THE MODE OF ACTION OF DRUGS UPON INTESTINAL MOTILITY

E. M. VAUGHAN WILLIAMS

Department of Pharmacology, Oxford University, Oxford, England

TABLE OF CONTENTS

I. INTRODUCTION

The motility of the intestine has been the object of fascinated, if unscientific, study from the earliest times; yet physiologists are still much worse informed about intestinal than about skeletal muscles. Writhing entrails were chosen for examination by Greek and Roman priests in their attempts to divine omens for the future, perhaps because an organ so resistant to death must have had a trace of divinity about it; perhaps also because less wastage was involved if only the least edible portions of their slaughtered animals were burned upon the altars. A sort of mystical and obscurantist odour seems to have lingered on even to the twentieth century around all studies of the bowel, for it is a strange fact that about few subjects have battles more bitter and persistent been waged than those that have concerned the mechanisms of intestinal movements and the actions of drugs thereon.

The chief difficulty has been, and still is, that the analytical method is hard to apply to so complex a tissue as the intestine. In almost every biological subject investigators have travelled away from one another in two opposite directions.

There is the school which seeks to divide and analyse, to separate tissues into smaller and ever smaller parts; to record from the nerve and the muscle; to record from the single nerve fibre and to penetrate the single muscle fibre with invisible electrodes; to follow chemical reactions with the miscroscope and electron microscope. On the other hand, there are those who try to examine under controlled conditions the most "natural" preparations possible; who perform elaborate operations so that they may measure responses without the complication of anaesthetics or psychological disturbances; who fear always that their experimental procedures distort the normal environment to an extent that could permit the escape of an important factor from their observation net.

Many intestinal studies have not exploited the advantages of either school. The tissue examined has often been a segment of intestine hung in an isolated organ bath, cut off from all central nervous and hormonal influences, yet in no way approaching the purity of a single tissue. Recently attempts have been made to achieve new preparations. In the direction of "natural conditions" a method has been developed of measuring quantitatively in unanaesthetised dogs the rate at which propulsive work is done by an intestinal loop, with nerves and blood supply intact, in transporting fluid under controlled conditions of temperature and pressure. In the opposite direction, Evans and Schild (67) have further developed the study of intestinal muscle completely free of nerves and ganglion cells, and some new procedures have been adopted in electrical recording. The penetration of intestinal muscle fibres by microelectrodes was first demonstrated by Vaughan Williams and Bülbring in 1951 (unpublished) who recorded membrane potentials up to 55 mV. Although progress has not been rapid in the past two years, further results concerning the membrane potentials of smooth muscles have recently been obtained (36).

II. THE ANALYTICAL APPROACH

1. Electrical Records. The analytical method has enabled very great advances to be made in the study of nervous conduction and neuromuscular transmission in the skeletal system, and it may be advantageous to discuss how far the behaviour of the intestine can be regarded as analogous. It is known that in peripheral nerve ionic currents, which are of greatest density at the nodes in medullated nerve, flow towards an active region from the inactive parts in advance of the impulse, so that these in turn become active. At the nerve terminals the currents are too small to excite the motor end-plate of the muscle, but acetylcholine is released, in amounts proportional to the number of calcium ions present (40). The amount of acetylcholine released, considered as an ionic charge, is also insufficient to depolarize the end-plate, but it has an effect such that permeability to all ions is greatly increased. As a result the membrane potential at the end-plate becomes approximately zero (69). Currents then flow towards the depolarised region, causing a change in the permeability of the muscle membrane to sodium; the membrane potential thus reverses, the inside becoming positive to the outside. Increased permeability to potassium follows, resulting in a restoration of the original membrane potential, *i.e.*, the inside becoming once more 90–95 mV negative to the outside (93).

Two further points should be mentioned. First, acetylcholine is not released only during a nerve impulse. Small amounts are released in quanta (70, 71) all the time at random intervals; upon the arrival of an impulse a large number of quanta are released simultaneously. Secondly, a considerable depolarisation of the end-plate region can exist without any measurable tension being developed in the muscle; conversely, under certain conditions, such as the presence of acetylcholine at the surface of a chronically denervated muscle (33, 129) the contractile mechanism of the muscle can be activated in association with electrical "silence."

In drawing an analogy between this mechanism and the behaviour of the intestinal system, there are several crucial questions to be asked.

Does the intestinal muscle have a steady membrane potential which is maintained during inactivity, and partly abolished or reversed during activity?

Is the development of tension normally associated with a change in potential?

Can the muscle contract spontaneously, or must it be activated by a "transmitter" from a nerve or other tissue or by a drug?

Is the muscle a "syncytium" so that it can be regarded as one large fibre, or are the individual fibres insulated from one another electrically?

Do the very small nerves in the intestine behave in the same way as in the skeletal system, *i.e.*, by the successive activation of limited areas, which exhibit refractoriness after the passing of an impulse, or do they behave in some other way?

To take the last question first, intestinal nerves are so fine and deeply embedded in other tissues that no records have been taken from them, nor are likely to be with methods at present available. Analogy with very small peripheral nerves (86), would imply that they behave in the same way as larger nerves. On the other hand, they may be like the fine nerve terminals at motor end-plates, and release small amounts of transmitter more or less continuously at random intervals. A third possibility is that they are influenced by electrotonic spread of current from depolarised or hyperpolarised ganglion cells, in response to which the rate of "spontaneous" release of transmitter might increase or decrease.

The question of the existence of a syncytium is of crucial importance to the interpretation of intracellular recordings, and so it is appropriate to discuss what it implies in electrical terms (Fig. 1).

In skeletal muscle the action potential in one muscle fibre cannot excite an adjacent fibre; many fibres are activated simultaneously because they are individually excited by their own nerves (3). They are themselves effectively insulated from one another. If a recording microelectrode is inserted into a fibre, and a pulse of current is passed through a second electrode in an adjacent fibre, no potential change is recorded from the first (Fig. 1, *A*). If, however, the stimulating electrode is in the *same* fibre, a potential change is recorded from which the electrical constants of the membrane can be calculated (Fig. 1, *B*) (69).

The syncytial hypothesis (62) implies that low-resistance bridges between neighbouring fibres exist, such that the insides of the fibres are in electrical

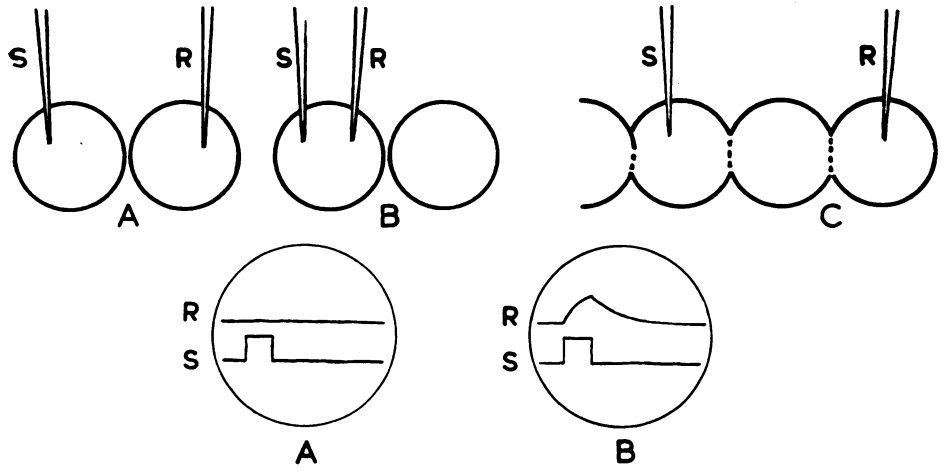

FIG. 1. Intracellular records to be expected from a muscle fibre with and without syncytial continuity with other fibres.

The small circles represent muscle fibres. The larger circles below show what would be recorded on an oscilloscope screen, when a brief pulse (seen on lower beam) is applied to the inside of a fibre through an intracellular electrode, S. *A*. If the recording electrode, R, is in a neighbouring fibre, no change in membrane potential is produced by the stimulus. *B*. When the recording and stimulating electrodes are in the same fibre, the membrane potential changes in the manner shown (69). *C*. If there are low-resistance bridges between fibres, then the recording electrode should register a potential change even if it is in a distant fibre.

continuity (Fig. 1, *C*). The *whole* of the membrane cannot have a low transverse resistance, because there could then be no membrane potential, since it would be short-circuited. The hypothesis involves the existence of special regions of muscle membrane, in continuity with the rest of the muscle surface, but with quite different electrical characteristics at the points where they touch another fibre.

Bozler (24, 29) believes that many smooth muscles including those of the stomach and intestine (27, 28) do constitute a syncytium in which conduction proceeds from muscle cell to muscle cell. His chief arguments are: (a) The action potentials recorded from one type of smooth muscle (the "unitary" type) are similar to those recorded from heart muscle (Fig. 2, *A* and *B*). (b) Conduction still occurs in muscles in solutions of cocaine and nicotine "which paralyse all known nervous mechanisms". (c) Although "protoplasmic bridges" have been but doubtfully demonstrated in some muscles of the unitary type, the existence of a membrane between cells does not exclude the possibility of a low-resistance pathway. The giant nerve fibres of the earthworm contain transverse membranous partitions which do not impede the conduction of impulses.

The strongest of these arguments is that conduction can be demonstrated in muscles which are thought to contain no nerves, and in nerve-containing muscles when nerves have been paralysed by cocaine. Yet there is always a possibility that very fine nerves have been missed; Prosser (123) has recently most elegantly demonstrated nervous conduction in muscle previously considered

of the unitary type. It is certainly reasonable to assume that cocaine would have the same effect on visceral nerve as it has on peripheral nerve, but here again neither this analogy nor the analogy with cardiac action potentials or with diaphragms in the nerves of earthworms constitute proof. Thus, though some of the evidence is certainly consistent with the existence of syncytial connections between cells, confirmation must await the evidence of direct intracellular recordings.

Although Magnus (106) did not observe spontaneous contractions in his plexus-free preparations, all later authors, including the most recent (67), who have worked with denervated intestinal muscle, have agreed that the fibres of intestinal muscle can contract in the absence of nervous stimulation. The possibility of the continuous release of a transmitter from tissues other than nerve, however, has to be considered, and is discussed in another section.

The relation between the state of the membrane potential and the activation of the contractile part of a muscle is still a matter for speculation even in skeletal muscle. Some new evidence has recently been obtained from studies of the slow muscle fibres of the frog skeletal system by Kuffler and Vaughan Williams (100, 101). These fibres are striated and indistinguishable without staining from the ordinary twitch fibres. Their interest in the present context is that they seem in some respects to be intermediate between smooth and skeletal muscles. The slow fibre surface is covered with fine nerve terminals, excitation of which gives rise to small junctional potentials of a few millivolts only, and *there is no conducted action potential along the fibre*. The membrane potential is only 60 mV, and never reverses during activity; indeed the membrane is never even completely depolarised, for the maximum recorded depolarisation, during a tetanus at 150/sec. was 33 mV, little more than half the membrane potential.

Some tension could always be recorded, however small the depolarisation. (In "twitch" muscle quite large subthreshold potentials can be produced without the development of any measurable tension.) Successive potentials during a tetanus summed with each other, so that a "plateau" of depolarisation was built up (Fig. 2, *D*), its height being a function of the frequency of stimulation. The behaviour of the slow fibres thus differed fundamentally from the twitch fibres in that the summing of successive phases of activity allowed a continuous gradation of response, unlike the discrete and staccato all-or-none activations of the twitch fibres, each followed by a refractory period. There are, now, therefore, two possible models of skeletal neuromuscular systems to which intestinal smooth muscle can be compared; the fact that there are already two renders more acceptable the possibility of a third quite different system in intestinal muscle unlike either of them.

