1924 (pt. 1).

13. Wood, H. C. Therapeutics, Its Principles and Practice, 12th ed., J. B. Lippincott and Co., Philadelphia, 1905.

14. Magnus, R. Versuche am überlebenden Dünndarm von Saugetieren. V Mitteilungen. Wirksamweise und Angriffspunkt einiger Gifte am Katzendarm. Archiv f. d. ges. Physiol. 108: 1, 1905.

15. Unger, M. Beitrage zur Kenntnis der Wirksamweise des Atropins und Physostigmins auf den Dünndarm von Katzen. Archiv f. d. ges. Physiol. 119: 273, 1907.

16. Magnus, R. Versuche am überlebenden Dünndarm von Saugetieren. VII Mitteilungen. Zur Wirkung Kleinster Atropinmengen auf den Darm. Archiv f. d. ges. Physiol. 123: 99, 1908.

17. Dubois-Reymond, E. Gesammelte Abhandlungen fur allgemeinen Muskel- und Nervenphysik. Two volumes. Veit u. Comp., Leipzig, 1875-1877. Cited from Goodman, L., and A. Gilman. The Pharmacological Basis of Therapeutics, The MacMillan Co., New York, 1941.

18. Elliot, T. R. The action of adrenalin. J. Physiol. 32: 401, 1905.

19. Dixon, W. E. On the mode of action of drugs. The Med. Mag. 16: 454, 1907.

20. Hunt, R. and R. de M. Taveau.. On the physiological action of certain choline derivatives and new methods for detecting choline. Brit. Med. J. 2: 1788, 1906.

21. Hunt, R., and R. R. Renshaw. Further studies on the methyl cholines and analogous compounds. J. Pharmacol. & Exper. Therap. 51: 237, 1934.

22. Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. J. Pharmacol. & Exper. Therap. 6: 147, 1914.

23. Wieland, W. Zur Kenntnis der Entstehung der Darmbewegung. Archiv f. d. ges. Physiol. 147: 153, 1912.

24. Le Heux, J. W. Cholin als Hormon der Darmbewegung. Archiv f. d. ges. Physiol. 173: 8, 1918.

25. Le Heux, J. W. Cholin als Hormon der Darmbewegung. II Mitteilung. Zur Erklarung der wechselnden Wirkung des Atropins auf den Darmbewegung. Archiv f. d. ges. Physiol. 179: 177, 1920.

26. Le Heux, J. W. Cholin als Hormon der Darmbewegung. III Mitteilung. Ueber die Beteiligung des Cholins an der Wirkung verschiedener organischer Sauren auf den Darm. Archiv f. d. ges. Physiol. 190: 280, 1921.

27. Rona, P. and P. Neukirch. Experimentale Beitrage zur Physiologie des Darmes. Archiv f. d. ges. Physiol. 146: 371, 1912.

28. Lipmann, Fritz. Metabolic generation and utilization of phosphate bond energy. Adv. in Enzymol. 1: 99, 1941.

29. Loewi, O. Über Humorale Übertragbarkeit der Herznervenwirkung. I Mitteilung. Archiv f. d. ges. Physiol. 189: 239, 1921.

30. Loewi, O Über Humorale Übertragbarkeit der Herznervenwirkung. II mitteilung. Archiv f. d. ges. Physiol. 193: 201, 1922.

31. Loewi, O., and E Navratil. Über Humorale Übertragbarkeit der Herznervenwirkung. X Mitteilung. Über das Schicksal des Vagusstoffes. Archiv f. d. ges. Physiol. 214: 678 , 1926.

32. Stedman, E., E. Stedman, and L. H. Easson. Cholin-esterase. An enzyme in the blood serum of the horse. Biochem. J. 26: 2056, 1932.

33. Dale, H. H. Nomenclature of fibres in the autonomic system and their effects. J. Physiol. 80: 10P, 1933.

34. Goodman, L., and A. Gilman. The Pharmacological Basis of Therapeutics, The MacMillan Co., New York, 1941.

35. Ganter, G. Studien am menschlichen Darm. Archiv f. d. Exper. Path. und Pharmakol. 103: 84, 1924.

36. Ganter, G., and K. Stattmüller. Studien am menschlichen Darm. III Mitteilung. Ztschr. f. d g. exper. Med. 42: 143, 1924.

37. Jackman, R. J., and J. A. Bargen. Influence of certain anti-spasmotic drugs on the intestine of man. Surg., Gynec., & Obst. 67: 63, 1938.

