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THE EFFECTS OF DRUGS, SUGARS AND ALLIED SUBSTANCES ON THE ISOLATED SMALL INTESTINE OF THE RABBIT

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The experiments described in this paper were carried out to analyse the stimulating action of sugars and related substances on the isolated intestine of the rabbit. In the course of the experiments the effects of acetylcholine, muscarine, eserine, phloridzin and atropine on the longitudinal and circular muscle layers of the intestine were examined in the presence and absence of glucose. Previous studies have usually been concerned only with the effect on the longitudinal muscle in the presence of glucose.

Rona & Neukirch in 1912 described the stimulating action of various sugars and related substances on the longitudinal muscle of the isolated intestine of the rabbit suspended in glucose-free Tyrode solution. Under these conditions the activity of the muscle diminished and disappeared. The subsequent addition to the solution of glucose, mannose or pyruvate revived and strongly stimulated the muscle. A weak action was obtained with galactose and lactate, whereas disaccharides were ineffective. Since the sugars disappeared from a solution into which a loop of intestine had been placed the stimulating effect was attributed to the ability of the substances to supply, in varying degree, the chemical energy necessary for the metabolism of the working muscle. The rhythmic activity and tone of the longitudinal muscle were regarded as being inherent properties of the muscle fibre. This concept does not become invalid if it is assumed that choline and acetylcholine which are continuously given off from the surviving intestine [Weiland, 1912; Le Heux, 1919; Feldberg & Rosenfeld, 1933; Donomae, 1934; Feldberg & Kwiatkowski, 1934] are the stimuli for augmenting the activity and tone of the muscle. In the absence of glucose the longitudinal muscle may become inexcitable to

the liberated choline and acetylcholine, normal excitability to them being restored by the addition of glucose or similarly acting substances. This explanation still attributes their stimulating action to an effect on the muscle fibre.

The sugars may, however, act by influencing the production and consequently the release of choline and acetylcholine. Magnus [1930] suggested that the stimulating action of pyruvate might be due to the formation of pyruvyl choline which was found to be more active than choline. He did not explain the stimulating action of the various sugars. The recent findings that these as well as pyruvate and lactate accelerate synthesis of acetylcholine in brain tissue in vitro [Mann, Tennenbaum & Quastel, 1938] and in a stimulated ganglion [Kahlson & MacIntosh, 1939] suggest such a mechanism as a possible explanation. Synthesis of acetylcholine in intestinal tissue has been demonstrated by Dikshit [1938] and, according to him, is mainly a function of the nerve plexus in the intestinal wall.

Our experiments were carried out in an attempt to evaluate the relative importance of the different mechanisms suggested above. According to Dikshit an intestinal preparation kept in the cold for a few days loses the property of synthesizing acetylcholine. A stimulating effect of glucose on such a preparation would therefore exclude the possibility of the stimulation being due to increased synthesis of acetylcholine. A negative result would be inconclusive, since the cold might prevent stimulation in any of several ways. In a second series of experiments the effect of glucose on the output of acetylcholine from the perfused intestine was examined. It might be argued that an increase in synthesis might not necessarily lead to an increase in output of acetylcholine. However, available evidence suggests that increased synthesis would stimulate the muscle only if acetylcholine were also liberated in increased amounts. A comparison of the stimulating action of glucose with that of acetylcholine or eserine in the absence of glucose should give further information on the problem.

There is at present no evidence that the stimulation of the intestine by various sugars and related substances is in each case due to the same mechanism and that they all act by 'replacing' glucose. If that were true stimulation should not occur when the substances are tested on preparations suspended in Tyrode solution containing glucose.

METHODS

Rabbits weighing 1·5–2·5 kg. were killed by a blow on the back of the neck. The abdomen was opened in the middle line and the procedure then adopted varied according to the experiment.