Action potentials have been recorded from smooth muscle by several authors (22, 23, 25, 26, 29, 57, 58) including intestinal muscle (27, 28, 30, 31, 124). Bozler recorded from his "unitary" type of muscle action potentials which resembled cardiac potentials. He used external leads and a differential amplifier. From theoretical considerations (43) he was able to reconstruct the shape of the monophasic potential. A tracing of such a reconstructed potential is compared with a

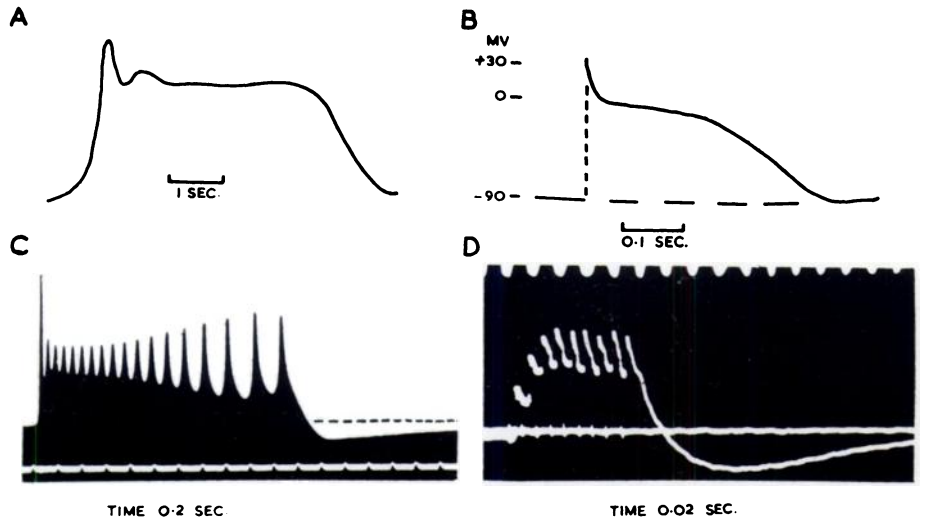

FIG. 2. Action potentials from different types of muscle. *A*. Action potential of stomach, derived graphically from differential recording with extracellular electrodes (27). *B*. Cardiac action potential recorded with intracellular electrode (168). *C*. Complex monophasic potential from a rat's ureter (26). *D* Intracellular record from a frog's slow fibre in response to nerve stimulation.

cardiac potential recorded by means of an intracellular electrode in Fig. 2. Some of the potentials from various types of smooth muscle, including intestine, recorded by Bozler exhibited "spikes" in the course of the plateau (Fig. 2, *C*). Their presence suggests that the plateau may in fact be built up of individual potentials which sum; this kind of plateau is seen in slow skeletal muscle fibres (Fig. 2, *D*). Smooth muscle fibres are so minute that even the smallest of external electrodes "sees" numerous fibres, so that any recorded potential is the algebraic sum of the activity of very many units. The relative failure of the electrophysiological technique as applied to intestinal muscle to add new knowledge, compared to the great success of such methods in the skeletal system, illustrates the great difficulty of interpreting records of this type from complex tissues. Here, again, better resolution may be obtained with intracellular electrodes.

Although it has so far been found impossible to study the activity of single units in the intestine, analysis can still be attempted by inference, especially from changes in behaviour brought about by drugs. A great deal of work has been done on these lines, but no very clear picture of intestinal function has emerged.

2. The Site of Drug Actions. Ganglion or Muscle? This is an old problem which has aroused much recent controversy. It has sometimes happened in the past, when results from different laboratories did not agree, that a study of the points of disagreement led to new insight, so that apparent contradictions were reconciled. It may be worth while, therefore, to review the recent work in some detail. Ambache (5) became interested in the depression by nicotine of the response of isolated mammalian intestine to certain drugs. He noted that, in the presence of

ganglion-paralysing doses of nicotine, from ten to one hundred times as much acetylcholine was required to stimulate the intestine. He concluded that part of the action of acetylcholine in the absence of nicotine was to stimulate ganglia, and thus to release further acetylcholine from postganglionic nerve endings.

Histamine, barium and potassium, on the other hand, were active in the presence of nicotine. These drugs could not, however, elicit a contraction from a preparation kept for several days at 0 to 2°C—a procedure said to inactivate all nervous structures. From this the conclusion was that histamine, barium and potassium, though not predominantly stimulating ganglion *cells*, owed most of their action in fresh preparations to stimulation of postganglionic axons or nerve terminals; they also, therefore, acted indirectly by releasing acetylcholine. Their action was abolished in the presence of strong (1:1000) calcium solutions; the abolition was said to be due to the depression by calcium ions of acetylcholine synthesis at the nerve endings. In support of this hypothesis Ambache noted that the action of all these drugs was increased by eserine, implying a potentiation of the acetylcholine which they "released" from the nerve endings. He measured the rate of synthesis of acetylcholine in strips of intestine, and found the rate was increased by the addition of histamine, barium or potassium.

Emmelin and Feldberg (61) confirmed several of these findings. They agreed that eserine potentiated the responses to histamine, barium and potassium, but thought the effect could be explained as a summing of the action of those drugs with the basic increase in activity due to the eserine. They confirmed that in preparations "denervated" by cooling for several days the action of barium (and to a less extent of potassium) was reduced to a greater extent than the action of acetylcholine. However, they were unable to find a similar difference between histamine or pilocarpine and acetylcholine; the actions of all three seemed to them to be equally depressed by cooling. They concluded that there was no reason to postulate stimulation of nervous elements by histamine and pilocarpine. They confirmed that potassium ions to some extent accelerated the rate of acetylcholine synthesis, but could not attribute any such activity to barium either in their original (61) tests, or in subsequent experiments carried out exactly according to Ambache's technique (73).

Their experiments with nicotine were of particular interest. They showed that the depression by nicotine of intestinal sensitivity to acetylcholine did not always occur, and that when it did, it was only temporary; full sensitivity returned even while nicotine remained in the bath. They showed also that nicotine depressed equally the responses to pilocarpine and to the "purely muscarine-like" drug 2268F, (ethylal of γ-trimethylammoniumpropanediol), both of which at that time were thought to have an insignificant effect on ganglia. Thus they concluded that nicotine had a non-specific depressant action on the responsiveness of the muscle itself. A similar depression was shown by Cantoni and Eastman (39) to occur after most drug-provoked maximal contractions.

Since drugs "without ganglionic action" were also potentiated by eserine, Emmelin and Feldberg argued that potentiation by eserine could not be taken as evidence for the release of acetylcholine from postganglionic fibres. Ambache (7),

however, in experiments on the perfused sympathetic ganglion of the cat, demonstrated that barium, pilocarpine and 2268F did in fact stimulate postganglionic neurones, so that the depression by nicotine of part of the activity of these drugs also could still be regarded as due to a paralysis of the intestinal ganglia. He and Rocha e Silva (9) showed that nicotine did not depress the contractions of the guinea-pig's ileum in response to Bradykinin, from which it was concluded that the depression by nicotine of the response to the other drugs mentioned could not be due to a reduction in the responsiveness of the muscle itself.

Further evidence was obtained by Feldberg (73) and by Feldberg and Lin (75) concerning the mode of action of histamine. These authors placed their preparations in solutions of cocaine sufficiently strong to ensure that when nicotine was added no contraction took place. They concluded that the cocaine had rendered the postganglionic axons inexcitable, and argued that histamine, which now produced a contraction as large as ever, could not be acting via the postganglionic fibres. A similar argument was applied in the case of hexamethonium. Since this was taken to be a purely ganglion-paralysing drug (115), its failure to reduce contractions in response to histamine was taken to disprove any postganglionic neuronal stimulation.

Interestingly enough, however, it was found that, in the presence of hexamethonium, contractions in response to acetylcholine *were* sometimes reduced to a small extent, so that by this criterion Ambache's original contention was confirmed, that some of the normal activity of acetylcholine could be attributed to an action on the ganglia (72).

Evans and Schild (67) had recourse to a surgical technique introduced by Magnus (106) and since employed by several authors (4, 85, 92). They developed the method further and made preparations of completely ganglion-free intestinal muscle. The preparations were examined histologically after use, and found to be without nerve cells. In many preparations they endeavoured to get rid not only of ganglion cells, but also of the terminal "nerve net" by removing the ganglia at a sterile operation several weeks before the experiments. A nerve-like structure persisted even after such operations, and the technique has been criticised on the grounds that some nerve terminals survive separation from their parent cells. Apart from the improbability of such a phenomenon, Evans and Schild have some evidence that these surviving structures are non-nervous. They found that barium increased the spontaneous contractions of the nerve-free muscle, but never caused the "spastic" type of response seen in the innervated preparations. It could, therefore, be concluded that though part of the activity of barium might be attributed to a direct action on smooth muscle fibres, Ambache's hypothesis was confirmed in so far as a great deal of the activity was due to excitation of nervous structures.

Nicotine was found to contract relaxed nerve-free preparations, but to relax them if they had already been made to contract (*e.g.*, by the application of acetylcholine). This type of relaxation, however, was easily distinguishable from, and briefer than the relaxation (and associated depression of response to other drugs) which followed the action of nicotine in innervated preparations. Thus

at least part of the "inhibitory" effects of nicotine in fresh preparations could evidently be associated with nervous activity; (the relevance of this point will be discussed in the section which follows). One extremely interesting finding was that in the ganglion-free preparations both the stimulating and brief relaxing effects of nicotine were antagonised by hexamethonium (68). Thus this drug, so often employed as a purely ganglion-blocking agent, was shown to have effects on the muscle itself as well.

3. Are There Intestinal Ganglia with Adrenergic Postganglionic Fibres? Inhibitory Effects. In preparations of mouse and rabbit intestine in which the activity of cholinergic fibres (6) had been abolished by botulinus toxin, Ambache (8) observed that nicotine caused relaxation, and inhibition of spontaneous contractions. The effect of 200 μg. of nicotine was equivalent to that of 1 μg. of adrenaline, and was antagonised by hexamethonium. He accordingly postulated the existence of postganglionic adrenergic fibres, left unaffected by botulinus toxin and sensitive to nicotine. Ambache and Edwards (10) showed that preparations of kitten (but not rabbit) intestine when immersed in solutions of atropine 10^{-7} or stronger, responded to nicotine with a pure relaxation. This certainly could not be the non-specific effect of a maximal contraction (39), because in the presence of atropine there was no contraction. Nor was it similar to the relaxing effect of nicotine on contracted nerve-free intestinal muscle described by Evans and Schild (67). Here again, therefore, their conclusion was that block of the cholinergic mechanisms unmasked the existence of adrenergic postganglionic fibres. Walder (167) found that both adrenaline and nicotine relaxed atropinised preparations of the muscularis mucosae of the human stomach. The nicotine effect was antagonised by hexamethonium. Further evidence for the existence of adrenergic postganglionic fibres was recently obtained by Rocha e Silva and others (133) who thought that the antagonistic effect exhibited by nicotine to the action of 5-hydroxytryptamine could be explained as due to the release by nicotine of sympathin from postganglionic neurones.

Motor Effects. King and Robinson (95) showed that nicotine caused contractions of the dog's muscularis mucosae in the presence of concentrations of atropine strong enough to antagonise acetylcholine. Ellis and Rasmussen (60), using rabbit intestine, found that, in the presence of atropine, nicotine (and piperidine) caused contractions. The effect was apparently mediated by nerves, because the contractions were abolished in solutions of cocaine or if further nicotine or large doses of adrenaline antagonists were given. Such high concentrations of antagonists were necessary, however, that the authors concluded that although the atropine-resistant action of nicotine involved the stimulation of postganglionic nerves, these could not be adrenergic. (Yet there are, of course, other situations known in which adrenaline antagonists abolish the action of externally applied "transmitter" without antagonising the effects of nerve stimulation, (134).)

A motor effect of adrenaline on guinea-pig intestine was demonstrated by Bernheim and Blochsom (19) and recently re-investigated by Munro (108, 109, 110). In atropinised ileal segments periarterial stimulation caused contractions,

as did adrenaline. The effects of adrenaline, but not of stimulation, were antagonised by ergotoxine and dibenamine. In some, but not all, preparations the motor effects of nerve stimulation were abolished by nicotine, tetraethylammonium and tubocurarine, implying that the motor impulses were relayed at ganglia in the intestinal wall. The nerve-stimulation experiments with ganglion-blocking agents were not, however, carried out on atropinised segments, so that the evidence for adrenergic postganglionic fibres coming from ganglia in the intestinal wall still rests upon the demonstration of an atropine-resistant response to nicotine. In those preparations in which contractions persisted in the presence of ganglion-blocking drugs, it was supposed that the nerves stimulated were sympathetic postganglionic fibres passing directly to the muscle fibres.

Summary. Since this section has dealt with a very controversial subject, it may be useful to summarize some of the evidence about the actions of the more important substances investigated.

Nicotine. Nicotine in fresh preparations of the intestine of nearly all species caused contractions of intestinal segments; an effect supposed to be due mainly to the stimulation of postganglionic neurones.

The contractions were followed by a phase of "inhibition," during which responses of the preparation to acetylcholine, pilocarpine, 2268F and, to a less extent, histamine, were depressed (39). Responses to Bradykinin, however, were not affected by nicotine.

Nicotine also caused contractions of ganglion-cell-free and chronically denervated preparations of circular intestinal muscle. When these preparations were already contracted nicotine relaxed them. This relaxation was briefer than, and different from the phase of inhibition observed after nicotine in the innervated preparation. It does not, therefore, support the hypothesis that this inhibitory phase is due to a generally depressant effect of nicotine on intestinal muscle.

The antagonistic effect of nicotine to the stimulant action of other drugs has been explained in three ways. (a) It has been taken as evidence that the other drugs have some action on ganglia, which is abolished by nicotine. (b) It has been ascribed to stimulation by nicotine of postganglionic adrenergic fibres; sympathin released at the nerve endings is responsible for the antagonism. (c) It has been ascribed to a generally depressant effect of nicotine on intestinal muscle.

The fact that nicotine is also apparently able to stimulate afferent nerves (34, 166) may prove to be relevant to its action on the intestine.

