THE INNERVATION OF THE FROG'S STOMACH. By WALTER E. DIXON, M.A., M.D. (Thirteen Figures in Text.)

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Historical. In recent years plain muscle has been the subject of many communications, and a considerable variety of graphic methods have been evolved in illustrating its contractions. In the case of cold-blooded animals Morgen[1], Bowditch[2], Schultz[3] and Woodworth[4] used 'stomach rings' as their method of studying contraction: Barbèra[5], Glaessner[6] and others used the stomach as a whole. Bottazzi and Grünbaum[7] used the entire œsophagus of the toad, whilst Stiles[8] applied the ring method to this organ.

The literature of plain muscle may be conveniently divided into three groups, (*a*) that dealing with the spontaneous movements, (*b*) that dealing with direct stimulation of the muscle (mechanical, chemical, thermal or electrical), and lastly that dealing with the innervation. Although observations on the innervation of the stomach are very numerous, only those directly concerned with these experiments will be mentioned here.

Goltz[9], working with curarised frogs, found that a half per cent. solution of salt placed in the mouth was rapidly passed to the stomach, but that this was not the case if either the vagi were cut or the nervous system destroyed. He concluded from this that the vagi contained inhibitory fibres. Langley[10] has conclusively shown this to be the case with mammals.

Waters[11] noticed that strong stimulation of the third, fourth, fifth and sixth nerves in the frog at their exit from the spinal cord led to contraction of the œsophagus, stomach, and sometimes of the small intestine.

Steinach[12] described motor effects in the frog's viscera as a result of stimulating posterior roots: according to this writer the œsophagus is innervated from the second and third spinal nerves and the stomach from the third, fourth and fifth. Horton-Smith[13] failed to confirm Steinach's results and asserted that all efferent fibres pass to the viscera either by the vagus or the anterior roots, and in the case of the stomach and œsophagus the innervation is from the vagus alone. Steinach[14] in a later communication confirms his previous work. In the same communication he asserts that the medulla contains inhibitory and excitatory fibres for the œsophagus and stomach, the impulses being transmitted through the vagi.

Structure. The muscular coat of the frog's stomach according to Ranvier[15] consists entirely of circular fibres which are the active agent in producing contraction and not the muscularis mucosæ. Other observers, Morgen[1], Winkler[16], Schultz[3] and Grützner[17] have discussed at length the question of longitudinal fibres. Without entering into this discussion it can be readily demonstrated that such longitudinal fibres as may be present are of an insignificant character: if longitudinal strips of the stomach are prepared and arranged so as to pull on a horizontal lever, then stimulating such preparations with the faradic current or applying to them a stimulating solution, such as 1 °/₀ digitalin, produces practically no result. Grützner has conclusively shown however that a fine band of longitudinal fibres is usually to be found in the inner curvature, whilst a few fibres are also demonstrable at the pyloric and cardiac ends, prolonged apparently from the duodenum and œsophagus respectively. In the following experiments it may be assumed therefore that circular fibres alone are being dealt with: it should be noted however that these tend to form a meshwork with one another, the angles always becoming greater towards the pylorus, so that the 'ring preparations' do not strictly represent parallel fibres.

Method. The principle adopted for recording the movements, has been to fill the stomach with water or normal saline solution at a small pressure, and to register the alterations in its internal capacity. The same principle has been previously used by Barbèra[5] and Glaessner[6]. In all experiments the brain and cord were completely destroyed, and two or three times the cord was removed, so that the question of reflex effect need not be considered. The animals were curarised when necessary by injection into the dorsal lymph-sac. If atropine were employed, 0·01 grm. of the sulphate was injected subcutaneously one or two hours before the experiment, and the animal was considered under the influence of the drug when the skin was quite dry. The frogs were of medium size and freshly caught in summer and autumn; the summer animals gave on the whole the most satisfactory results; spring animals, which Schultz says are quite unsuitable for experiments on plain muscle, were not tried. The use of starved animals as recommended by Glaessner and others gave distinctly inferior results to normal animals, and frogs kept in captivity for two months I found to be quite useless.

The cœlom was well opened in the medium line, the sternum removed and a ligature passed round the pylorus; this was generally tied at once, but in the earlier experiments a cannula was inserted into the stomach from the duodenum and made tight. A free incision was now made into the œsophagus; the stomach was grasped gently and its contents forced out through the œsophageal opening; in this manipulation absorbent wool, moistened with Ringer's solution, was the medium by which the pressure was applied. This operation does no harm to the stomach, and frequently the best results were obtained with those stomachs which were found filled with food. A short

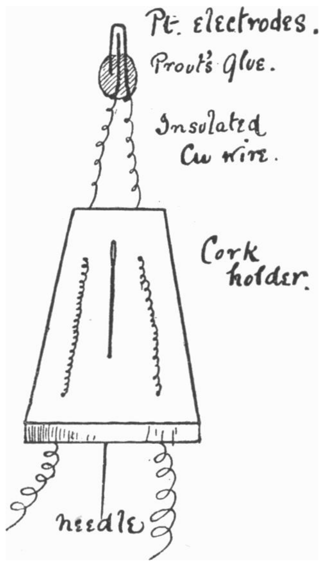

Fig. 1. Represents the form of electrodes commonly used.