Suspension of an isolated piece of intestine. A piece of 7–12 cm. from the duodenum or the upper third of the small intestine was used. When the response of the longitudinal muscle alone was examined it was suspended in such a way as to leave both ends open, the oral end being attached to a thread leading to a Lovatt Evans frontal writing lever. For studying the effect on the circular muscle layer a volume record was taken according to Trendelenburg [1917]. The caecal end was tied over a glass cannula connected by rubber tubing to the lower end of a 1 l. aspirator half-filled with the same solution as used for the bath. The upper end, attached by thread to the lever, was kept open for 30–60 min., during which time the inside of the gut was frequently washed out from the aspirator. The upper end was then closed by a ligature and the pressure inside the gut brought to 2½–3 cm. water by raising the bottle. The upper opening of the aspirator was connected by rubber tubing to a small Krogh volume recorder. In the figures the upper tracing is the record of the intestinal volume, a diminution of which causes an upward stroke. The lower tracing is made by the suspension lever, an upwards stroke indicating a shortening of the gut. Some caution is necessary in the interpretation of the changes in volume. Shortening of the preparation may lead to some reduction in volume, but any reduction in volume which occurs without shortening must be due to a contraction of the circular muscle. The simultaneous record from the longitudinal muscle will avoid misinterpretation from this source. A strong but localized constriction of the circular muscle will have little influence on the volume, and reductions of equal extent may be brought about either by a strong contraction of part of the circular layer or by weak contraction of the whole wall. Therefore the effects on the circular muscle layer were further observed through the glass wall of the tank and the inside vessel. Tyrode solution, with and without glucose, was used, air bubbled through it and the temperature kept between 32 and 37° C. The volume of the bath was 35, 45 or 60 c.c. When no volume record was taken a 14 c.c. bath was often used. It was emptied by overflow and the substances were added with a syringe.

On cooled preparations the reactions on the longitudinal muscle only were examined. Pieces of the washed intestine were kept in glucose-free

Tyrode solution (at −1° C.) for 4–80 hr. Before use they were placed for 30–60 min. in glucose-free Tyrode solution at room temperature. The lumen was always full of secreted contents which were washed out and if necessary pressed out with a glass rod, before the preparations were suspended in the 14 c.c. bath.

Perfusion of the isolated intestine. A cannula of the type devised by Gaddum and modified by MacIntosh [1938] for perfusing the superior cervical ganglion in cats was tied into the peripheral end of the superior mesenteric artery which was divided near its origin. Perfusion was started at once with aerated Locke or Tyrode solution containing eserine in a concentration of 1 in 100,000 and at a temperature between 37 and 39° C. The upper and lower third of the small intestine were tied off and removed, leaving for perfusion a piece varying in length between 70 and 140 cm. A cannula was tied into the portal vein for collecting the venous outflow and into the lumen at each end of the perfused part the inside of which was washed out with about 50 c.c. of the perfusion fluid. After ligating all vascular connexions with the rest of the body the perfused part was transferred to a perfusion chamber which consisted of an 11 cm. glass funnel immersed in an electrically heated water-bath. An inverted Petri dish was fixed with plasticine into the inside of the funnel leaving room for fluid to run down the funnel. The perfused intestine was spread on the dish, avoiding kinking, and the perfusion cannnula was fixed in position. The venous cannula was connected to a fine rubber tube and the venous effluent collected and assayed separately from the fluid oozing from the outside and inside of the intestinal wall which was collected by the funnel. The funnel was covered with a glass plate and a thermometer was placed in the chamber so obtained. Its temperature was kept well over 30 and below 40° C. The rate of inflow was 2–3 c.c./min. From the beginning of the perfusion to the collection of the first sample 30–40 min. elapsed.

The samples were assayed for acetylcholine on the frog's rectus muscle suspended in diluted eserinized Locke solution. The muscle was sometimes further sensitized by adding urethane to the solution [Emmelin, 1939]. The high choline content of the samples as well as the presence of another interfering principle made it necessary, in order to obtain the true equivalent for acetylcholine, to make the control solution not in saline but in part of the sample itself after its acetylcholine had been destroyed by NaOH and then again neutralized. After treatment with NaOH the sample was further assayed against choline on the frog rectus and the values obtained compared with the choline estimates found by acetylation.