Adrenaline. The effect of adrenaline is predominantly to relax segments of intestine. In some preparations, however, especially of lower guinea-pig ileum, adrenaline causes contractions, which persist after atropine and cocaine, but which are antagonised by dibenamine, ergotoxine and nicotine.

Acetylcholine. The effects of small amounts of acetylcholine are antagonised by nicotine, tubocurarine and sometimes by hexamethonium. Some authors (5, 7, 72) have considered the antagonism implies that part of the action of acetylcholine in fresh preparations is due to stimulation of intestinal ganglia as well as muscle. The threshold to acetylcholine of nerve-free preparations is 10 to 100 times higher than that of the innervated preparation (65). On the other hand,

after treatment with eserine, the thresholds of the innervated and denervated preparations are the same. The explanation of the threshold difference could be, therefore, not that acetylcholine stimulates the ganglia of the innervated muscle, but that in the denervated muscle it is more easily destroyed (67).

Barium. Barium increased the activity of nerve-free intestinal muscle, but stimulated innervated preparations differently and more vigorously. It stimulated the superior cervical ganglion, as did pilocarpine and the "muscarinic" drug 2268F. These ganglionic actions were antagonished by *atropine.* On the innervated preparation of guinea-pig ileum its action was diphasic. The more rapid phase was antagonised by atropine, and the slower phase by hexamethonium (151).

Histamine. There is some evidence that histamine can stimulate ganglia as well as smooth muscle (152*a*). Though different authors obtained contrary results from experiments upon preparations of cooled intestine, histamine can stimulate the superior cervical ganglion if it is circulated with blood. Some of the actions of histamine are antagonised by hexamethonium (96); this is discussed in the section which follows.

4. Analysis by Drug Antagonism. It is, perhaps, appropriate at this point to consider whether the fact that workers in different laboratories have arrived at such varied conclusions, suggests some fundamental limitation of the techniques employed. In several of the pharmacological analyses already described, the assumption has been made that because a drug A (*e.g.*, hexamethonium) "paralyses" a certain structure (the autonomic ganglia) as tested by the failure of the ganglia to respond to drug B (nicotine), then in the continued presence of A the same structure will still be inexcitable by a third drug C (*e.g.*, histamine). To take another example, a similar assumption was made with regard to the action of Bradykinin. Because this substance was able fully to stimulate intestinal smooth muscle in the presence of nicotine, it was assumed that the muscle fibres themselves must be just as fully excitable by other drugs in the presence of nicotine. The fact that certain drugs were *less* active in the presence of nicotine was taken to show that they could not normally be acting directly on the muscle, but on the ganglia, since it was said to be at this point only that nicotine could antagonise them. If the assumption were valid that a particular drug would eliminate a particular type of response, an indefinitely extended pharmacological analysis could be made, different sites of action being eliminated one by one, until the preparation was bathed in a mixture of drugs. An antihistamine could be injected, to eliminate effects of histamine-release, followed by a ganglion-blocking agent to block ganglia; nicotine would then be added to confirm that the ganglia were well and truly blocked; an adrenaline antagonist might then be included to abolish "sympathetic effects", so that by the time the drug to be investigated was given, there might well be little left to eliminate but the cat. This may be a frivolous exaggeration, but the principle is relevant.

Though it is convenient to classify drugs by their main effects as "sympatholytics" and "ganglion-blocking" agents etc., it is a commonplace that they often have other very powerful, and sometimes unwanted, effects as well. Local

anaesthetics have cardiac actions comparable to those of quinidine (48); hexamethonium, until recently considered faithfully specific, can act upon more than one structure; conversely, postganglionic neurones can be activated by a wide variety of drugs, even including histamine. U. Trendelenburg (152a) found that although histamine, added to a perfusion fluid, failed to stimulate the perfused superior cervical ganglion, if the drug was injected into the lingual artery (the ganglion being left with its normal circulation intact), a contraction of the nictitating membrane followed. The contraction was abolished by removal of the ganglion. Histamine will also cause the release of adrenaline from the suprarenal glands. Furthermore, Kottegoda (96) has shown that although histamine continues to exert an undiminished effect upon isolated rabbit auricles in the presence of a hexamethonium concentration sufficient to block the action of nicotine, yet if the hexamethonium concentration is increased sufficiently the action of the histamine also is abolished.

Schild (139) has suggested that drug antagonisms should be described in terms of one particular drug blocking the action of another particular drug on a specified preparation, because the antagonisms are often only relative. Neoantergan is an antihistamine, but so is atropine, though it is 1,000 times weaker. Similarly, neoantergan is in a sense "atropine-like," since it will block the effect of acetylcholine on the guinea-pig ileum if its concentration is 40,000 times that of an equipotent solution of atropine. These are extreme examples, but the ratios are often much closer. Hexamethonium is a "ganglion-blocking agent," but the fact that a dose sufficient to block the action of nicotine does not block drug X does not prove that X does not act on ganglia; it may merely imply that the antagonism of hexamethonium towards nicotine is relatively more powerful than its antagonism to X.

In view of these considerations it may seem that "analysis by drug antagonism" could never be expected to yield the certainty and precision that can be achieved by physical methods; but this does not, of course, exclude its great usefulness for the limited purpose of breaking down drug activity in the whole animal into relative activity on different organs.

5. The Transmitter of Intestinal Muscle. Acetylcholine. "Local hormones" have lately received much attention (37). The idea has been advanced that substances locally produced may affect the function of tissues, especially those exhibiting rhythmical activity, in a manner analogous to the "driving" of various organs by the hormones proper produced in distant parts of the body. It has been suggested that the heart-beat, for example, and the pendulum movements of the intestine may be initiated in this way. If so, there are two possibilities with regard to the site of origin of the "hormone" or activator. First, it could be secreted by another nearby tissue, such as a nerve terminal or secretory cell. Alternatively, it could be produced by the cell which is itself excited. This would lead to the rather curious phenomenon of one part of a cell extruding from itself a substance which then becomes re-attached and stimulates the cell's own function by a sort of parthenogenesis. Though the idea of a single cell being specialised both for secretion and contraction is unusual, different parts of the surface of cells certainly do have very different properties. Skeletal muscle fibres are excitable by

acetylcholine in the end-plate region only (35, 99); and the opposite sides of the cells lining the kidney tubule, for example, have obviously different functions. The internal architecture of single units as revealed by electron microscopy (143) can be considerably complicated.

Much effort has been devoted to investigating which types of cell do in fact secrete acetylcholine in the intestine. Dikshit (49) measured the rate of synthesis of acetylcholine in the different layers of the intestinal wall. He concluded that the synthesis was achieved mainly by tissues to which the ganglionic plexuses adhered when the layers were separated. A contrary conclusion was reached by Feldberg and Lin (74, 76) who found no correlation between the number of ganglion cells present and the rate of synthesis. Their experiments left no doubt that the glandular layer, without nerve cells, was capable of synthesizing as large amounts of acetylcholine as any other layer, and suggested that part of the function of acetylcholine might be to control the rate of production of intestinal secretions. The authors pointed out, however, that the number of nerve axons would not necessarily be correlated with the frequency of occurrence of nerve cells, so that the possibility still existed that nerve terminals were mainly responsible for the synthesis. A recent clinical finding may be relevant. Hirchsprung's disease is a condition of great dilatation of the large bowel. A segment of the recto-sigmoid is unable to propel onwards the material delivered to it from the descending colon. In consequence, the normal bowel proximal to the abnormal portion becomes distended, and hypertrophies in its attempts to force the faeces through the paralysed section. Histological examination of the pathological recto-sigmoid has shown it to be devoid of ganglion cells (20). These are evidently necessary, therefore, for normal bowel function, even though the intestinal muscle can still contract without them.

In additon to acetylcholine there are many other naturally occurring substances which stimulate contractions of intestine, and some of them have from time to time been put forward as possible transmitters or agents exercising a control over the level of normal intestinal activity.

Substance P. This substance, originally found by Euler and Gaddum (66), has recently been studied in detail by Pernow (117, 118). It occurs in alcoholc extracts of several organs, including intestine and brain, and is precipitated by ammonium sulphate. By adsorption, elution and other procedures, activity could be increased from 3 units/mg. in crude extracts to 3,000 units/mg. in the purified extracts. Substance P was not antagonised by nicotine, unless the dose of nicotine was very large; it was even less sensitive than histamine, for example, to antagonism by nicotine. Atropine, antihistamines, and cocaine in concentrations strong enough to abolish the action of nicotine, did not abolish the responses to substance P.

Substance P is destroyed by boiling in concentrated hydrochloric acid, but histamine is not. Histamine, on the other hand, is destroyed by nitrites, in which substance P is stable (83). Substance P is distinguished from acetylcholine by being hydrolysed by trypsin, but not by cholinesterases. Bradykinin (131) and Darmstoff (164) are also stable in trypsin solutions.

The occurrence of substance P is not correlated with the presence of ganglion

cells, because its concentration is the same in normal colon as in the pathological rectosigmoid of Hirschprung's disease, which contains no ganglion cells (59). Its distribution in the wall of the intestine of the dog has been studied in detail by Douglas, Feldberg, Paton and Schachter (53). Since only 15–20 % of it occurs in the muscularis externa, its rôle as a normal activator of intestinal contractions is doubtful.

5-Hydroxytryptamine (Enteramine, Serotonin). Any naturally occurring substance with general smooth-muscle-stimulating properties is of immediate interest by reason of its possible significance in asthma or hypertension. Two groups of investigators have in the last few years been on the tracks of such a substance, and it has recently turned out that they have been hunting the same hare. Rapport (127) and Reid and Rand (128) were mainly concerned with the vasoconstrictor actions of a substance found in serum, serotonin. Erspamer and Boretti (64) and Erspamer and Asero (63) investigated a substance, Enteramine, occurring in extracts of the salivary glands of octopoda. Both substances have been identified as 5-hydroxytryptamine, which has, among other actions, a powerful stimulating effect upon the intestine.

Gaddum (82) found that the contractions of guinea-pig's ileum produced by 17 μg/l. of 5-hydroxytryptamine were not reduced by atropine at a concentration of 1 mg./l. Rocha e Silva, Valle and Picarelli (133), however, disagreed with this, since they found that 0.4 μg. atropine in a 15 ml. bath (27 μg/l.) *did* abolish the effect of 5 μg. of 5-hydroxytryptamine, at pH 7.4. They suggested that the atropine used by Gaddum was ineffective because it might have been alkaline. Gaddum tested the efficacy of the atropine by showing that it had abolished the action of acetylcholine. According to Rocha e Silva atropine is ten times stronger as an antagonist of acetylcholine than as an antagonist of 5-hydroxytryptamine, and it might at first appear that this would explain the discrepancy. The solutions of atropine used by Gaddum were, however, thirty times stronger than those found adequate to block 5-hydroxytryptamine by Rocha e Silva, so that there does appear to be a direct conflict of evidence.

Feldberg and Toh (77) studied the distribution of 5-hydroxytryptamine in the wall of the digestive tracts of dogs and rabbits. There was none in the muscularis externa of the dog, but the mucosa was rich in the substance, especially in the pyloric region. These findings would not suggest that its normal rôle was concerned with the motility of the external muscle. Dalgliesh, Toh and Work (47) demonstrated that the activity of enteramine was in fact due to two substances, one of them 5-hydroxytryptamine, the other a related substance containing an indole ring.

Darmstoff and Bradykinin. Both Darmstoff, believed by Vogt (163) to be concerned with the control of intestinal motility and possibly relevant to the atropine resistant contractions seen on vagal stimulation, and Bradykinin, a smooth-muscle-stimulating substance investigated principally by Rocha e Silva (131, 132) and others, can be distinguished from acetylcholine, histamine and substance P. Their rôle in normal intestinal physiology is uncertain, but they are of interest as adding to the growing list of naturally occurring substances which

can stimulate intestinal muscle. Analysis of the mode of action of drugs upon the intestine thus increases in difficulty, as more substances are discovered which might be "released" from sites as yet unknown. The prospect of obtaining adequate information by "pharmacological analysis" and the selective blocking of possible mediators is bleak.

III. THE APPROACH TO NATURAL CONDITIONS

1. Choice of Method. A full understanding of the mode of action of drugs has obviously to be based upon a knowledge of the functioning of individual units. When, as in the case of the intestine, the fundamental physiology is still in question, the investigator has to be content with guesses based on evidence from heterogeneous preparations. From the clinical point of view what matters is the effect of drugs on whole animals—patients; and the best experimental procedure may be the one which departs as little as possible from natural conditions. Isolated organs are of limited value in this respect, since they are cut off from central influences, which are known to be of considerable importance in intestinal physiology. Radiologists are only too keenly aware how much mental attitudes affect gastrointestinal motility. Indeed the power of emotion to upset the autonomic system has been recorded by various observers from Aristophanes, who described the embarrassing consequences of terror (12) to Venning and Jungmann (162) who watched the X-ray appearances of a patient's stomach when a pistol was fired behind his back.