38. Herrin, R. C. The effect of atropine and pilocarpine on emptying time of the stomach. Am. J. Physiol. 115: 104, 1936.

39. Elsom, K. A., and J. L. Drossner. Intubation studies of the human small intestine. Am. J. Dig. Dis. 6: 589, 1939.

40. Gruber, C. M., W. W. Greene, C. S. Drayer, and W. M. Crawford. Studies on the effect of morphine sulphate, atropine sulphate, and hyoscine hydrobromide upon the intact intestine of un-anesthetized dogs. J. Pharmacol. & Exper. Therap. 38: 389, 1930.

41. Quigley, J. P., W. H. Highstone, and A. C. Ivy. The action of morphine, papaverine, atropine, pilocarpine, pituitrin, pitocin, and pitressin on intestinal propulsive activity determined in the unanesthetized dog by the bolus method. J. Pharmacol. & Exper. Therap. 51: 308, 1934.

42. Krueger, H. The effect of morphine on the pressures developed by the intestinal musculature. J. Pharmacol. & Exper. Therap. 51: 85, 1934.

43. Youmans, W. B. Similarity of the effects of adrenalin and inhibitory sympathin in intestinal motility: sensitization by denervation. Am. J. Physiol. 123: 424, 1938.

44. Youmans, W. B., H. F. Haney, and K. W. Aumann. Relation of the groups of the adrenalin molecule to its cardio-inhibitory action. Am. J. Physiol. 130: 190, 1940.

45. Brouha, L. and S. J. G. Nowak. The role of the vagus in the cardio-accelerator action of atropine in sympathectomized dogs. J. Physiol. 95: 439, 1939.

46. Feldberg, W., and P. Rosenfeld. Der Nachweiss eines acetylcholinartigen Stoffes im Pfortaderblut. Archiv f. d. ges. Physiol. 232: 212, 1933.

47. Dale, H. H., and W. Feldberg. Chemical transmission of vagal effects to the stomach. J. Physiol. 81: 320, 1934.

48. Bunting, H. W., W. J. Meek, and C. H. Maasko. Chemical transmission of vagal effects to the small intestine. Am. J. Physiol. 114: 100, 1935.

49. Bacq, Z. M., and M. Goffart. L'acetylcholine libre et combinee du tractus digestif. Schweiz. Med. Wochenshr. 71: 310, 1941 (pt. 1).

50. Dikshit, B. B. Acetylcholine formation by the tissues. Quart. J. Exper. Physiol. 28: 243, 1938.

51. Feldberg, W., and C. M. Solandt. Effects of drugs, sugars, and allied substances on the isolated small intestine of the rabbit. J. Physiol. 101: 137, 1942.

52. Nachmansohn, D., and A. L. Machado. The formation of acetylcholine. A new enzyme: "Choline Acetylase". Neurophysiol. 6: 397, 1943.

53. Gasser, H. S. Plexus-free preparations of the small intestine. A study of their rhythmicity and of their response to drugs. J. Pharmacol. & Exper. Therap. 27: 395, 1926.

54. Van Esveld, L. W. Verhalten von plexushaltigen und plexusfreien Darmsmuskelpraparaten. Archiv f. exper. Path. u. Pharmakol. 134: 347, 1928.

55. Alvarez, W. C. An Introduction to Gastro-Enterology. Paul B. Hoeber, Inc., New York, 1940.

FIGURE 1

These are balloon-mercury-manometer records of intestinal motility taken simultaneously from two normally innervated Thiry fistulae in the same dog. Where indicated by the arrow, a subcutaneous injection of 0.1 milligram of atropine sulphate per kilogram of body weight was made.

The numerals indicate the heart rate.