Results

The suspended fresh preparation of the intestine

Acetylcholine

In the presence of glucose. The immediate shortening of the isolated gut caused by small doses of acetylcholine occurs even when both ends are closed and the inside pressure raised to 3 cm. of water. Usually the shortening is accompanied by a strong but evanescent contraction of the circular muscle starting at the upper end and frequently passing over the whole preparation. This causes a large reduction in volume lasting a few seconds, after which the volume returns to an intermediate position in which it remains as long as the shortening persists (see Figs. 3, 4). This remaining reduction in volume, if small, may be accounted for by the shortening, if pronounced, it may indicate some tonic effect on the circular muscle. In a few experiments the shortening appeared to proceed without any visible effect on the circular muscle, but even in these instances, after washing out the acetylcholine, the circular muscle usually passed through a period of increased activity. With larger doses of acetylcholine left in contact with the gut for several minutes, the contraction of the longitudinal muscle was well maintained but might be interrupted by slight evanescent lengthening of the gut as a result of a strong contraction of the circular muscle. This effect was never as pronounced as that produced by prolonged action of eserine or muscarine.

In the absence of glucose. The excitability of the longitudinal muscle to acetylcholine and other stimulating substances decreases and can be lost, whereas that of the circular is much better retained. The first sign of decreased excitability of the longitudinal muscle was its inability to remain contracted during the 60–90 sec. the acetylcholine was left in contact with the gut. During this period the muscle relaxed and sometimes returned to its original length. If rhythmic activity had been present prior to the acetylcholine it sometimes disappeared for a minute or two. In several instances this happened after the acetylcholine had been washed out. It was not associated with activity of the circular muscle. Later on the contractions of the longitudinal muscle to acetylcholine became smaller and eventually the muscle might fail to contract at all, stoppage of its spontaneous rhythm sometimes being the sole response. This stage was reached in some preparations more slowly than in others. Its appearance was accelerated by shortening the intervals between successive administrations of acetylcholine and by increasing their dose. On the other hand, there was usually some recovery by

lengthening the intervals. The preparation, a record of which is shown in Fig. 1, had been suspended for over an hour in a glucose-free Tyrode solution and subjected during the last 20 min. to repeated doses of 1 μg. of acetylcholine at short intervals, the last two responses being shown at A and B. The contractions are not well sustained, and that at B is weaker and of shorter duration than that at A. After a rest of 5 min. acetylcholine caused strong and maintained contraction (at C), but recovery was evanescent as seen from the subsequent responses (D–F). An interval of 8 min. caused recovery (at G) which was more pronounced than that at C. The interpolation, once or twice, of a large dose of acetylcholine was even more effective in decreasing the excitability than was shortening the intervals. It usually resulted in a period of complete inexcitability

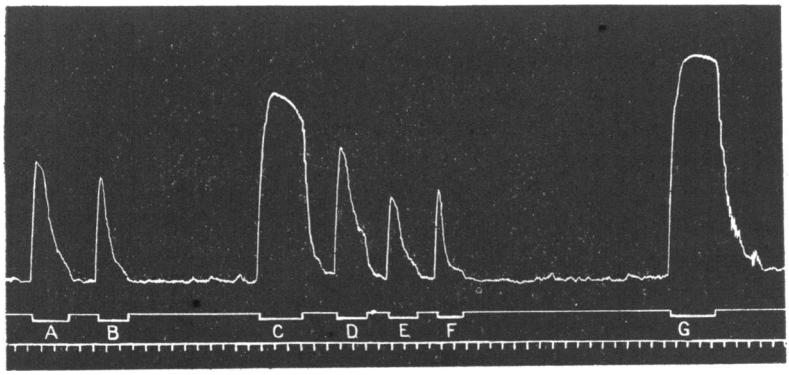

Fig. 1. Rabbit's intestine in 35 c.c. glucose-free Tyrode solution; lumen left open at both ends. A–G = 1 μg. acetylcholine chloride. Time in 30 sec.

of the longitudinal muscle (see Fig. 5 A, B). The figure shows also that some recovery took place after a rest of over 10 min., when 10 μg. of acetylcholine were given at C.

The absence of glucose from the Tyrode solution did not abolish the effect of acetylcholine on the circular muscle. It thus became possible to obtain records of contractions of the circular muscle without interference by shortening of the intestine. Relatively large doses were necessary to produce the effect, and it is therefore possible that the lack of glucose had diminished the excitability of the circular muscle. Small effects were obtained with 5–10 μg. and large effects with 50–100 μg. in a 45 c.c. bath (Figs. 2 A, 5 A–C). There was some reduction in the responses if large doses were given repeatedly at short intervals. Strong contractions of the circular muscle were often associated with lengthening of the gut

(Fig. 5 B), and if a contraction of the longitudinal was present it was cut short by the contraction of the circular muscle (Fig. 5 A).