straight cannula with a long neck was now inserted through the œsophageal opening and tied in the stomach. Sometimes the cannula was inserted from the mouth, but this method was not generally employed. The cannula was connected by rubber tubing to the lower part of an upright glass tube, which was capable of being readily raised or lowered, the upper part of which was connected to a **T**-piece and so to a recording tambour. The glass tube was about half-filled with salt solution, and hence the stomach movements were communicated through the water manometer to the tambour. The water pressure giving the best results varies considerably in different animals. The pressure generally employed varied between 10 and 20 cms.: it was frequently necessary however to begin with rather a high pressure, which was reduced later if necessary.

Care should be taken that no air gets into the stomach and that it is completely emptied before the cannula is tied in, otherwise it is very apt to block. If the cannula is tied too high the cardiac orifice may become tightly constricted, preventing the inflow of fluid, and if too low, so that the ligature grips the stomach fibres, the results are impaired.

Drying was prevented by allowing Ringer's solution to drop regularly and slowly on the surface. The experiments were performed in a room the temperature of which varied between 15° and 17° C.

Spontaneous movements.

In the large majority of experiments well-defined spontaneous movements are present, and in those cases where the stomach is quiescent these may readily be brought out by stimulating the fourth ramus, the vagus, applying any stimulating solution to the stomach, or sometimes even by suddenly altering the pressure of the fluid in the manometer. These rhythmical movements have been described in practically all forms of involuntary muscle since Engelmann[(18)] in 1869 drew attention to them in the rabbit's ureter. In the frog's stomach, recording as already described, the registered movements differ in no essential respect from descriptions published by other observers, except perhaps they exhibit a greater regularity than is found with the 'ring preparations.'

A single 'wave' shows a phase of contraction which commences slowly but rapidly accelerates, a short period of complete contraction, followed by a more lengthy phase of relaxation, which begins sharply and gradually tails off. The phase of relaxation is always longer than that of contraction. These waves are very slow, generally less than one per minute, and are usually produced by the contraction and relaxation of localised rings of the stomach muscle. The rings generally show little tendency to spread, but when relaxation of one ring is complete a fresh contraction ring in another part of the stomach appears, and thus the recorded waves are the result of a series of disconnected ring contractions. Rarely the contraction rings move in a peristaltic-like manner, but when this does occur the direction is usually from the pyloric to the cardiac end of the stomach. Sometimes the stomach, especially when it is well distended, contracts and expands as a whole, in which case the movements are hardly perceptible to the naked eye, but are well shown up graphically.

The origin of automatic movements in plain muscle has been a subject of considerable debate. Engelmann, Sertoli, Bottazzi, and Straub have ascribed them as purely muscular, whilst Ranvier, Morgen, Schultz, and Barbèra have contended that they originate in nerve cells. An attempt to solve the question was made in a number of experiments in which the stomachs were treated with cocaine or nicotine. Cocaine as a $\frac{1}{2}$ % solution in normal saline was first employed in the manometer tube instead of simple saline solution, but in the majority of cases this failed to completely paralyse the nervous mechanism so that a solution of 0·05 % or 0·1 % was also applied externally; by this means complete paralysis of the nerve elements could be obtained as shown by the fact that excitation of the fourth ramus or vagus nerves gave no response, and the direct application of 1 % pilocarpine failed to elicit any contraction. Such a stomach still shows spontaneous movements, but these differ somewhat from the normal ones in that they are not so well pronounced and have no regular sequence (Fig. 2)[1]. Nicotine solutions used in the manometer

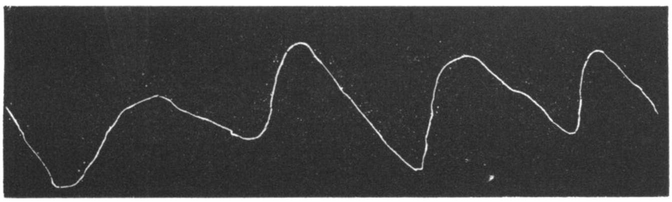

Fig. 2. Shows 'wave production' in a stomach the nerve elements of which have been paralysed with cocaine. Time 1 cm. = $\frac{1}{2}$ minute.

tube also fail to paralyse the nervous mechanism, but the outward application of a 0·3 % solution of nicotine produces in a short time complete paralysis. The automatic movements obtained by this treatment with nicotine resemble those of the cocaine stomach.

[1] All tracings should be read from left to right.

That the muscle itself is uninjured by this treatment is evident from the fact that 0·1 % solution of $BaCl_2$ still gives the typical contraction. These automatic movements are therefore probably myogenic in origin.