During the past fifty years and more an enormous number of papers has been published concerning the action of two drugs in universal use, morphine and posterior pituitary extract; yet authors have still disagreed about the explanation of their various observed effects. It is hoped it will be possible to show that nearly all the contradictions were apparent only, and resulted from differences in the experimental methods used, each tending to bring out a particular aspect of an action which was in fact complex. Since the choice of method has proved of such importance in the interpretation of studies of drug action, it is proposed to review in some detail the various attempts made so far to achieve the ideal technique for measuring intestinal motility.

X-rays. There is no doubt that X-ray photography provides the most "natural" kind of study of intestinal movements. A study of the motility of the normal human small intestine was recently carried out by McLaren, Ardran and Sutcliffe (105), using serial radiography at two exposures per second. Apart from the elaborate apparatus required, the method is unsuitable for pharmacological studies owing to the great difficulty of obtaining significant pictures; the authors emphasized the enormous labour involved in studying literally hundreds of photographs in order to secure enough information for unequivocal interpretations. Unless some cinematographic apparatus is employed, the observer has, in the main, to rely upon his subjective impressions from a screen, taking occasional direct photographs at intervals; (cinematograph records of a screen give poor definition). These recording difficulties are encountered whatever X-ray method is employed; *i.e.*, whether the conventional barium meal is used, or some other

technique, such as the infiltration of the intestinal wall with radio-opaque material (which incidently gives no information concerning propulsion) (145).

In animals which can be quickly killed propulsion can be studied by measuring the distance travelled down the gut in a given time by carbon suspensions (155). Experimental X-ray studies of the passage of barium meals were made by Cannon as long ago as 1902 (38), and many authors have since used this type of method for pharmacological investigations. In order to obtain some degree of objectivity "transit times" have been measured by noting the moment at which the barium reaches anatomically identifiable points. Unfortunately, normal transit times, in humans at any rate, can vary tenfold, and are accordingly of little value in comparative studies. Although X-ray methods do not disturb physiological conditions unless the procedure causes alarm, this advantage is outweighed by all the aforementioned difficulties of interpretation and of adequate objective recording.

Fistulae. The Use of Balloons and Boluses. The preparation of intestinal fistulae to collect digestive secretions from conscious dogs was originated by Thiry (150). He separated a portion of intestine from the rest of the alimentary tract (whose continuity was restored by anastomosis) brought out one end of it through the abdominal wall, closed the other end, and left the resected segment, with its blood supply intact, lying freely in the abdominal cavity. Vella (161) improved upon this by exteriorising both ends of the loop, and such "Thiry-Vella" fistulae were used for many years by various workers for the collection of secretions. In 1921 Plant (119) employed a Thiry-Vella loop to study intestinal *motility* by introducing a balloon attached to a manometer (a method of recording originated in 1899 by Bayliss and Starling for acute experiments (14)). Similar preparations have been, and still are extensively used to obtain records of intestinal activity.

All methods which employ intraluminal balloons, however, are open to the objection that the contractions recorded may be influenced by the presence of the balloon. The contents of the small intestine being fluid or semi-fluid, a balloon with its associated tube must be regarded as an abnormal object for the lumen to contain.

Furthermore, balloons cannot give a record of intestinal propulsion. In order to function at all the balloon must remain *in situ*, whereas the contents of the intestine are normally propelled along. An attempt to overcome this difficulty was made by Rowlands, Chapman, Taylor and Jones in 1950, who employed a series of four balloons in a row, swallowed together with a barium meal by human volunteers (136). These investigators endeavoured to correlate the records of the simultaneous activity of the four balloons with the observations of bowel movements seen by X-rays. In a later paper (41) it was emphasized how great was the variability of the results, even when only placebos were given. The disadvantages attending the use of balloons led Reid (130) to develop a method, originally introduced by Gottlieb (88), and further elaborated and fully described by Quigley, Highstone and Ivy (126), whose technique was to insert an artificial bolus (made of cork or sponge rubber) into a Thiry-Vella loop, and to measure the time it took to emerge from the distal end. It was found necessary to instil several millilitres of liquid paraffin into the loop, but even with adequate lubrica-

tion the bolus was held up in a kink or pocket in many of their preparations, which had then to be discarded. Perhaps the greatest disadvantage of the method, however, was that the bolus took several minutes to traverse the loop; consequently "readings" were infrequently obtained, and all but long-lasting effects were missed.

This last disadvantage was avoided by the method of Templeton and Adler (149) who recorded the tension developed by a dog's colon in its effort to propel forwards a bolus inserted through a caecostomy and held back by a thread attached to a spring. Though both these methods (in contrast to the balloons) to some extent recorded propulsive efforts of the intestine, neither of them gave any information about the state of intestinal "tone" or the amplitude of individual contractions.

The Transport of Fluid. Since the normal contents of the intestine are fluid, it would be reasonable to employ, as a means of estimating intestinal propulsion, an apparatus to measure the rate at which fluid could be transported through an intestinal segment. Baur (13) employing isolated segments of intestine removed from anaesthetised guinea-pigs, and Sollmann and Rademakers (144), using segments from rabbits, devised methods of recording the rate at which fluid was passed from one end of the segment to the other. Quigley, Highstone and Ivy (126) recognised the advantages of employing fluid instead of boluses to measure intestinal propulsion, and gave an extensive trial to such a method in unanaesthetised dogs. The chief difficulties which led them to abandon the attempt were (a) the problem of achieving a water-tight joint between the fistula and recording apparatus without compressing or damaging the ends of the loop. (b) The construction of a suitable recording apparatus. (c) The error introduced by the subtraction of fluid from the recording system by absorption, or the addition of fluid to it by secretion.

The first difficulty was claimed to have been solved by Doster-Virtue and Virtue (50) who achieved a "water-tight union" by sewing the gut wall to a ring of bone through which a catheter passed into the lumen. The bone was invaginated into the intestine which was then sewn to the abdominal wall. These authors had abandoned this method a year later, however, (51) but introduced a new method employing a steel mesh in place of bone. The method was tried by Gregory (89) but was not found to be adequate. Gregory achieved satisfactory unions with silver cannulae, and rather similar cannulae were used for a time by the author, but later abandoned in favour of perspex cannulae (157). Gregory's technique permitted measurement of the amplitude of contractions of the part of the loop near the cannula, and of the resistance of the loop to the passive flow of fluid introduced at a fairly high pressure into the proximal end. As Gregory pointed out, however, it gave no information about propulsion. A further possible disadvantage was that, since there was no cannula at the distal end, resistance to flow could be influenced by contractions of the voluntary muscles of the abdomen. Similar objections applied to the method of Borchardt (21) who measured the rate of passive flow of fluid through a Thiry-Vella loop, while recording contractions simultaneously with a balloon.

A method of measuring the transport of fluid, under controlled conditions of

temperature and pressure, by cannulated Thiry-Vella loops in conscious dogs was described by Vaughan Williams and Streeten (147, 157). Since that time the method has been considerably developed, with regard both to the operative technique of fixing the cannulae in position, and to the apparatus, which now permits the avoidance of the errors due to absorption or secretion of fluid by the loops (156). It may, therefore, be of interest to give a brief description of the method in its final form.

Technique of Operation. Bitches of 8–10 kg. were used. Deprived of food, but not water, overnight, and given atropine 0.1 mg./kg. and morphine, 1.0 mg./kg. preoperatively, they were anaesthetised with ether, a McGill's cuffed endotracheal tube being passed immediately after induction. After a midline incision, a segment of intestine 4–6 in. long, with a pedicle carrying nerves and blood vessels left intact, was separated from the alimentary tract, whose continuity was restored by a side-to-side anastomosis (Fig. 3, *D*). Cannulae machined from a solid rod of perspex (Fig. 3, *A*, *B*) were sewn into each end of the segment, and with the aid of a detachable trocar (Fig. 3, *C*), were passed through separate stab incisions in the abdominal wall. After recovery, the cannulae gave permanent access to both ends of the loop, which itself lay in the abdominal cavity. It was found that connective tissue grew through the openings in the flange F of the cannula (Fig. 3, *B*), forming a firm and water-tight seal. During recordings a perspex washer, W, was made to compress the abdominal wall against the flange F by means of the detachable steel collar and flange S (Fig. 3, *G*). The latter served the dual purpose of providing an anchor for the recording apparatus, and of preventing the dog from biting its cannula in the intervals between experiments, when it was fastened flush with the mouth of the cannula and no longer compressed the abdominal wall.

Recording Apparatus. Tyrode solution was allowed to flow from a constant-level reservoir of the Marriotte bottle type on a stand of adjustable height, through a heat exchanger which raised its temperature to 37°C just before it entered the proximal cannula. The activity of the loop then pumped the fluid into an outflow recorder whose height was also adjustable. The outflow recorder was usually set one or two centimetres above the level of the inflow reservoir, so that propulsive work had to be done in raising the Tyrode solution through a known vertical distance. The recorder was operated by a relay to empty itself approximately every 10 ml. In this form the set-up was quite suitable for making quantitative comparisons of the propulsive work done by the loop under the influence of drugs (157).

If it was desired to know also the state of "tone" of the loop, *i.e.*, the volume of the contents of the lumen at a given pressure, or to control errors due to absorption or secretion by the loop, a second recorder could be attached to measure simultaneously the rate at which fluid entered the loop. It was reset at intervals to approximately its original position by the 10 ml. of fluid ejected from the outflow recorder; special precautions had to be taken to maintain the air inside this closed circuit at constant temperature and pressure (156). A comparison of inflow and outflow records then permitted both loop volume changes and absorption-secretion differences to be calculated. Volume changes were represented by fairly rapid alterations of the rate of inflow relative to the outflow, according as the loop dilated or contracted as a whole. Changes due to secretion or absorption were small, and were detected as a steady "drift" of one recorder against the other. The amplitude of the individual contractions at both ends of the loop was recorded by means of piston recorders connected to side-arms at the inflow and outflow tubes.

2. The Mode of Action of Morphine. The long controversy concerning the action of morphine on the bowel has been the product partly of a rather loose use of words such as "stimulants" and "inhibitors," partly of a very wide choice of experimental methods. The dispute began in 1882 when Nothnagel (113) failed

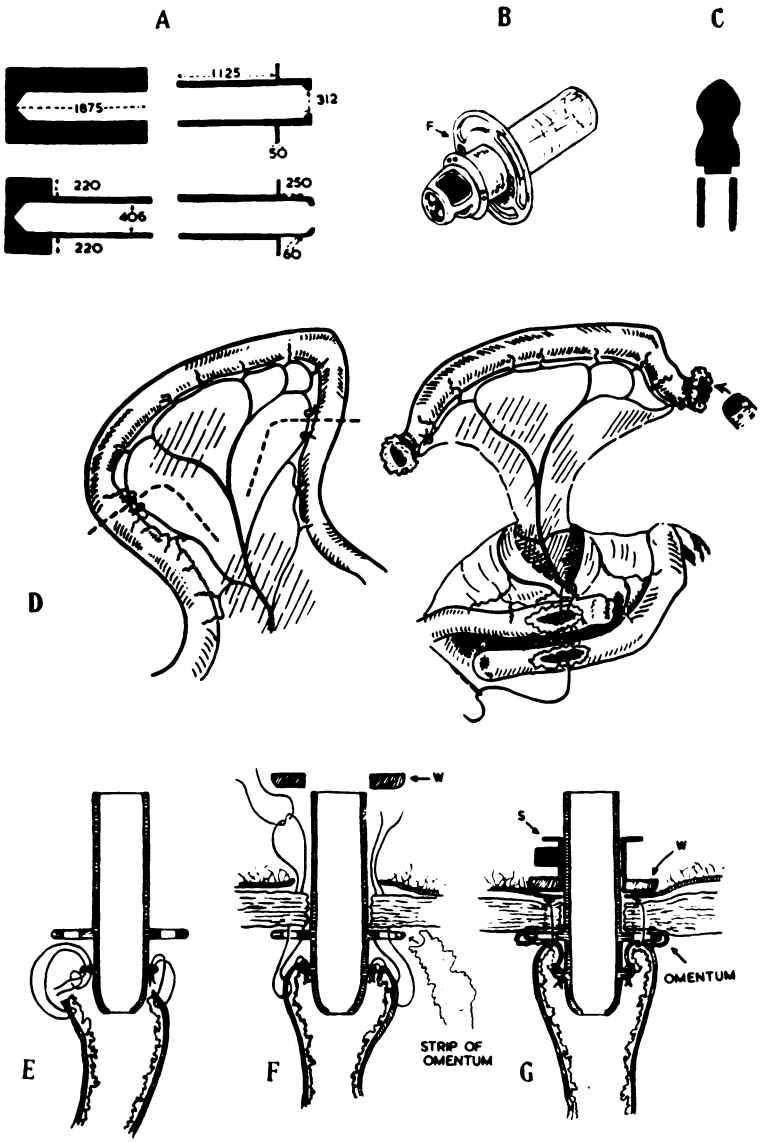

FIG. 3. Preparation of cannulated Thiry-Vella loops. *A*. Machining of cannula from solid 1 inch Perspex rod. Dimensions in thousandths of an inch. *B*. Completed cannula. *C*. Perspex trocar, fitting into mouth of cannula, to facilitate its passage through the abdominal wall. *D*. Preparation of loop and side-to-side anastomosis. *E–G*. Method of fixing cannula to abdominal wall.

to confirm Nasse's observation in 1865 that morphine "stimulated" rabbit intestine (112), and it has continued to the present day. The literature up to 1940 has been reviewed by Krueger, Eddy and Sumwalt (97). The difficulty has been to reconcile the well-known constipating action of morpine with the fact

that immediately after an injection of the drug faeces are sometimes passed and vigorous movements can be seen in bowel exposed at operation or revealed by X-rays.