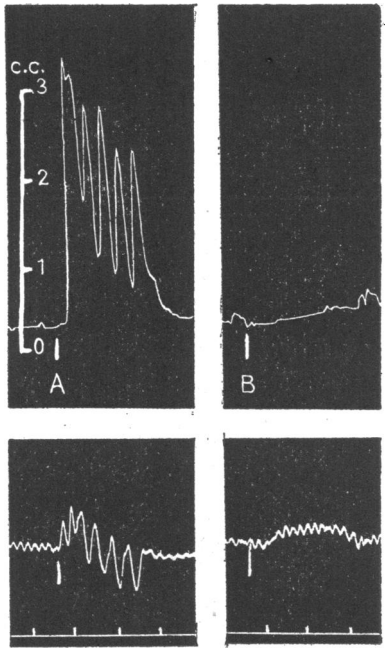

Fig. 2. Rabbit's intestine in 45 c.c. glucose-free Tyrode solution. A and B = 100 μg. acetylcholine chloride for 1 min.; between A and B 5 μg. of atropine. In this and the following figures the vertical scale indicates the changes in intestinal volume in c.c. Time in 30 sec.

Muscarine

The fact that relatively large doses of acetylcholine were needed to contract the circular muscle in the absence of glucose might suggest a nicotine-like effect on the nerve cells. Its abolition by small doses of atropine (see later) would be no evidence against this interpretation. We have therefore, at the suggestion of J. H. Gaddum, examined the effect of muscarine which is devoid of a nicotine-like action. It stimulated the longitudinal as well as the circular muscle. In the absence of glucose the effect on the longitudinal but not on the circular muscle became weaker and ultimately disappeared, the factors favouring this condition being the same as those described for acetylcholine. On the circular muscle muscarine had a relatively greater effect than acetylcholine which, in the presence of glucose, became apparent when large doses were compared.

In Fig. 3 the effects on the two muscle layers of 1 μg. of acetylcholine (A) and of 0·2 c.c. of 1 in 400 of the solution of muscarine at our disposal (B) correspond approximately. D and E are the effects of 10 and 50 μg. of acetylcholine respectively, and C that of a dose of muscarine 20 times stronger than that given at B. It causes a greater reduction in volume than 50 μg. acetylcholine. The contraction of the ring muscle is so strong as to produce lengthening of the gut which subsides after washing out the muscarine. In observing the preparation the impression is gained

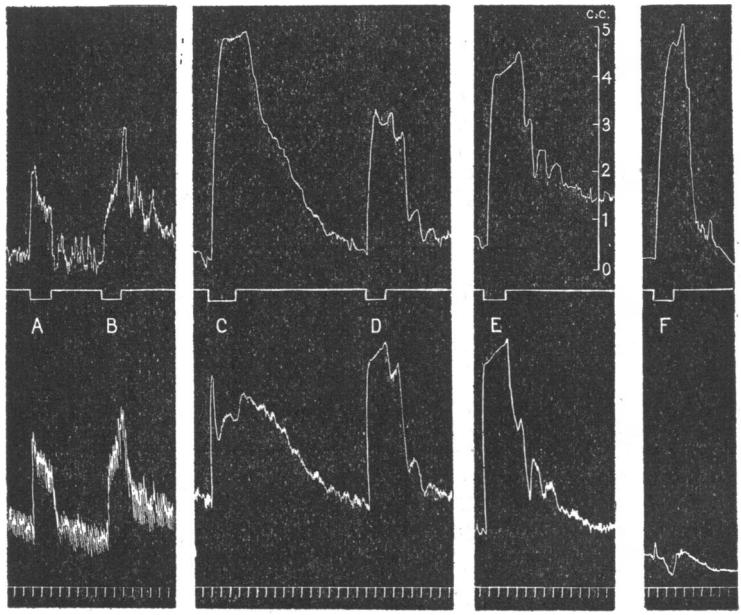

Fig. 3. Rabbit's intestine in 60 c.c. Tyrode solution. A, D and E = 1, 10 and 50 μg. acetylcholine chloride. B, C and F = muscarine. F is from a different preparation in glucose-free Tyrode solution. Time in 30 sec.

that the lengthening is brought about passively by the contraction of the circular muscle. Trendelenburg [1917] observed similar changes in the two muscle layers, when the pressure in the lumen of the intestine was raised, but he attributed the lengthening to active inhibition of the longitudinal muscle co-ordinated with the strong contraction of the circular muscle.