That waves are however largely influenced by the nervous system is easily demonstrable. If they are completely absent a weak stimulation of the fourth ramus produces a regular sequence such as is shown in Fig. 3, and if they are ill-defined and irregular they are augmented

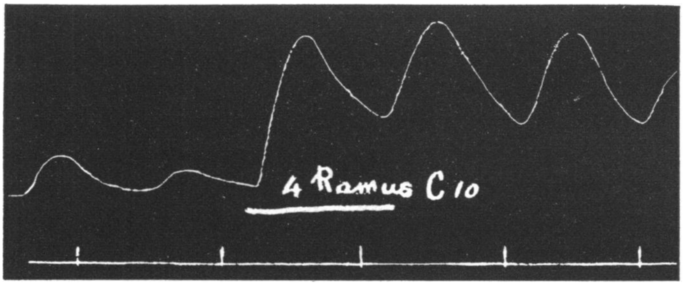

Fig. 3. Wave production neurogenic in origin. Time = minutes.

and made more regular. The progress of a regular sequence of [illegible] produced either in this manner or by the direct application of a little dilute pilocarpine solution to the stomach, can be destroyed by painting with a 0·1 % solution of cocaine. From this it would appear that although spontaneous movements may occur in the absence of a local nerve mechanism, any regular sequence of movements is probably the direct outcome of nerve activity. In experiments where cocaine is employed it should be remembered that this drug is a general protoplasmic poison with some selective action for nerve tissue, and if too strong a solution is employed the muscle fibres will be affected. Thus 1 % cocaine solution applied directly to the stomach is generally sufficient to eliminate all movements, and in a short time will even destroy the muscle fibres, as shown by the inability of barium to produce a contraction.

EFFECT OF STIMULATING THE SYMPATHETIC.

Curari and atropine were occasionally employed but neither drug in any way modified the effects of stimulation. Battelli[19] however has stated that atropine paralyses these fibres in mammalia, but his results when stimulating the splanchnic were not sufficiently constant to warrant this conclusion, which is in opposition to that of other observers.

To prevent current escape electrodes were employed on the principle of those of Werigo and fashioned out of platinum wire and sealing-wax. These will be sufficiently explained by a reference to Fig. 1. An ordinary Du Bois Reymond's coil was employed, worked by a single dry cell: with the electrodes on the tongue the secondary current could just be felt when the coil was at 15 cms. Mechanical and chemical stimulation of nerves failed to give good results.

The sympathetic was well isolated between the first spinal nerve and the trunk of the vagus, and stimulated in this position, but such excitation produced practically no effect on the stomach: in two cases only a very slight increase of tonus occurred, of such an insignificant nature as to be within the limits of experimental error: it may therefore be assumed that no fibres from the sympathetic reach the stomach through the vago-sympathetic.

The rami communicantes of the spinal nerves were then stimulated, from above downwards. To reach these the viscera were gently moved over to the animal's left side, the liver, ovary, and oviducts being carefully removed. The peritoneum on the posterior body wall was then incised and cut away and the rami placed on the electrodes near the spinal column. Moderate faradic stimulation of the third, fourth and fifth gave rise to an increase of tonus and the production of well-defined waves (Fig. 4). The fourth ramus always produced the maximum effect. There is a latent period of about eight seconds, followed by a contraction beginning slowly but rapidly accelerating, which may either reach its maximum at once, or after one or more small relaxations, the height of contraction being roughly proportional to the intensity of the stimulation. An excitation lasting for about 30 seconds produces an increase of tonus which continues above the normal usually for 5 or 6 minutes. Relaxation (tonic) is very gradual and is accompanied by well-defined contraction rings: exaggeration of these rings is an almost invariable adjunct of alteration in tonus (Fig. 2). The results of stimulation of the third and fifth rami vary a good deal in different animals, and although moderate stimulation nearly always gives some effect (wave production), it sometimes gives no increased tonus. Stimulation of the fifth rami generally produces a greater motor effect than stimulation of the third. The effect of excitation of the fourth ramus is wonderfully constant, and in 30 or 40 experiments with freshly caught animals I have had no negative results.