Attempts to submit the conflict to the arbitration of controlled experiment have led to contrary verdicts. Many investigators studying intestinal behaviour in whole animals have agreed that morphine increases the "tone" of the intestine, usually measured with balloons and defined as a reduction in volume of the lumen, or as a raised resistance to distension (for detailed references, see 157, 158). Increased "tone" has also been described as a prolonged shortening of longitudinal or circular muscle, or of both; studies making measurements of these types of contraction also showed (137) that tone was increased by morphine. Such a rise in tone, however, is not necessarily related to any improvement in the ability of the intestine to propel its contents onwards, although it has frequently been assumed that it must be. Plant and Miller (120), for example, in whose paper may be found an excellent review of early work, concluded from their experiments that "the action of morphine on the small intestines of dogs . . . is to stimulate practically all phases of muscular activity: there is an increase in tone, and in the frequency and amplitude of peristaltic waves."

Quigley, Highstone and Ivy, employing the "bolus" technique already described, found that although morphine at first quickened the passage of an artificial bolus forcibly introduced into a Thiry-Vella loop, its main long-term effect was to depress activity, so that the bolus resided for long periods in the loop without being expelled. Under the influence of morphine more force was required than usual to push the bolus into the mouth of the loop. The findings of Kanan (94) and Weisel, Youmans and Cassels (169) with similar preparations were essentially in agreement. Templeton and Adler (149), recording the propulsive force exerted by a Thiry-Vella loop upon a bolus held back by a string attached to a tension recorder, likewise found an early increase in tension followed by a prolonged reduction in propulsive effort.

Methods involving the observation of the passage of radio-opaque materials, observed by X-ray (107, 140) in cats and dogs, or of carbon suspensions in rats have all shown an increase in the time taken for the substances to traverse the gut. The number of faecal pellets passed by rabbits in a 7-hour period was shown to be reduced by morphine (137), and the constipating effect of morphine in dogs and rats was confirmed by Scott, Chen, Kohlsteadt, Robbins and Israel (141).

In man delayed gastric emptying under morphine has been frequently observed (1, 111, 114, 138, 165) by X-ray methods. The delay has sometimes been associated with an increase in the size and the apparent vigour of contractions, though their frequency was unchanged (11). The rate at which intestinal contents have been discharged from an ileostomy in man was found by Forster (79, 80) to be greatly diminished after morphine; a result confirmed by Adler, Atkinson and Ivy (2) in their studies of a human ileostomy and four colostomies.

All the evidence obtained from methods which measured any form of propulsive effort, therefore, has shown that, though there may be a brief initial spurt of activity, the main effect of morphine is to cause a prolonged depression of

intestinal propulsion. In apparent contradiction of this finding has been the universal agreement that morphine "stimulates" the bowel, in so far as its "tone" is raised. Indeed, the actual work in gram centimetres done by an intestinal segment is found to be increased after morphine, if the work is measured as the ability of the segment to hold up a column of water connected to a balloon inside the lumen (98). But if the work is measured in terms of fluid transported, a contrary result is obtained.

The method of Vaughan Williams (156) permitted the simultaneous measurement in a conscious dog of the internal volume or tone of a Thiry-Vella loop and of the work done in gram centimetres in terms of fluid transported. Cannulae at both ends of the loop prevented obstruction to the flow of fluid by contractions of the abdominal muscles. The loop had to act as a pump, since the level of the inflow reservoir was below the outflow. The temperature and pressure of the fluid were constant, and the rate of inflow was measured as well as the rate of outflow.

It was found that even small doses of morphine caused quite prolonged reductions in the amount of propulsive work done by the loop. An immediate and prolonged increase in "tone" was demonstrated by the fact that the fluid already in the loop was expelled *in both directions*. In other words, the whole loop closed down tightly, and the failure of transport was due to an increase in resistance to the entry of fluid. Perhaps the most interesting finding was that if the pressure of the fluid at the proximal cannula was progressively raised (158), a point was reached when it at last forced its way into the loop (equivalent to the forcible introduction of boluses in the experiment of Quigley, Highstone and Ivy). At this moment the contractions became much more vigorous than before the morphine, and fluid was again transported. This is consistent with the observation that the amplitude of contractions is a function of the intraluminal pressure (147).

Thus it is clear that the previous work emphasising the "stimulant" action of morphine is perfectly consistent with a failure of transport. If the whole bowel closes down tightly, in the initial phase hard objects such as faeces or an artificial bolus may be expelled from the rectum or from an intestinal loop. A segment of bowel which is artificially and forcibly dilated by an object, such as a balloon, which it cannot expel, may continue to exhibit vigorous contractions. Similarly, non-absorbable fluids like barium may be forced from regions where the muscle is strong, to regions where it is weak or where resistance to further propulsion is aided by a flexure. Transport over a distance, however, is prevented by the increased resistance of each segment to the entry of material from above so that the overall effect is a failure of propulsion. The constipating and "stimulant" actions of morphine are thus reconciled.

3. The Action of Posterior Pituitary Extracts. The extensive and extended controversy about morphine has been rivalled by an almost equally active dispute concerning the effect of posterior pituitary extracts upon the bowel. In the early childhood of endocrinology, when it was the fashion to observe the effects of extracts of glandular and other tissues, Fodera and Pittau in 1909 noted that

injections of hypophyseal extracts into dogs caused them to defaecate (78). In the same year Dale (46) independently reported that extracts of the posterior lobes of ox pituitaries, boiled in acetic acid, filtered, and neutralized before use, caused an isolated strip of dog intestine to contract. The extract became of clinical interest when Bell (17) who had observed that it caused defaecation in pithed rabbits, tried it on patients with paralytic ileus, and reported universally successful results. Numerous commercial preparations then became available.

Experimental work with these extracts followed, but many of the conclusions drawn were contradictory. Roth in 1917 suggested that the main cause of the discrepancies was that some extracts were strongly acid or contained preservatives (135). Detailed reviews of the early literature were compiled by Geiling (87) by Gruber and Robinson (91), and by P. Trendelenburg (152).

A second difficulty in interpreting results was the problem of contamination of the extracts with histamine. Macdonald (104) using neutralized, preservative-free extracts could find no stimulating action upon cat intestine, and suggested that the activity previously described was due to the presence of histamine. Gaddum (81) confirmed that cat intestine was insensitive to pituitary preparations, but showed that isolated rabbit intestine was powerfully contracted by extracts which were certainly histamine-free. The activity was contained in the pressor fractions of the extracts.

So far it was clear that posterior pituitary preparations, even when the three complicating factors of pH and preservatives, of contamination with histamine, and of species differences in sensitivity, had been eliminated, still contained an active principle capable of stimulating isolated segments of intestine, as well as causing defaecation in pithed rabbits, in normal dogs, and in patients with ileus. It was, therefore, confidently expected to be active on preparations which were designed to reveal intestinal activity *in vivo*. A contrary result, however, was obtained by many investigators (for detailed review see 159), most of whom studied records obtained with intraluminal balloons either swallowed or inserted into fistulae. On the other hand, authors who had observed the passage of radio-opaque meals, or the expulsion of intestinal contents from fistulae, universally reported an *increase* in intestinal activity. Thus a fourth contribution to confusion was the employment of unsuitable methods for recording motility. A fifth and final complication was that different regions of the gut were not equally sensitive to the drug. Puestow (124), observing by eye the movements of various parts of the human bowel in hernial sacs, gained the impression that the small intestine was relaxed and inhibited by pituitrin and pitressin, but that the colon was stimulated. Larson and Bargen (102), recording from various parts of colonic fistulae in dogs, were of the opinion that pituitrin contracted the middle but not the upper or lower parts of the colon. Gaddum (81) had noted that the colon was more sensitive than the small intestine.

The method of Vaughan Williams and Streeten permitted the measurement of intestinal propulsive work for long periods, and it was found that this was remarkably constant for hours at a time. It was thus possible to estimate both the immediate and the longer-term effects of drugs by comparing the total output of

work after a drug injection with the mean level of work recorded in the hour before injection. It was found that injections of pituitrin caused an immediate loss of tone of the intestinal wall and a dilatation of the lumen (159). This continued for a time which was dependent upon the dose given and to some extent on the pressure of the inflowing fluid; but it was always followed by a period of increased activity which more than compensated for the "loss" of work during the phase of relaxation. Thus the total effect of the drug was to raise the output of propulsive work done by the loop, even though the immediate effect was inhibitory. It is clear from an examination of the tracings of some of the authors who reported that posterior pituitary extracts inhibited intestinal activity (91, 125) that attention was concentrated upon the early phase of relaxation, and the subsequent increase in activity, visible towards the end of their published records, escaped notice.

It may be of interest at this point to mention the mode of action of eserine, whose net effect is also to increase the output of propulsive work. Eserine, like pituitrin, provokes a preliminary phase of "inhibition," in the sense that the output of work is at first diminished. But whereas, after pituitrin, the gut has lost its tone and is relaxed during the inhibitory phase, after eserine the tone is increased during the inhibition, and fluid is expelled from the loop in both directions. Thus pituitrin dilates the gut, but increases the output of propulsive work; eserine constricts, and increases the output of work; and morphine constricts, but decreases the output of work.

In our experiments pituitrin was found to be active on jejunal as well as on ileal segments, so that even though there may be regional variations in sensitivity, the action of the drug is not confined to the large bowel *in vivo*. Pitocin had no effect upon intestinal propulsion.

4. Interpretation of Drug Activity. The activity of the gut is normally subject to so many influences that it is extremely difficult to decide on which tissue, or upon how many tissues a drug may be acting. The concentration of morphine required to affect the activity of dog intestine *in vitro* is more than one hundred times the calculated threshold concentration *in vivo*. This does not *prove*, however, that morphine is exciting central mechanisms *in vivo* (*e.g.*, the vagus); an alternative possibility is that normal centrifugal impulses (absent from *in vitro* preparations) arrive at their usual frequency but are potentiated peripherally. Proof was obtained, however, in cross circulation experiments (141) that the morphine substitute methadone exerts an effect on gastro-intestinal motility through the vagus. Morphine (18, 55), pethidine (32) and methadone (56) all inhibit the cholinesterase of brain tissue, but such *in vitro* findings are not, of course, necessarily relevant to the *in vivo* actions of the drugs.

The frequency of intestinal contractions seems to be very precisely controlled. It is higher in the jejunum than in the ileum, but for any one segment of gut it is extremely constant from hour to hour and day to day, and under varied conditions, *e.g.*, at different intraluminal pressures (147). The frequency in one segment is decreased by separation from the segment above, implying the removal of accelerating influences (52); in isolated gut the frequency is increased by cocaine

(75) which suggests the removal of decelerating influences. Each segment is also controlled by nerves arriving in arterial sheaths, but these have now been shown (54) to extend very little beyond the area which each artery supplies with blood. They do, however, have an undoubted influence on motility.

The exact function of the intrinsic nerve plexuses of the gut is still unknown, but it is generally supposed that they are responsible for the "peristaltic reflex" (contraction above a distended region), since this reflex is present in isolated segments, and is abolished by cocaine in concentrations which do not prevent "myogenic" contractions. On the other hand, contractile responses to stretch have been observed in nerve-free intestinal muscle. In Bayliss and Starling's classical descriptions (15, 16) there was a region of inhibition below a distending stimulus as well as a contraction above. Bozler (30, 31), however, found no evidence for inhibition below such a stimulus in his electrical records, though the action potentials were more numerous above it.

Intestinal activity is influenced not only by nervous impulses, but also by anoxia (154), and by abnormal electrolyte concentrations (see below). Further, some or all of the naturally occurring substances already discussed (substance P, 5-hydroxytryptamine, etc.) may play a rôle in normal physiology which could be affected by drugs. Ignorance of the details of normal intestinal activity is so great that from the practical and clinical point of view investigation of the effects of new drugs upon intestinal motility would seem likely to be most profitable if carried out on preparations involving whole animals.

IV. CLINICAL APPLICATIONS OF RECENT RESEARCH

A survey of the present position regarding the action of "spasmolytics" is outside the province of the present article, but brief mention may be made of some recent work. The activities of Trasentin and Trasentin H were compared with that of atropine by Tripod (153). The effect of amphetamine sulphate and its isomers upon intestinal motility was studied by Van Liere and Stickney (155) in rats and in dogs. Craver *et al.* (44) investigated the action of N,N-dimethyl-oxyacetamidine in dogs with Thiry-Vella loops (balloon recorders); they found that it was active in doses of 0.1 mg./kg. and both relaxed tone and inhibited contractions.