Direct observation of the preparation also showed that muscarine was more active on the circular muscle than was acetylcholine. At C the circular muscle became completely contracted occluding the lumen of the

whole piece of gut. The effect at F was obtained with twice the dose of muscarine given at C, but on a different preparation suspended in Tyrode solution free from glucose. There was no stimulation of the longitudinal muscle which lengthened during contraction of the circular muscle. It was not possible, in the absence of glucose, to obtain contractions of the circular muscle as strong as those produced by muscarine even by increasing the dose of acetylcholine to several 100 μg. On the other hand, if the excitability of the longitudinal muscle was partly depressed, acetylcholine often caused a relatively strong contraction of this muscle, whereas that produced by muscarine was small or absent. The effect of muscarine on the longitudinal muscle appears, therefore, to be relatively weaker than that of acetylcholine.

Eserine

In the presence of glucose. Fig. 4 shows the interaction of the stimulating effect of eserine on the two muscle layers. Stimulation starts after a latency which varied between 10 and 30 sec. and the contraction proceeds gradually. With small doses of eserine even longer latencies were observed. The stimulation of the circular muscle usually began a few seconds later and overcame the shortening of the gut. The figure illustrates the sudden onset of periods of maximal contractions of the circular muscle spreading over the whole wall and causing lengthening of the gut which shortens again with partial relaxation of the circular muscle. The interaction of the two muscles repeated itself four times, the last occurring after the eserine had been washed out. Sometimes the circular muscle remained strongly contracted as long as the eserine was left in contact. This caused a trough-like depression on the record of the longitudinal muscle.

In the absence of glucose. The stimulating effect of eserine on the longitudinal muscle was weak or absent, whereas that on the circular muscle was retained (Fig. 5). The latency appeared to be longer and the contraction disappeared more quickly after washing out the eserine, indicating some impairment in the functioning of the circular muscle.

Glucose

Effect on the longitudinal muscle in the absence of glucose. A preparation suspended with the lumen left open at both ends relaxed progressively for 20–60 min. There was usually first an increase in the amplitude of the rhythmic contractions due to more complete relaxation followed by a decrease in amplitude due to less extensive contraction. In addition, the

tone diminished independently of these changes. Glucose added to the relaxed preparation had a stimulating effect and restored the excitability of the muscle to subsequent administration of acetylcholine. The stimulation by glucose started after a latency of 15–60 sec., the rhythmic movements increased in amplitude but not in frequency and the tone