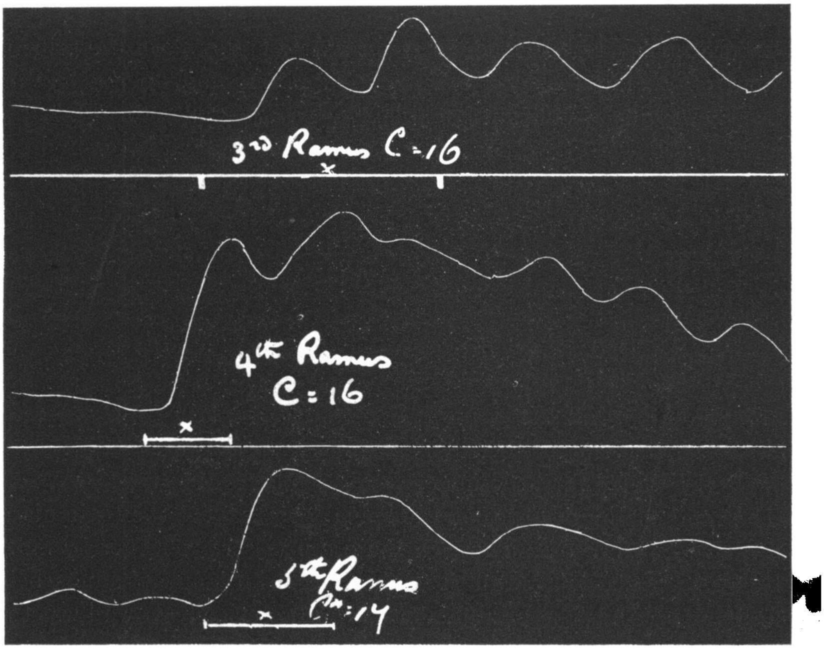

Fig. 4. Reduced by one-third. Stimulation applied during the period marked by the crosses. Time 1 cm. = ¼ minute.

The main factor to notice in these experiments is the increase of tonus, superadded to which are the lesser contractions and relaxations giving rise to the waves. It must be remembered however that the graphic representation of these waves depends upon the amount of fluid which can be forced from the stomach by any one contraction, and hence they are more likely to be shown up when the tonic contraction is small, rather than large, in which case the internal capacity of the stomach is almost obliterated, for example, they are better shown in Fig. 4 after stimulating the third than the fourth ramus. The strength of current necessary to produce these effects varies considerably in different frogs. Sometimes a faradic current with the secondary coil at 15 will give a very marked effect, as in Fig. 4, whilst at other times a more prolonged stimulation with the coil at 10 or 12 cm. may be necessary to get the same effect (Fig. 2). In the earlier experiments the rami on both sides were stimulated, and as they invariably produced similar effects stimulation was confined in the later experiments usually to the right rami on account of their easier dissection.

Fatigue sets in early in these preparations when stimulating the sympathetic, and subsequent excitation applied to the fourth ramus of a nerve-stomach preparation already fatigued produces after the usual latent period a very short contraction, and the normal tonus is rapidly reached again whilst the stimulus still continues (Fig. 5). Further

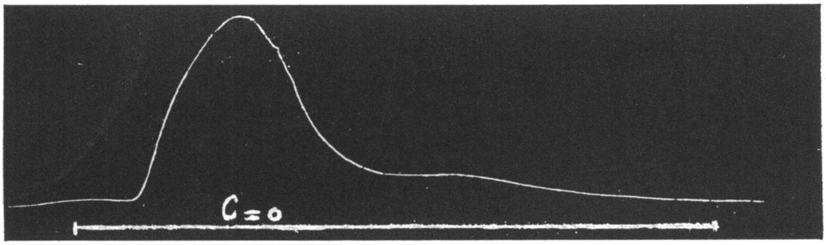

Fig. 5. Fatigue effect. Shows the effect of a long stimulation of a fatigued preparation. Time 1 cm. = ½ minute.

stimulations after an interval produce progressively diminishing effects. The stomach fibres still respond normally to direct excitation.

EFFECT OF STIMULATING THE VAGUS.

The graphic effects produced on stimulating the vagus nerve do not exhibit quite the same constancy which obtains on stimulating the sympathetic, but this lack of constancy is apparent rather than real and is the result of slight alterations in the conditions. In experiments with the vagus the heart was usually excised. Atropine, administered in the fashion already described, does not paralyse the peripheral ends of the vagus in the stomach. Curari was employed in about half the experiments.

The electrodes were placed in three positions, on (*a*) the common vago-sympathetic, (*b*) the intracranial roots, (*c*) that portion of the medulla containing the vagus nucleus; in the latter case the cerebral hemispheres were destroyed by pliers, leaving the medulla uninjured. Stimulation in all these positions gave similar results, and as it has been shown already that the sympathetic nerve in the neck contains no fibres for the muscular walls of the stomach, most of the following observations were obtained by stimulating the vago-sympathetic nerve.

When analysing results it is even more important in vagal than in sympathetic excitation to take into consideration the previous condition of the stomach. The normal tonicity, which is a very variable factor in different frogs, the manometer pressure, the state of auto-contraction are all conditions which may considerably modify the results of vagal excitation. When the tonus is moderately high, so that the internal volume of the stomach is comparatively small, the usual effect of stimulation is to produce a diminution in tonus (Fig. 6). This does

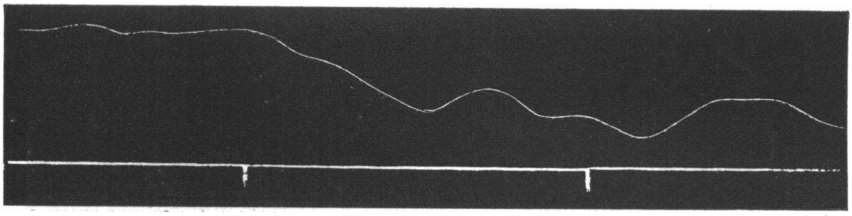

Fig. 6. Shows typical effect of stimulating the left vagus. Coil at 10 cms. Time 1 cm. = ¼ minute.