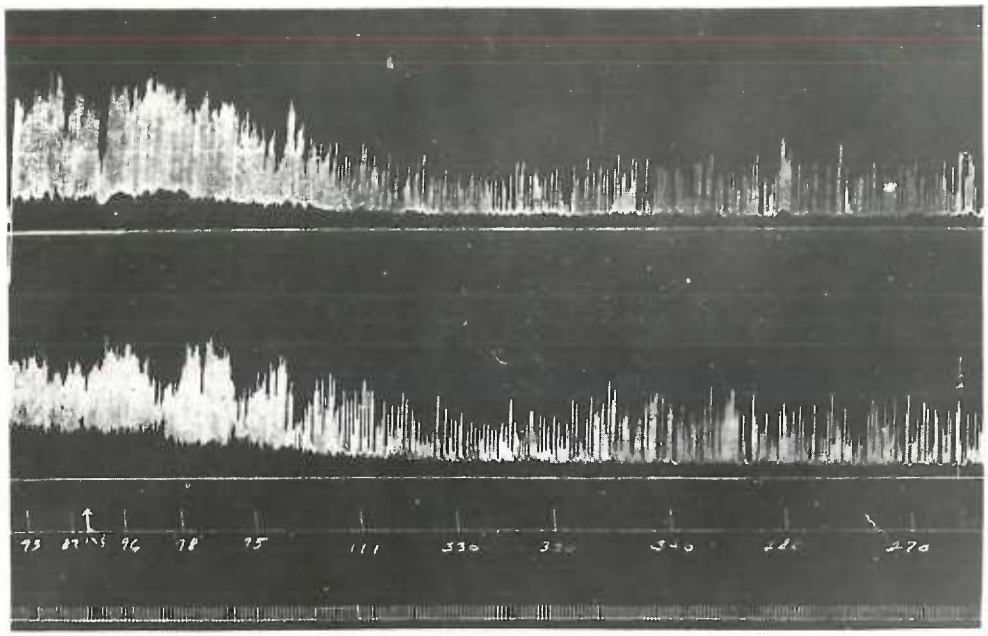
Time in ten second intervals is recorded by the lowermost tracing.

FIGURE 2

In these tracings the upper recording is from an extrinsically denervated intestinal loop, the lower from a normally innervated loop. Where indicated by the arrow, a subcutaneous injection of 0.1 milligram of atropine sulphate per kilogram of body weight was made.

The numerals indicate the heart rate.

Time in 5 second and one minute intervals is indicated by the tracing second from the bottom.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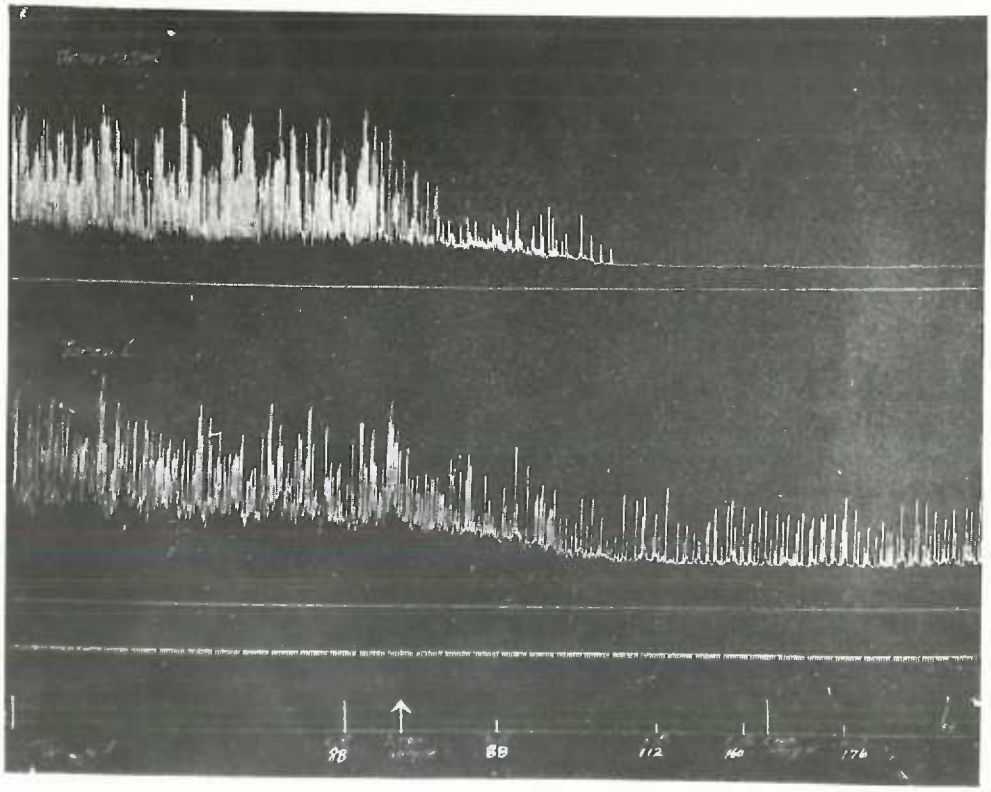