In the following section attention will be confined to three topics: first, the choice of analgesics for surgical patients; second, the use of morphine as a clinical aid in radiology; third, the treatment of paralytic ileus.

1. Analgesics for Surgical Patients. Morphine is still the most widely used analgesic for severe pain, but its prolonged inhibitory effect on intestinal propulsion is a definite disadvantage in surgical patients, especially after abdominal operations. For this reason a comparison (157) was recently made of the effects upon intestinal propulsion of morphine and of the synthetic analgesics pethidine (90) and methadone. It was found that the method of measuring the actual propulsive work done permitted straight-line curves to be constructed for each drug when work "lost" as a result of the action of the drug was plotted against the log of the dose. The curves were nearly parallel, and there was no overlap of experimental

points from different curves, so that reasonable confidence was placed in the calculation that the number of molecules of active base of morphine, methadone and pethidine required to produce an equal inhibitory effect on propulsion were in the ratio 1:2.5:75. Since methadone is about twice as powerful an analgesic as morphine (42), and pethidine one-tenth as powerful, the results show that a dose of methadone of equivalent analgesic potency to a given dose of morphine has only one-fifth its inhibitory activity on the gut. Similarly, pethidine has a clinical advantage of 7.5:1 over morphine, so far as inhibition of the gut is concerned, though it is well known that it is unable to control very severe pain. Thus in patients in whom difficulty or delay in establishing bowel movements is feared, pethidine would seem to be the best analgesic when the pain is not severe, and methadone when it is.

2. The Use of Morphine as an Aid to X-Ray Examination. The very action of morphine which is disadvantageous in surgical patients has been put to good use in X-ray observations of the stomach and upper intestine (45, 121, 122). It has been proved of such value in difficult examinations of ulcers of the duodenal cap, and in patients with anastomoses, that some radiologists never subject such cases to fluoroscopy except under morphine. The drug is usually injected intravenously; but its action is not connected with the release of histamine, for the following reasons. First, the morphine action is too prolonged; secondly, the amounts given cause no fall in blood pressure; thirdly, the morphine is also effective when given subcutaneously; fourthly, if a very small dose of histamine is given with the morphine, the subjective effect on the patient is immediate and profound, and quite unlike the effect of morphine alone.

The first visible difference between the patient given morphine and the untreated patient is that the stomach "stiffens up" as it were, and vigorous contractions propel the barium into the duodenum. The walls of the duodenum also have an increased tone and squeeze tightly on the barium which is forced into pockets and crevices. The barium then tends to remain longer in the duodenum, being held up by the increased resistance to its passage into the jejunum. The result is that an ulcer crater is filled which would often otherwise have been missed. An analogy would be a football with a split in its leather; if the pressure inside it is increased by pressing on it or blowing it up, the rubber bulges out of the split and eventually bursts. Fortunately, however, no perforations of ulcers under morphine have been recorded.

The use of morphine has proved especially valuable in examining patients with anastomoses. After gastrectomy or gastro-enterostomy the barium often passes from the stomach directly into the jejunum without filling the duodenal loop. Under morphine the jejunum closes down tightly, and offers so much resistance to the entry of the barium that the latter's path of least resistance is into the duodenum, which is then filled and dilated, and the radiologist is delighted (45).

3. The Treatment of Paralytic Ileus. Although Bell (17) reported no failures in his treatment of cases of paralytic ileus with posterior pituitary extracts, similar good fortune has not attended the ministrations of all physicians. Seed, Falls and Fantus (142), for example, found that although a perfectly satisfactory response

could be obtained in uncomplicated post-operative cases, the drug was of no value once ileus was established. Indeed, the same may be said of all commonly used intestinal stimulants; namely, that they work in normal people but fail in most cases of ileus. Streeten (146) observed that the recovery of some of his cases of ileus coincided with the reappearance of chloride in the urine, and thought that the intestinal failure might be associated with low plasma chlorides. A similar suggestion had been put forward in 1935 by Levy and Nora (103) who had found low plasma chlorides in their ileus patients. The problem was difficult to approach experimentally, since in spite of numerous endeavours, no condition resembling ileus had yet been produced in animals.

An attempt was therefore made to investigate the effect of low chlorides on intestinal propulsion in the dogs which had already been provided with cannulated Thiry-Vella loops for the morphine experiments. It was found possible to train these animals to lie quietly, with their loops "plugged in" to the recording apparatus, while as much as a litre or more of sodium-chloride-free isotonic solution was run into the abdominal cavity, and later drained off again. In this way several grams of sodium chloride could be removed from the animal on successive days, while records of intestinal propulsion were obtained as the depletion progressed. Estimations of blood and plasma sodium, potassium and chloride were made at frequent intervals. It was found (160) that a condition closely resembling paralytic ileus supervened when the plasma sodium and chloride had fallen by about a third. Contrary to expectation, however, the onset of paralysis did not coincide with a large and sudden fall in chloride or in sodium, both of which fell fairly slowly, but with a sudden rise in the plasma potassium. It seemed that a point in the depletion was reached at which the cells could no longer retain potassium. It appeared to be the loss of intracellular potassium that was the more important factor, and not the rise in plasma potassium, because injections of potassium sufficient to produce marked cardiac effects did not paralyse the gut. Both factors, loss of intracellular potassium and rise in plasma potassium, would in any case operate in the same direction, that of lowering the ratio of intracellular to extracellular potassium.

Severe anoxia is also able to cause paralysis (154), and it is possible that this may have contributed to the paralysis to a small extent in these experiments. It was not, however, the main factor, because control experiments in which severe anoxia was induced, showed that in spite of the added depressant effect of an anaesthetic, the arterial O_2 had to fall to 12.8 ml./100 ml., before intestinal transport was affected. In the sodium-depleted dogs complete paralysis was induced when the arterial oxygen content was much higher, 15.8 ml./100 ml., and there was no anaesthetic. Moreover, intestinal propulsion was considerably depressed when the depletion had not progressed far enough to produce any cardiac or respiratory effects at all.

Further depletion caused circulatory failure and death. The intestine did appear to be more sensitive to electrolyte loss than other tissues, however, because at a time when the loop was completely paralysed the heart and skeletal muscles were still functioning adequately. Partial recovery of intestinal propulsion was

obtained in a matter of minutes simply by the injection of some sodium chloride; a few hours after the injection the normal pre-depletion rate of propulsion was attained, and the plasma potassium had returned to normal.

Some further experimental results are relevant. Salt depletions were always accompanied by a sharp fall in the eosinophil count, indicating a degree of "stress" sufficient to produce an output of ACTH. This is, of course, also an invariable accompaniment of abdominal operations in patients. Injections of ACTH have been shown to produce a loss of intracellular potassium (116) and the output of ACTH may well have been responsible, at least in part, for the observed rise in plasma potassium in our experiments. Further, desoxycorticosterone acetate administration has caused depression of gastro-intestinal motility in rats (170) and experimentally produced potassium deficiency has led to gastro-intestinal disturbances (84). All these facts support the hypothesis that derangement of the ratio of extracellular to intracellular electrolyte concentrations could contribute to intestinal paralysis in man. Such a derangement might be brought about or aggravated by an abnormally prolonged output of ACTH in patients who develop ileus. A segment of human intestine, which contracted vigorously in Tyrode solution, became paralysed when placed in a solution containing the same electrolyte concentrations as those found in the plasma of a case of paralytic ileus.

On the basis of these experimental findings, Streeten and Ward-McQuaid (148) treated a number of patients on the assumption that electrolyte deficiencies were of primary importance in the production of ileus. Electrolyte levels and balances were determined, and the appropriate quantities of electrolyte replaced. They found evidence during ileus of adrenocortical hyperactivity, with prolonged eosinopenia, excessive potassium loss in the urine, and retention of sodium chloride and water. On recovery the eosinophil count rose, and there was a diuresis of water and sodium, and a retention of potassium. The therapeutic results of the regime were most encouraging.

It would appear, therefore, that the use of stimulant drugs in ileus is of limited value. The gut does not contract because its "ionic battery" has run down, as it were, and "it is no good throwing the switch if the battery is flat." If one may be permitted to speculate upon the mechanism of the failure, it is tempting to draw an analogy with what is known of skeletal muscle. The muscle fibre contains much potassium and little sodium inside, so that there is both a potassium and a sodium concentration gradient across the cell wall, opposite in sign. The resting or membrane potential is found to be reduced if the concentration gradient of potassium is reduced; conversely, the action potential is affected by a change in the ratio of intracellular to extracellular concentrations of sodium. It is possible that sodium chloride depletion, aggravated by adrenocortical hyperactivity, can so derange the balance of ionic forces that potassium begins to leave the cell. Consequently, the intracellular concentration falls and the extracellular concentration rises. Since excitation depends upon the flow of ionic current when the permeability of the membrane to ions is increased by the transmitter, the ratio of intracellular to extracellular ions could be so reduced that even when the transmitter short-cir-

cuited the membrane, there would be an insufficient voltage gradient to make the necessary current flow. Thus a drug such as eserine, whose effect was merely to increase the action of the transmitter, would be useless.

The preceding paragraph is pure speculation. It is not even known to what extent the functioning of smooth muscle is analogous to that of skeletal muscle. If reliable measurements of smooth muscle membrane potentials can be obtained, however, a means will become available of testing the effects of different ionic concentrations and their relation both to normal activity and to the effect of drugs. Yet from the practical point of view there does appear already to be sufficient evidence to justify the belief that the correction of electrolyte deficiencies should come first in the management of cases of ileus, for until these are made good, very little assistance can be expected from the employment of stimulating drugs.

REFERENCES

1. Abbott, W. O. and Pendergrass, E. P.: Intubation studies of the human small intestine. V. The motor effects of single clinical doses of morphine sulphate in normal subjects. Am. J. Roentgenol., **35**: 289–299, 1936.
2. Adler, H. F., Atkinson, A. J. and Ivy, A. C.: Effect of morphine and dilaudid on the ileum and of morphine, dilaudid and atropine on the colon of man. Arch. Int. Med., **69**: 974–985, 1942.
3. Adrian, E. D.: The spread of activity in the tenuissimus muscle of the cat and in other complex muscles. J. Physiol., **60**: 301–315, 1925.
4. Alvarez, W. C. and Mahoney, L. J.: The myogenic nature of rhythmic contractions of the intestine. Am. J. Physiol., **59**: 421–430, 1922.
5. Ambache, N.: Interaction of drugs and the effect of cooling on the isolated mammalian intestine. J. Physiol., **104**: 266–287, 1946.
6. Ambache, N.: The peripheral action of Cl. botulinum toxin. J. Physiol., **108**: 127–141, 1949.
7. Ambache, N.: The nicotinic action of substances supposed to be purely smooth-muscle stimulating. J. Physiol., **110**: 145–163, 164–172, 1949.
8. Ambache, N.: Unmasking, after cholinergic paralysis by Botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexus. Brit. J. Pharmacol., **6**: 51–67, 1951.
9. Ambache, N. and Rocha e Silva, M.: Analysis of certain interactions of nicotine with bradykinin and histamine. Brit. J. Pharmacol., **6**: 68–74, 1951.
10. Ambache, N. and Edwards, J.: Reversal of nicotine action on the intestine by atropine. Brit. J. Pharmacol., **6**: 311–317, 1951.
11. Ardran, G. M.: Personal communication, 1954.
12. Aristophanes: The frogs. Athens, B.C. 405.
13. Baur, M.: Die Peristaltik bei dauernder Füllung. Arch. exper. Path. u. Pharmakol., **100**: 95–111, 1923.
14. Bayliss, W. M. and Starling, E. H.: The movements and innervation of the small intestine. J. Physiol., **24**: 99–143, 1899.
15. Bayliss, W. M. and Starling, E. H.: The movements and innervation of the large intestine. J. Physiol., **26**: 107–118, 1901.
16. Bayliss, W. M. and Starling, E. H.: The movements and innervation of the small intestine. J. Physiol., **26**: 125–138, 1901.
17. Bell, W. B.: The pituitary body and the therapeutic value of the infundibular extract in shock, uterine atony, and intestinal paresis. Brit. M. J., **2**: 1609–1613, 1909.
18. Bernheim, F. and Bernheim, M. L. C.: Action of drugs on the cholinesterase of the brain. J. Pharmacol. & Exper. Therap., **57**: 427–436, 1936.
19. Bernheim, F. and Blocksom, B. H.: Action of epinephrine on the intestine following stimulation by parasympathetic drugs. Am. J. Physiol., **100**: 313–316, 1932.
20. Bodian, M., Stephens, F. D. and Ward, B. C. H.: Hirschsprung's disease and idiopathic megacolon. Lancet, **1**: 6–11, 1949.
21. Borchardt, W.: Gibt es nervöse Chemorezeptoren in der Dünndarmschleimhaut? Zugleich ein Beitrag über die Einwirkung der Psyche auf Tonus und Bewegung des Dünndarms (nach Versuchen an Hunden mit Vella-Fisteln). Arch. ges. Physiol., **215**: 402–426, 1927.
22. Bourdillon, R. B.: Vaginal potentials in rabbits. J. Physiol., **97**: 138–152, 1939.
23. Bourdillon, R. B. and Lowell, O. M.: Slow vaginal potentials. J. Physiol., **98**: 480–486, 1940.
24. Bozler, E.: Electric stimulation and conduction of excitation in smooth muscle. Am. J. Physiol. **122**: 614–623, 1938.
25. Bozler, E.: An analysis of the excitatory and inhibitory effects of sympathetic nerve impulses and adrenaline on visceral smooth muscle. Am. J. Physiol., **130**: 627–634, 1940.