not begin until after a latent period considerably longer than that of sympathetic stimulation, and is commonly half a minute; as the tonus diminishes, the usual course of events is an initial short period without waves, followed by a period of much augmented waves, so that a stomach which before stimulation shows very slight or no automatic movements,

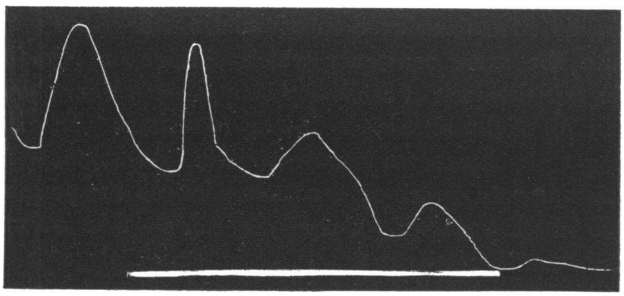

Fig. 7. Effect of stimulating the right vagus when the wave production is well developed at the start. One normal wave only is shown. Coil at 12 cms. Time 1 cm. = ½ minute.

afterwards is in a condition of well-marked ring contraction, although the tonus is less. This effect can often be well shown in those cases where the tonus is initially low by first producing an artificial increase by stimulating the fourth ramus; when the tonus is constant, stimulation of the vagus will produce the effect just described, except that the increased 'nervous waves' as the result of sympathetic excitation are commonly diminished in amplitude (Fig. 7).

When the tonus is feeble, however, *i.e.* where the stomach is considerably distended with a small manometer pressure, an apparently different result may occur on stimulation; after a lengthy latent period relaxation begins as before, soon followed by a marked ring contraction which rapidly relaxes till the normal tonus is reached again. This contraction, which is sometimes extremely well marked as shown in Fig. 8, is never tonic but marks the first of a series of well-defined waves.

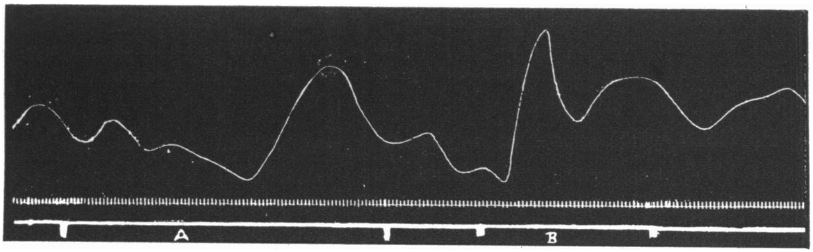

Fig. 8. Reduced by one-third. Stomach recording regular contractions, one only being shown. During the period marked *A* the left vagus was stimulated with the secondary coil at 5 cms. During the period *B* the splanchnic was stimulated with the same strength of current. Time = seconds.

In this case there is practically no alteration in the registered tonus. In Fig. 8 another point of some importance is brought out. The large contraction on vagal stimulation there shown was the result of a ring contraction which showed a tendency to spread from the pyloric to the cardiac end of the stomach, and the curve is therefore represented as a gradual rise and fall. With the sympathetic stimulation, however, which is also shown in the same figure, there was a rapid tonic contraction of the stomach which registered itself as a steep curve, and the increase of tonus was permanent. Lastly, this augmented wave production has occurred not infrequently without any primary fall of tonus as registered by the graphic method, but in these cases the initial tonus was always low. Exaggerated waves of this description are shown in Fig. 9, in which the stomach was much distended with a low manometer pressure and practically motionless before the stimulation.

As in the case of the heart, strong stimulation of one vagus may give negative results whilst moderate stimulation of the other may be sufficient to produce a decided effect. The left vagus usually produces a greater effect than the right. In a few cases stimulation gave negative results, but in no case was an increase of tonus observed.

There can be no doubt from these results that the vagi contain tonic inhibitory fibres to the stomach muscle.

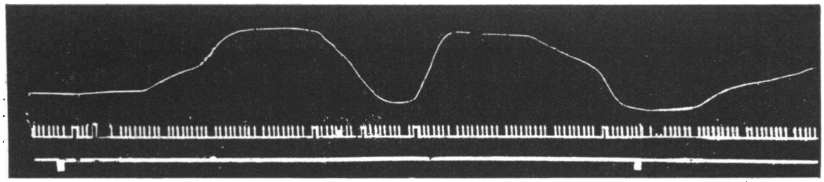

Fig. 9. Reduced by one-third. Stomach with very little tonus, that is considerably dilated, and exhibiting no movements before excitation. Shows effect of stimulating left vagus. Coil at 10 cms. Manometer pressure = 11 cms. Time = seconds.