88
88
112
160
176

FIGURE 3

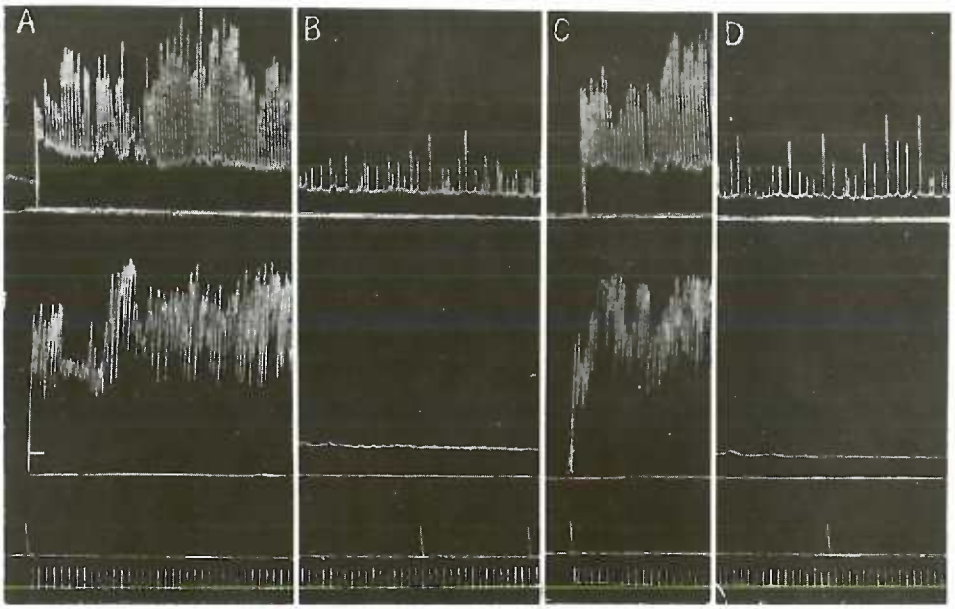
In this illustration the lower recording is from a completely extrinsically denervated fistula, while the upper recording is from a fistula having only the sympathetic part of its extrinsic innervation intact. As indicated in the accompanying legend, records A & B show the effect of atropine, while records C & D show the effect of scopolamine.

FIGURE 4

While this record was taken with the use of scopolamine, it does not differ in any detail from similar records taken with atropine. The upper recording is from a fistulae completely extrinsically denervated, while the lower recording is from a fistulae having only its postganglionic sympathetics intact. The injection of 0.1 mgm. scopolamine hydrobromide per kilogram body weight was made where indicated by the arrow.

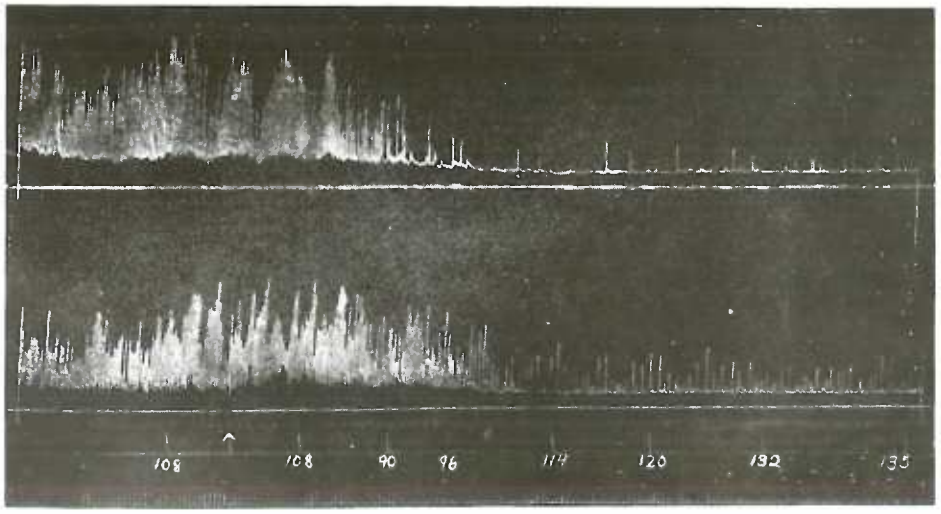
The numerals indicate the heart rate.

Time is in ten second intervals.



Effect of Atropine and Scopolamine on the Motility of a Completely Denervated Intestinal Segment (Lower Record) Compared with Effect on a Segment Having Only Its Vagal Innervation Destroyed (Upper Record)

Dog #3. A. Before injection of atropine. B. Maximal inhibition obtained following injection of atropine, 0.1 mgm. per kgm. C. Normal motility several hours later D. Maximal inhibition obtained after injection of scopolamine, 0.1 mgm. per kgm.