26. Bozler, E.: The action potentials accompanying conducted responses in visceral smooth muscles. Am. J. Physiol., **136**: 553–560, 1942.
27. Bozler, E.: The action potentials of the stomach. Am. J. Physiol., **144**: 693–700, 1945.
28. Bozler, E.: The relation of the action potentials to mechanical activity in intestinal muscles. Am. J. Physiol., **146**: 496–501, 1946.
29. Bozler, E.: Conduction, automaticity and tonus of visceral muscles. Experientia, **4**: 213, 1948.
30. Bozler, E.: Myenteric reflex. Am. J. Physiol., **157**: 329–337, 1949.
31. Bozler, E.: Reflex peristalsis of the intestine. Am. J. Physiol., **157**: 338–342, 1949.
32. Brindley, C. O.: Demerol and cholinesterase. Fed. Proc., **3**: 5, 1944.
33. Brown, G. L.: The actions of acetylcholine on denervated mammalian and frog's muscle. J. Physiol., **89**: 438–461, 1937.
34. Brown, G. L. and Gray, J. A. B.: Some effects of nicotine-like substances and their relation to sensory nerve endings. J. Physiol., **107**: 306–317, 1948.
35. Buchthal, F. and Lindhard, J.: The physiology of striated muscle fiber. Ejnar Munksgaard, Copenhagen, 1939.
36. Bülbring, E.: Smooth muscle potentials recorded in the taenia coli of the guinea-pig. J. Physiol., **123**: 55P, 1954.
37. Burn, J. H.: A discussion on the action of local hormones. Proc. Roy. Soc. B, **137**: 281–320, 1950.
38. Cannon, W. B.: The movements of the intestines studied by means of the Röntgen rays. Am. J. Physiol., **6**: 201–277, 1902.
39. Cantoni, G. L. and Eastman, G.: On the response of the intestine to smooth muscle stimulants. J. Pharmacol. & Exper. Therap., **87**: 392–399, 1946.
40. Castillo, J. del and Stark, L.: The effect of calcium ions on the motor end-plate potentials. J. Physiol., **116**: 507–515, 1902.
41. Chapman, W. P., Rowlands, E. N., Taylor, A. and Jones, C. H.: Multiple-balloon-kymograph recording of variations in motility of the upper small intestine in man during long observation periods before and after placebo administration. Gastroenterology, **15**: 341–355, 1950.
42. Christensen, E. M. and Gross, E. G.: Analgesic effects in human subjects of morphine, meperidine and methadon. J. A. M. A., **137**: 594–598, 1948.
43. Cole, K. S. and Curtis, H. C.: Electric impedance of the squid giant axon during activity. J. Gen. Physiol., **22**: 649–670, 1938.
44. Craver, B. N., Barrett, W., Cameron, A., Earl, A. E. and Yonkman, P. Y.: Pharmacological properties of a new antispasmodic N, N-dimethyloxyacetamidine HCl, SU198. Amer. J. Digest. Dis., **18**: 241–245, 1951.
45. Crone, R. S.: Some radiological observations on the effect of morphine sulphate on the stomach after gastroenterostomy and partial gastrectomy. Gastroenterologia, 1954, (In press).
46. Dale, H. H.: The action of extracts of the pituitary body. Biochem. J., **4**: 427–447, 1909.
47. Dalgliesh, C. E., Toh, C. C. and Work, T. S.: Fractionation of the smooth muscle elements present in extracts of gastro-intestinal tract. J. Physiol., **120**: 298–310, 1953.
48. Dawes, G. S.: Synthetic substitutes for quinidine. Brit. J. Pharmacol., **1**: 90–112, 1946.
49. Dikshit, B. B.: Acetylcholine formation by tissues. Quart. J. Exper. Physiol., **28**: 243–251, 1938.
50. Doster-Virtue, M. E. and Virtue, R. W.: A method of preparing isolated intestinal loops in the dog. Proc. Soc. Exper. Biol. & Med., **45**: 813–816, 1940.
51. Doster-Virtue, M. E. and Virtue, R. W.: An improved closure for the Doster-Virtue intestinal loop. Proc. Soc. Exper. Biol. & Med., **48**: 326, 1941.
52. Douglas, D. M.: The decrease in frequency of contraction of the jejunum after transplantation to the ileum. J. Physiol., **110**: 66–75, 1949.
53. Douglas, W. W., Feldberg, W., Paton, W. D. M. and Schachter, M.: Distribution of histamine and substance P in the wall of the dog's digestive tract. J. Physiol., **115**: 163–176, 1951.
54. Downman, C. C. B.: Distribution along the small intestine of afferent, vasoconstrictor and inhibitory fibres in the mesenteric nerve bundles. J. Physiol., **116**: 228–235, 1952.
55. Eadie, G. S.: The inhibition of cholinesterase by morphine in vitro. J. Biol. Chem., **138**: 597–602, 1941.
56. Eadie, G. S., Bernheim, F. and Fitzgerald, D. B.: The inhibition of the cholinesterase of rat brain by methadon. J. Pharmacol. & Exper. Therap., **94**: 19–21, 1948.
57. Eccles, J. C. and Magladery, J. W.: The excitation and response of smooth muscle. J. Physiol., **90**: 31–67, 1937.
58. Eccles, J. C. and Magladery, J. W.: Rhythmic responses of smooth muscle. J. Physiol., **90**: 68–99, 1937.
59. Ehrenpreis, T. and Pernow, B.: On the occurrence of substance P in the recto-sigmoid in Hirschsprung's disease. Acta physiol. scandinav., **27**: 380–388, 1953.
60. Ellis, E. and Rasmussen, R.: The atropine-fast nicotine stimulation of the rabbit's intestine and of the muscularis mucosae of the dog's intestine. J. Pharmacol. & Exper. Therap., **103**: 259–268, 1951.
61. Emmelin, N. and Feldberg, W.: The smooth muscle contracting effects of various substances supposed to act on nervous structures in the intestinal wall. J. Physiol., **106**: 482–502, 1947.
62. Engelmann, T. W.: Beiträge zur allgemeinen Muskel- und Nervenphysiologie. Arch. ges. Physiol., **3**: 246–326, 1870.
63. Erspamer, V. and Asero, B.: Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature, London, **169**: 800, 1952.
64. Erspamer, V. and Boretti, G.: Identification and characterization by paper chromatography of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of octopoda and in other tissue extracts of vertebrates and invertebrates. Arch. internat. Pharmacodyn., **88**: 296–332, 1951.

65. Esveld, L. W. van: Verhalten von plexushaltigen und plexusfreien Darmmuskelpräparaten. Arch. exper. Path. u. Pharmakol., **134**: 347–386, 1928.
66. Euler, U. S. von and Gaddum, J. H.: An unidentified depressor substance in certain tissue extracts. J. Physiol., **72**: 74–87, 1931.
67. Evans, D. H. L. and Schild, H. O.: The reactions of plexus-free circular muscle of cat jejunum to drugs. J. Physiol., **119**: 376–399, 1953.
68. Evans, D. H. L. and Schild, H. O.: Reactions of nerve-free and chronically denervated plain muscle to drugs. J. Physiol., **122**: 63P, 1953.
69. Fatt, P. and Katz, B.: An analysis of the end-plate potential recorded with an intracellular electrode, J. Physiol., **115**: 320–370, 1951.
70. Fatt, P. and Katz, B.: Spontaneous subthreshold activity at motor nerve endings. J. Physiol., **117**: 109–128, 1952.
71. Fatt, P. and Katz, B.: Statistical aspects of transmission at a single nerve-muscle junction. J. Physiol., **120**: 32P, 1953.
72. Feldberg, W.: A discussion on the action of local hormones. Proc. Roy. Soc. B, **137**: 285–292, 1950.
73. Feldberg, W.: Effects of ganglion-blocking substances on the small intestine. J. Physiol., **113**: 483–505, 1951.
74. Feldberg, W. and Lin, R. C. Y.: The effect of cocaine on the acetylcholine output of the intestinal wall. J. Physiol., **109**: 475–487, 1949.
75. Feldberg, W. and Lin, R. C. Y.: The action of local anaesthetics and d-tubocurarine on the isolated intestine of the rabbit and guinea-pig. Brit. J. Pharmacol., **4**: 33–44, 1949.
76. Feldberg, W. and Lin, R. C. Y.: Synthesis of acetylcholine in the wall of the digestive tract. J. Physiol., **111**: 96–118, 1951.
77. Feldberg, W. and Toh, C. C.: Distribution of 5-hydroxytryptamine (serotonin, enteramine) in the wall of the digestive tract. J. Physiol., **119**: 352–362, 1953.
78. Fodera, F. A. and Pittau, L.: Etudes sur l'"hypophysis cerebri". Arch. ital. Biol., **52**: 370–374, 1909.
79. Forster, A. C.: Effects of morphine on the motility of the human ileum. Proc. Soc. Exper. Biol. & Med., **38**: 332–333, 1938.
80. Forster, A. C.: The production of hyper- and hypomotility of the musculature of the small bowel in the human. Ann. Surg., **112**: 370–377, 1940.
81. Gaddum, J. H.: Some properties of the separated active principles of the pituitary (posterior lobe). J. Physiol., **65**: 434–440, 1928.
82. Gaddum, J. H.: Tryptamine receptors. J. Physiol., **119**: 363–368, 1953.
83. Gaddum, J. H. and Schild, H. O.: Depressor substances in extracts of intestine. J. Physiol., **83**: 1–14, 1934.
84. Gardner, L. I., MacLachlan, E. A., Terry, M. L., McArthur, J. W. and Butler, A. M.: Chloride diarrhea and systemic alkalosis in potassium deficiency. Fed. Proc., **8**: 201, 1949.
85. Gasser, H. S.: Plexus-free preparations of the small intestine. A study of their rhythmicity and their response to drugs. J. Pharmacol. & Exper. Therap., **27**: 395–410, 1926.
86. Gasser, H. S. and Erlanger, J.: The role played by the sizes of the constituent fibres of a nerve trunk in determining the form of its action potential wave. Am. J. Physiol., **80**: 522–547, 1927.
87. Geiling, E. M. K.: The pituitary body. Physiol. Rev., **6**: 62–123, 1926.
88. Gottlieb, R.: Wirkung des Morphins auf den Darm. Deutsche med. Wchnschr., **36**: 1549, 1910.
89. Gregory, R. A.: Some factors influencing the passage of fluid through intestinal loops in dogs. J. Physiol., **111**: 119–137, 1950.
90. Gruber, C. M., Hart, E. R. and Gruber, C. M., Jr.: The pharmacology and toxicology of the ethyl ester of 1-methyl-4-phenylpiperidine-4-carboxylic acid (demerol). J. Pharmacol. & Exper. Therap., **73**: 319–334, 1941.
91. Gruber, C. M. and Robinson, P. I.: The influence of pituitary extract, "vasopressin" and "oxytocin", upon the intact intestine in anaesthetised dogs. J. Pharmacol. & Exper. Therap., **36**: 203–225, 1929.
92. Gunn, J. A. and Underhill, S. W. F.: Experiments on the surviving mammalian intestine. Quart. J. Exper. Physiol., **8**: 275–296, 1914.
93. Hodgkin, A. L.: The ionic basis of electrical activity in nerve and muscle. Biol. Rev., **26**: 339–409, 1951.
94. Kanan, M. A.: Action of morphine sulphate on intestinal motility and its modification by atropine sulphate. Proc. Soc. Exper. Biol. & Med., **36**: 506–508, 1937.
95. King, C. E. and Robinson, M. H.: The nervous mechanisms of the muscularis mucosae. Am. J. Physiol., **143**: 325–335, 1945.
96. Kottegoda, S. R.: Personal communication, 1954.
97. Krueger, H., Eddy, N. B. and Sumwalt, M.: The pharmacology of the opium alkaloids. U. S. Public Health Rep., Suppl. 165, 1941.
98. Kreuger, H., Howes, H. and Gay, H.: The effects of morphine and its derivatives on intestinal movements. IV. Dihydropseudocodeine and dihydroallopseudocodeine. J. Pharmacol. & Exper. Therap., **55**: 288–318, 1935.
99. Kuffler, S. W.: Specific excitability of the end-plate region in normal and denervated muscle. J. Neurophysiol., **6**: 99–110, 1943.
100. Kuffler, S. W. and Vaughan Williams, E. M.: Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate. J. Physiol., **121**: 289–317, 1953.
101. Kuffler, S. W. and Vaughan Williams, E. M.: Properties of the "slow" skeletal muscle fibres of the frog. J. Physiol., **121**: 318–340, 1953.
102. Larson, L. M. and Bargen, J. A.: Action of cathartics on isolated dog's colon. Arch. Surg., **27**: 1130–1145, 1926.