Previous writers are in agreement that stimulation of the vagus results in "peristaltic movements," and so far my observations agree with these, but as their results were observed entirely with the naked eye, without the assistance of a graphic method, the simultaneous diminution of tonus was not properly appreciated. If the vagus contains motor fibres it is quite obvious that they are of a kind altogether different from those contained in the sympathetic, in that they never increase tonus.

Similar augmentation of intensity of the contraction rings which obtain on vagal stimulation may be induced either by stimulating the sympathetic electrically, by the application to the stomach of certain drugs which act on nerve tissue, or by merely painting the sympathetic ganglia with nicotine. It appears therefore that any alteration in the local nerve mechanism in the stomach produces alteration in the automatic contractions. It may also be noted that suddenly increasing or diminishing the manometer pressure, thereby giving rise to an increased or diminished internal stomach capacity respectively, often induces augmented movements, but it is probable that these are myogenic, in that I have seen them in the cocainised stomach.

The vagus therefore must be regarded as a direct inhibitory nerve to muscle fibre; its excitation electrically also gives rise to an increase of force of the normal contraction rings or when these are absent it may call them into existence.

THE ACTION OF NICOTINE ON THE SYMPATHETIC GANGLIA.

Langley[20] has shown recently that the application of nicotine to the sympathetic ganglia in the cat produces initial stimulation of the nerve-cells; the evidence as to the point of action in the subsequent paralysis being incomplete. In order to see if such application produced any effect in the frog, the fourth sympathetic and cœliac ganglia, the fourth rami, and the splanchnic nerve were isolated in a well-pithed animal. The administration of curari did not in any way modify the results.

An initial stimulation of the fourth ramus and the splanchnic was recorded, and each being found to produce on stimulation tonic contraction of the involuntary muscle of the stomach, the cœliac ganglion was carefully painted with a 0·1 % solution nicotine in normal saline (Fig. 10). It is convenient for this purpose to arrange the stomach as far as possible on the animal's left, cutting through any unimportant structures which aid in the arrangement. It is difficult however to avoid some nicotine reaching the fourth ganglion and some nerve fibres. Such an application commonly results in an immediate partial inhibition of automatic movements for about two minutes, followed by one or more greatly augmented ring contractions; these quickly pass off and the normal condition of affairs is reached in a few minutes: less commonly there is an increase of tonus lasting 2 or 3 minutes. Subsequent stimulation of the fourth ramus produces either a slightly diminished or a normal contraction, and paralysis is never seen. If the same ganglia are painted with a 1 % solution of nicotine the same stimulating effect is seen as before except that after a short time stimulation of the fourth rami produces no effect whilst excitation of the post-ganglionic fibres still give rise to contraction. This effect I was at first inclined to regard as evidence of paralysis of the ganglia, but Professor Langley suggested that the result was more probably due to a direct action on nerve fibre in the absence of an active blood supply, and the fact that a considerable time is required, sometimes 10 minutes, before the paralysis is complete, and that more dilute solutions produce only the stimulation stage, seem to suggest that this is the true explanation. By the injection of nicotine directly into the circulation I have been unable to produce complete paralysis.

The effect of immersing the stomach in dilute solutions of nicotine has been already discussed; 0·1 % solution will not produce a block to sympathetic excitation in 10 minutes but 0·3 or 0·4 % soon leads to complete paralysis, as shown by the negative results obtained on stimulating either the fourth rami or fibres past the cœliac ganglion.

ACTION OF DRUGS.

The action of drugs on plain muscle and its automatic contractions has been already described by many observers so that reference to very few will be made here. The method usually adopted by investigators has been the simple one of dropping solutions of the drug dissolved in Ringer's fluid directly on the preparation. Glaessner has however compared the effect of various drugs on the frog's stomach when applied externally and when placed in the interior of the stomach, and has obtained results varying according to the method of application.

In these experiments two methods have been principally adopted; first injecting the drug directly into the circulation, and secondly by immersing the whole preparation in a bath of fluid which can be gradually changed to the fluid containing the drug. In the former method a small burette without a stopcock was fitted by fine india-rubber tubing to a special cannula with a very small nozzle. The apparatus is filled with the fluid to be injected, which is kept in the

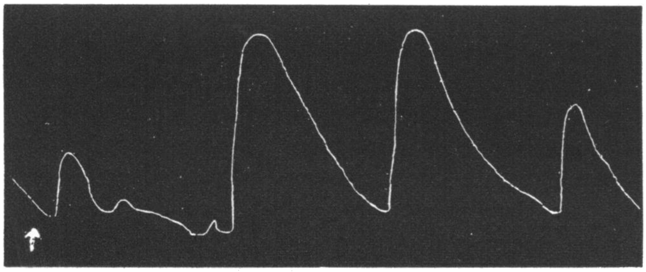

Fig. 10. Shows the effect of painting the cœliac ganglion with 0·1 % nicotine solution at the point marked by the arrow. Before painting the automatic contractions were represented by waves similar to that shown on the left of the figure. There was no subsequent paralysis. Time 1 cm. = 1 min.

cannula by bull-dogs placed on the rubber tubing. One of the hepatic veins is next dissected out and the cannula tied in. It is evident that on removing the bull-dogs some of the fluid in the burette will find its way into the heart and thus by the general circulation to the stomach. By this method it is possible to simultaneously observe the contractions of the heart and the movements of the stomach.