Effect of Scopolamine on the Motility of a Completely Denervated Intestinal Segment (Upper Record) Compared with Effects on a Segment (Lower Record) Retaining Its Connections with the Pre-Aortic Ganglia Which Have Been Decentralized by Vagotomy, Splanchnicotomy, and Lumbar Ganglionectomy

TABLE I

After Dikshit, Quart. J. Exper. Physiol. 28: 243, 1938

This table shows the acetylcholine production in the various tissues of the same species, the dog. The tissues were incubated in an eserinized Locke's solution for five hours, the total acetylcholine content of a sample being determined at the beginning and end of the incubation period.

TABLE II

After Dikshit

This shows species variation of acetylcholine production by tissue incubation technique as well as variation in acetylcholine production in various parts of the digestive tract of the same species.

TABLE I.—SHOWS THE AMOUNT OF A.CH. FORMED IN 5 HOURS BY 1 G. OF FRESH TISSUE OF DOG.

Tissue.		μg. per g. of fresh tissue.		
		(i) Suspension in Locke's.	(ii) Extraction in trichloracetic acid.	(iii) Difference.
Nervous tissue	brain cortex	1·0	0·4	0·6
	basal ganglia	6·0	2·0	4·0
	spinal cord	1·5	0·5	1·0
	sympathetic ganglia	6·0	2·0	4·0
	vagus nerve	6·0	5·5	0·5
	sciatic nerve	1·25	1·2	0·05
Cardiac and plain muscle	small intestine	5·0	1·0	4·0
	urinary bladder	4·0	1·0	3·0
	auricle	2·0	0·8	1·2
	ventricle	0	0	0
	aorta	0	0	0
	vena cava	0	0	0
	uterus	0	0	0
Glands	pancreas	1	0	1
	liver	0	0	0
	spleen	0	0	0
	testis	0	0	0
	ovaries	0	0	0
	kidneys	0	0	0
	supra renals	0	0	0
Other tissues	lungs	0	0	0
	skeletal muscle	0	0	0
	blood	0	0	0

0 : < than 0·25 μg.

TABLE II.—SHOWS A.CH. FORMATION BY THE GASTRO-INTESTINAL TRACT OF DIFFERENT SPECIES. THE FIGURES REPRESENT μG. OF A.C. FORMED IN 5 HOURS BY 1 G. OF FRESH TISSUES.

Species.	Stomach.	Small intestines.	Large intestine.
Dog . . .	2	5	3
Cat . . .	1·5	4	2
Rabbit . .	1·6	5	1·5
Guinea-pig . .	3	10	4·5

FIGURE 5

After Dikshit, Quart. J. Exper. Physiol. 28: 243, 1938

This record illustrates the method and results obtained by Dikshit for acetylcholine production in the relatively plexus-free circular muscle layer of the dog intestine as compared with the longitudinal muscle layer.

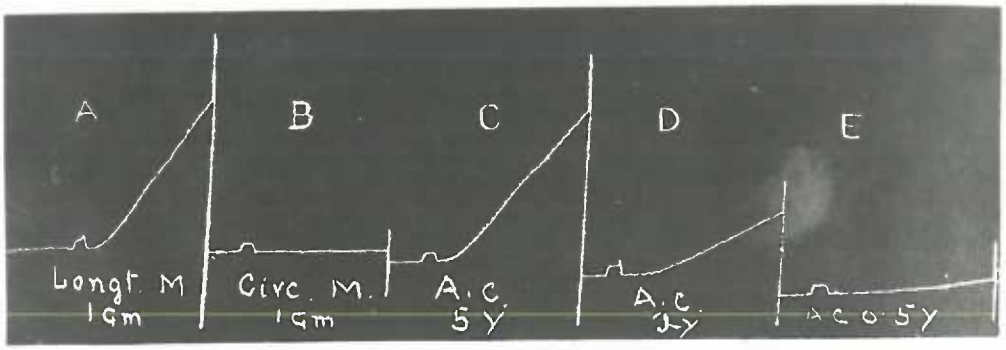


FIG. 2.—Contractions of eserinised frog's rectus muscle. Duration of contraction, 75 sec.

A: 1 g. of longitudinal muscles of dog's intestine suspended in warm eserinised Locke's solution and oxygenated for 5 hours.
B: 1 g. of the circular muscles of the same piece of intestine, suspended as in A.
C, D, and E: Acetylcholine 5 μg., 2 μg., and 0·5 μg. respectively.