103. LEVY, M. AND NORA, G.: Occlusion intestinale paralytique post-operative tardive. Arch. mal. app. dig., **25:** 190–193, 1935.
104. MACDONALD, A. D.: The action of pituitary extracts on intestinal muscle. Quart. J. Exper. Physiol., **15:** 191–200, 1925.
105. MCLAREN, J. W., ARDRAN, G. M. AND SUTCLIFFE, J.: Radiographic studies of duodenum and jejunum in man. J. Fac. Radiologists, **2:** 148–164, 1950.
106. MAGNUS, R.: Die Beziehungen des Darmnervensystems zur automatischen Darmbewegung. Arch. ges. Physiol., **102:** 349–363, 1904.
107. MAGNUS, R.: Die stopfende Wirkung des Morphins. Arch. ges. Physiol., **122:** 210–250, 1908.
108. MUNRO, A. F.: The effect of adrenaline on the guinea pig ileum. J. Physiol., **112:** 84–94, 1951.
109. MUNRO, A. F.: Potentiation and reversal of the adrenaline motor response in the guinea pig ileum by autonomic drugs. J. Physiol., **118:** 171–181, 1952.
110. MUNRO, A. F.: Effect of autonomic drugs on the responses of isolated preparations from the guinea pig intestine to electrical stimulation. J. Physiol., **120:** 41–52, 1953.
111. MYERS, G. N. AND DAVIDSON, S. W.: An investigation of the effects of certain substitutes for morphine and heroin upon the passage of food along the alimentary tract of the human subject. J. Hyg., **38:** 432–445, 1938.
112. NASSE, O.: Zur Physiologie der Darmbewegung. Zentralbl. med. Wiss. **3:** 785–787, 1865.
113. NOTHNAGEL, H.: Über die Einwirkung des Morphins auf den Darm. Virchows Arch. path. Anat., **89:** 1–8, 1882.
114. ORR, T. G. AND CARLSON, H. E.: Effects of morphine on the movements of the small intestine and sphincter muscles. Arch. Surg., **27:** 295–305, 1926.
115. PATON, W. D. M. AND ZAIMIS, E. J.: Paralysis of autonomic ganglia by methonium salts. Brit. J. Pharmacol., **6:** 155–168, 1951.
116. PEARSON, O. H. AND ELIEL, L. P.: Postoperative alkalosis and potassium deficiency. J. Clin. Invest. **28:** 803, 1951.
117. PERNOW, B.: Substance P distribution in digestive tract. Acta physiol. scandinav., **24:** 97–102, 1951.
118. PERNOW, B.: Studies on substance P. Acta physiol. scandinav., **29:** Supp. 105, 1953.
119. PLANT, O. H.: The effect of carminative volatile oils on the muscular movements of the intestine. J. Pharmacol. & Exper. Therap., **16:** 311–325, 1921.
120. PLANT, O. H. AND MILLER, G. H.: Effect of morphine and some other opium alkaloids on the muscular activity of the alimentary canal. I. Action on the small intestine in unanaesthetized dogs and man. J. Pharmacol. & Exper. Therap., **27:** 361–383, 1926.
121. PORCHER, P.: L'hyperkinesie gastrique provoquée. Technique nouvelle de l'exploration complémentaire de l'estomac et du bulbe duodénal. J. radiol. et electrol., **27:** 393–401, 1946.
122. PORCHER, P.: Quelques reflexions après mille morphines (methode personelle d'exploration gastro-duodénale). Arch. mal. app. digest., **35:** 59–64, 1946.
123. PROSSER, C. L.: Conduction in non-striated muscle. Abstr. Comm. XIX Internat. Physiol. Congress, Montreal, 1953, p. 688.
124. PUESTOW, C. B.: Studies on the origin of the automaticity of the intestine: the action of certain drugs on isolated intestinal transplants. Am. J. Physiol., **106:** 682–688, 1934.
125. QUIGLEY, J. P. AND BARNES, B. O.: Action of insulin on the motility of the gastro-intestinal tract. VI. Antagonistic action of posterior pituitary lobe preparations. Am. J. Physiol., **95:** 7–12, 1930.
126. QUIGLEY, J. P., HIGHSTONE, N. H. AND IVY, A. C.: 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:** 308–320, 1934.
127. RAPPORT, M. M.: Serum vasoconstrictor (Serotonin). V. The presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. J. Biol. Chem., **180:** 961–969, 1949.
128. REID, G. AND RAND, M.: Physiological actions of partially purified serum vasoconstrictor (Serotonin). Australian J. Exper. Biol. & M. Sci., **29:** 401–415, 1951.
129. REID, G. AND VAUGHAN WILLIAMS, E. M.: The development of sensitivity to acetylcholine in denervated muscle. J. Physiol., **109:** 25–31, 1949.
130. REID, P. E.: Effect of hypertonic sodium chloride intravenously on intestinal peristalsis. Proc. Soc. Exper. Biol. & Med., **29:** 220–222, 1931.
131. ROCHA E SILVA, M.: Bradykinin—Mechanism of its release by trypsin and kallikrein. Arch. internat. pharmacodyn., **88:** 271–282, 1951.
132. ROCHA E SILVA, M., BERALDO, W. T. AND ROSENFELD, G.: Bradykynin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Am. J. Physiol., **156:** 261–273, 1949.
133. ROCHA E SILVA, M., VALLE, J. R. AND PICARELLI, Z. P.: A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. Brit. J. Pharmacol., **8:** 378–388, 1953.
134. ROSENBLUETH, A. AND CANNON, W. B.: The adequacy of the chemical theory of smooth muscle excitation. Am. J. Physiol., **116:** 414–429, 1936.
135. ROTH, G. B.: Pituitary Standardization. Hygienic Laboratory Bull., **109:** 29, 1917.
136. ROWLANDS, E. N., CHAPMAN, W. P., TAYLOR, A. AND JONES, C. H.: Multiple balloon kymograph recording of the comparative action of morphine and placebos on the motility of the upper small intestine in man. Surg., Gynec. & Obst., **91:** 129–137, 1950.
137. SATO, M.: Über den Mechanismus der Morphinverstopfung. Tohoku J. Exper. Med., **26:** 83–103, 1935.
138. SCHAPIRO, N.: Über die Wirkung von Morphium, Opium und Pantopon auf die Bewegungen des Magen-Darm Tractus des Menschen und des Tieres. Arch. ges. Physiol., **151:** 65–96, 1913.

139. SCHILD, H. O.: pA, a new scale for the measurement of drug antagonism. Brit. J. Pharmacol., **2**: 189–206, 1947.
140. SCHWENTER, J.: Über Verdauungsversuche mit Opium, Morphium, Pantopon und morphinfreiem Pantopon. Fortschr. Geb. Röntgenstrahlen, **19**: 1–7, 1912.
141. SCOTT, C. C., CHEN, K. K., KOHLSTEADT, K. G., ROBBINS, E. B. AND ISRAEL, F. W.: Further observation on the pharmacology of 'Dolophine' (methadon, Lilly). J. Pharmacol. & Exper. Therap. **91**: 147–156, 1947.
142. SEED, L., FALLS, F. H. AND FANTUS, B.: Pitressin (beta-hypophamine) in laparotomies. Surg., Gynec. & Obst., **64**: 895–905, 1937.
143. SJÖSTRAND, F. S.: Electron microscopy of mitochondria and cytoplasmic double membranes. Nature, London, **171**: 30–32, 1953.
144. SOLLMANN, T. AND RADEMAEKERS, A.: Magnesium sulphate on the peristalsis and the propulsion in small intestines. Arch. internat. Pharmacodyn., **31**: 39–69, 1926.
145. STEGGERDA, F. R. AND GIANTURCO, C.: A method for visualising different organs in the normal unanaesthetised animal. Anat. Rec., **67**: 405–406, 1937.
146. STREETEN, D. H. P.: The effects of sodium and chloride lack on intestinal motility and their significance in paralytic ileus. Surg., Gynec. & Obst., **91**: 421–434, 1950.
147. STREETEN, D. H. P. AND VAUGHAN WILLIAMS, E. M.: The influence of intraluminal pressure upon the transport of fluid through cannulated Thirty-Vella loops in dogs. J. Physiol., **112**: 1–21, 1951.
148. STREETEN, D. H. P. AND WARD-MCQUAID, J. N.: Relation of electrolyte changes and adrenocortical activity to paralytic ileus. Brit. M. J., **2**: 587–592, 1953.
149. TEMPLETON, R. D. AND ADLER, H. F.: The influence of morphine on transportation in the colon of the dog. Am. J. Physiol., **131**: 428–431, 1940.
150. THIRY, L.: Über eine neue Methode, den Dünndarm zu isolieren. Sitz. Ber. Akad. Wiss. Wien. **50**: 77–96, 1864.
151. TOH, C. C.: Experiments on the Barium contraction of the guinea-pig's ileum. J. Physiol. **114**: 33P, 1951.
152. TRENDELENBURG, P.: Hypophyse. Die Hormone, **1**: 98–184, 1929, (Springer, Berlin).
152a. TRENDELENBURG, U.: Personal communication, 1954.
153. TRIPOD, J.: Vergleisch der parasympathicolytischen Wirksamkeit von Atropin, Trasentin-H, Trasentin. Helvet. physiol. et pharmacol. acta, **7**: 135–148, 1949.
154. VAN LIERE, E. J.: The effect of anoxia on the alimentary tract. Physiol. Rev., **21**: 307, 1941.
155. VAN LIERE, E. J., STICKNEY, J. C., NORTHUP, D. W. AND BELL, R. O.: Effect of *dl*-amphetamine sulfate and its isomers on intestinal motility. J. Pharmacol. & Exper. Therap., **103**: 187–189, 1951.
156. VAUGHAN WILLIAMS, E. M.: The measurement of secretion and absorption by intestinal loops in conscious dogs. J. Physiol., **112**: 32–33P, 1951.
157. VAUGHAN WILLIAMS, E. M. AND STREETEN, D. H. P.: The action of morphine, pethidine and amidone upon the intestinal motility of conscious dogs. Brit. J. Pharmacol., **5**: 584–603, 1950.
158. VAUGHAN WILLIAMS, E. M. AND STREETEN, D. H. P.: The mode of action of morphine upon the intestine. Brit. J. Pharmacol., **6**: 263–272, 1951.
159. VAUGHAN WILLIAMS, E. M. AND STREETEN, D. H. P.: The action of posterior pituitary extracts upon propulsion in the small intestine of conscious dogs. Brit. J. Pharmacol., **7**: 47–57, 1952.
160. VAUGHAN WILLIAMS, E. M. AND STREETEN, D. H. P.: Loss of cellular potassium as a cause of intestinal paralysis in dogs. J. Physiol., **118**: 149–170, 1952.
161. VELLA, L.: Neues Verfahren zur Gewinnung reinen Darmsaftes und Feststellung seiner physiologischen Eigenschaften. Moleschott's Untersuchungen zur Naturlehre des Menschen und der Thiere, **13**: 40–74, 1888.
162. VENNING, P. AND JUNGMANN, H.: Radiological investigation of stomach changes following a loud auditory stimulus. Brit. J. Radiol., **25**: 202–208, 1952.
163. VOGT, W.: Über die stoffliche Grundlage der Darmbewegungen und des Vagusreizes am Darm. Arch. exper. Path. u. Pharmakol., **206**: 1–11, 1949.
164. VOGT, W.: Über die Beziehung des Darmstoffs zur Substanz P. Arch. exper. Path. u. Pharmakol., **210**: 31–35, 1950.
165. VELDEN, R. VON DEN: Zur Pharmakologie der Magenmotilitat. München. med. Wchnschr., **56**: 1667, 1909.
166. WADA, M., ARAI, T., TAKAGAKI, T. AND NAKAGAWA, T.: Axon reflex mechanism in sweat responses to nicotine, acetylcholine and sodium chloride. J. Appl. Physiol., **4**: 745, 1952.
167. WALDER, D. N.: The muscularis mucosa of the human stomach. J. Physiol., **120**: 365–372, 1953.
168. WEIDMANN, S. AND DRAPER, M. H.: Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol., **115**: 74–94, 1951.
169. WEISEL, W., YOUMANS, W. B. AND CASSELS, W. H.: Effect on intestinal motility of cyclopropane anaesthesia alone and after morphine-scopolamine premedication. J. Pharmacol. & Exper. Therap., **63**: 391–399, 1938.
170. WINTER, H. A., HOFF, H. E. AND DSO, L.: Effect of potassium deficiency upon gastro-intestinal motility. Fed. Proc., **8**: 169, 1949.