It is necessary that all drugs in solution should be very dilute when applied externally to the stomach, otherwise "salt-action" may complicate the results. For example if a 3 % solution of NaCl or KBr, is applied to the stomach externally, marked tonic contraction is produced, but the effect is different if the salts are injected directly into the circulation, or placed in the interior of the stomach. Again, many drugs, for example, urethane and the acids, which in small amounts produce gradual relaxation of involuntary muscle, give rise to the opposite effect, viz., tonic contraction when applied in a more concentrated solution externally. It is important therefore to have the solution containing the drug to be experimented with as nearly as possible isotonic.

Lactic acid has the same effect on the stomach that it has on plain muscle in other parts of the body and on the heart. Very small amounts such as 1 in 10,000 are frequently sufficient to check automatic waves and produce considerable relaxation in from 15 to 30 minutes. Stronger solutions produce the same effect more rapidly. If a much stronger solution (1 in 500 and upwards) is applied directly to the exterior of a fresh stomach a different result is produced; there is now a rapid increase of tonus usually without any wave production which is followed by a very gradual relaxation (Fig. 11). These stronger solutions

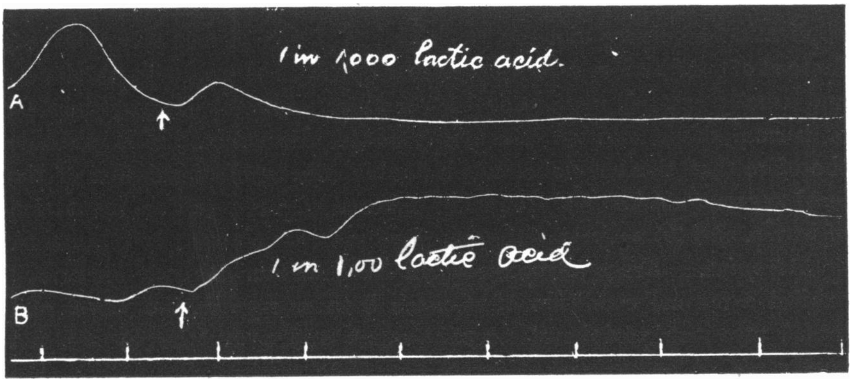

Fig. 11. Shows the action of lactic acid when applied to the stomach externally. The acid was applied at the arrows. *A* shows the diminution in tonus and cessation of movements as the result of a dilute solution. *B* shows the increased tonus produced by stronger solutions. Time = ½ minute.

added however to a stomach, already undergoing relaxation as the result of an earlier application of a more dilute solution of the acid, augment the relaxation. This difference of result, according to the strength of the solution employed, applies also to many other bodies.

Pilocarpine applied externally (1 in 5000) increases the amplitude of the waves, but has little effect on tonus. Injected into the hepatic vein as a $\frac{1}{2}$ % solution pilocarpine both increases the tonus and augments the waves. The effect quickly passes off and a diminution of tonus ultimately results (Fig. 12). If a solution of 1 in 500 is applied

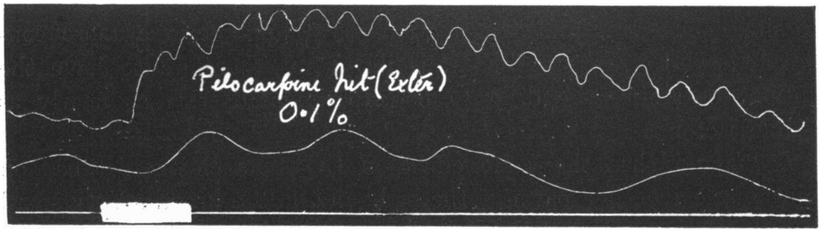

Fig. 12. Reduced by one-third. Action of pilocarpine nitrate on the stomach. The upper tracing represents the effect when the drug is applied externally. The lower tracing shows the result of injecting a few drops of a $\frac{1}{2}$ % solution in the hepatic vein. Time, upper tracing 1 cm. = 1 minute. Lower tracing 1 cm. = $\frac{1}{2}$ minute.

externally a rapid increase of tonus ensues superadded to which are a series of prominent waves.

If pilocarpine is applied to a stomach the nervous mechanism of which has been paralysed in the manner already described by the application of either nicotine or cocaine, a different result is obtained. Instead of contraction, relaxation is now quickly but gradually produced, and usually extends over some two or three minutes. The application of normal saline solution does not produce this effect. It is probable therefore that this substance has a double effect, (*a*) stimulation of the local nervous mechanism producing increase of tonus and augmentation of waves, and (*b*) a direct action on plain muscle resulting in relaxation.

Atropine. Frogs atropinised in the manner already described exhibit a normal condition of stomach both as regards the waves and tonus, also the peripheral nerves (vagus and sympathetic) are not paralysed. A solution of atropine, 1 in 5000, applied externally to the stomach produces no effect. A solution of 1 in 250 of the alkaloid rapidly kills the irritability of the muscle, so that, a short time after such an application, stimulating solutions produce no effect. A $\frac{1}{2}$ % solution applied directly to a normal stomach produces rapid relaxation, but a similar solution applied to the muscle in tonic contraction from the use of pilocarpine, barium or any other drug produces no immediate result.

Barium may be taken as a representative of a large group of drugs which act directly on the muscle fibre. They produce their effect equally well on the atropinised frog as on the non-atropinised, and also when the nervous tissue has been completely paralysed by the direct application of cocaine or nicotine to the stomach. If a few drops of a solution of barium chloride are injected into the circulation there is a considerable latent period before any effect is observed on the stomach. During this period however the heart shows the effect of the barium; it first accelerates, but soon slows again to the normal rate, and subsequently tonic contraction of the ventricle results (Fig. 13). The

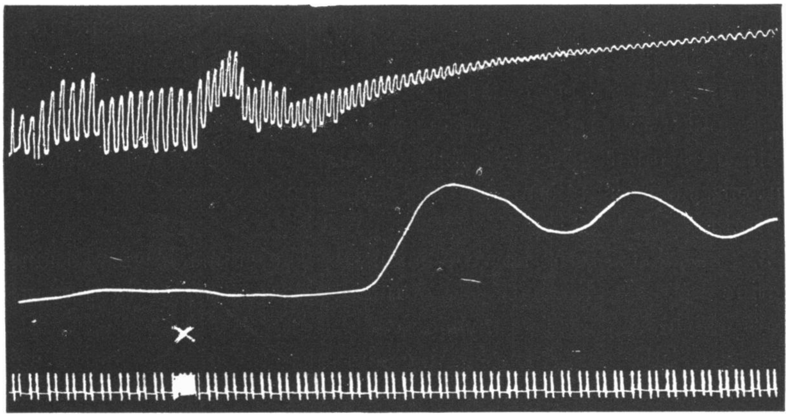

Fig. 13. Upper tracing represents the frog's heart recording by the suspension method. Lower tracing represents the stomach movements. Shows the effect of injecting a few drops of a solution of barium chloride into the hepatic vein. The cross marks the point of injection. Time = seconds.

tonus of the stomach is considerably increased and waves are brought out. The external use of barium on the stomach results in very similar effects.

Strychnine applied directly to the stomach in amounts from 1 in 15,000 upwards increases the amplitude of the waves. Stronger solutions such as 1 in 5000 also produce an increase of tonus.

Colchicine has practically no action, but oxdicolchicine gives rise to marked tonic contraction. Jacoby[21] has shown that the action of both these alkaloids is the same on mammalian plain muscle.

Suprarenal extract increases the tonus and exaggerates the waves. The effects are well shown on injection. If applied to a stomach paralysed with cocaine or nicotine relaxation results, of even a more decided character than with pilocarpine.

Nicotine. One in 10,000 stimulates the contractions and in larger amounts increases the tonus. When it is injected into the hepatic vein as a 0·2 or 0·3 % solution it markedly augments the automatic movements without affecting tonus.

Sodium Hydrate in such minute quantities as 1 in 20,000 stimulates the spontaneous contractions.

CONCLUSIONS.

1. In all experiments dealing with the frog's stomach two factors must be considered, (*a*) the tonus, (*b*) the spontaneous movements.

2. The automatic movements as recorded by this method consist generally of localised contraction rings which do not travel. The larger and more regular movements, especially those produced as a result of sympathetic excitation or the application of stimulating solutions, such as pilocarpine and suprarenal extract, are neurogenic in origin.

3. Paralysis of the nerve elements of the stomach with cocaine or nicotine does not eliminate the ordinary slow and very irregular movements which are therefore myogenic.

4. Stimulation of the rami communicantes of the fourth spinal nerves produces tonic contraction together with augmented and more regular automatic contractions. A similar but smaller effect is obtained on stimulating the third and fifth rami. This corresponds to the origin from the spinal nerves described by Waters and Steinach.

5. The vagus contains inhibitory fibres to tonus. Excitation of the vagus also commonly results in augmented automatic movements. Increase of tonus is never observed.

6. Nicotine 0·1 % applied to the cœliac and fourth sympathetic ganglia produces an initial stimulation as shown by the augmentation of the automatic contractions and sometimes by increase of tonus: it is not followed by paralysis.

7. By the use of the method here described drugs may be injected into the circulation. Effects on the stomach so obtained are not always the same as those produced by the direct external application of the drug.

8. Pilocarpine and suprarenal extract produce increased tonus and wave production on direct application, but give rise to immediate relaxation in a stomach the nerve mechanism of which has been paralysed by cocaine or nicotine.

9. Frogs which have been kept in confinement for more than a month are useless for innervation experiments.

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