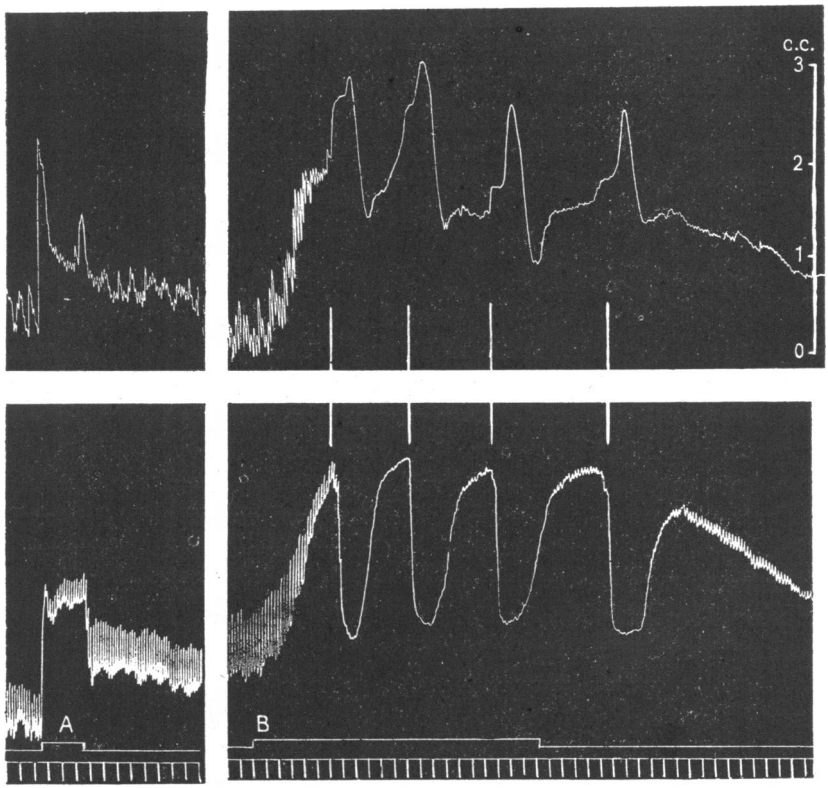

Fig. 4. Rabbit's intestine in 45 c.c. Tyrode solution. A = 1 μg. acetylcholine chloride; B = 10 μg. eserine sulphate. The four vertical white lines indicate corresponding points on the upper and lower tracings. Time in 30 sec.

became stronger. During the latency there was often some inhibition in tone and activity. Small stimulating effects consisting of an increase in activity only were sometimes observed on the addition of 1–2 mg. of glucose to a 35 c.c. bath. In some preparations, particularly from the lower end of the ileum, stimulation affected mainly the rhythmic activity, in others the effect was mainly on the tone (Fig. 6). The stimulating effect increased with repeated administration of glucose, the first doses often

increasing activity only, the later ones the tone (Fig. 7). It will be seen from both figures that when only the rhythmic activity was stimulated, the shortening increased with each rhythm, but the base-line was not affected. In the experiment of Fig. 7 the first dose of glucose was given

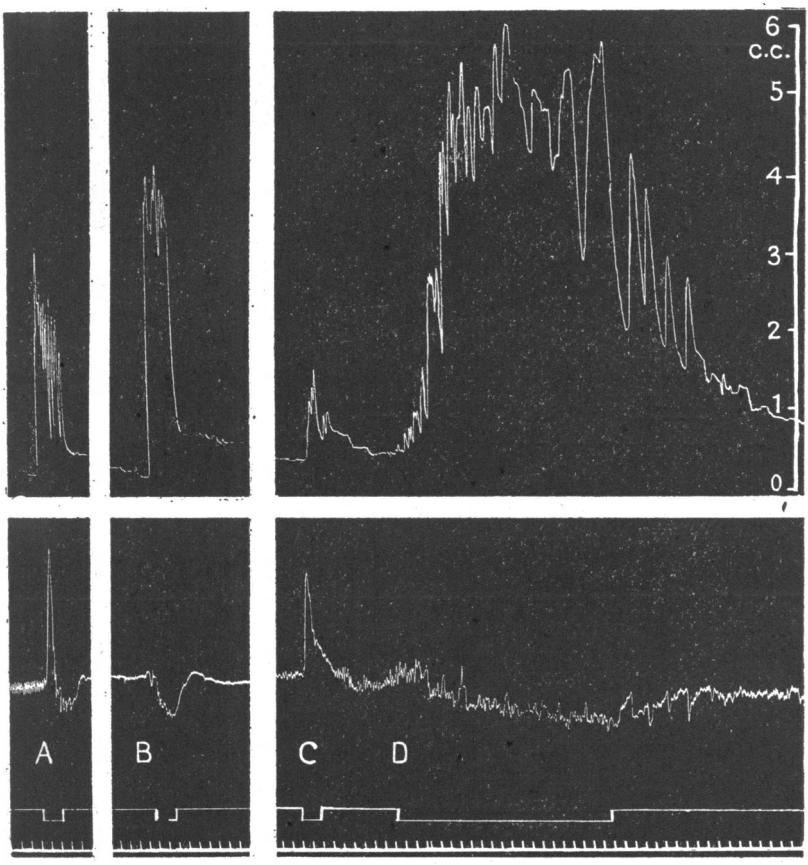

Fig. 5. Rabbit's intestine in 60 c.c. glucose-free Tyrode solution. A, B and C = 50, 100 and 10 μg. acetylcholine chloride; D = 20 μg. eserine sulphate. Time in 30 sec.

25 min. after the preparation had been suspended. In preparations suspended for a longer time even a first dose of glucose produced a strong stimulating effect, but some increase in the response was obtained with repeated administration.

The restoration of the excitability of the muscle by glucose was usually studied with acetylcholine, but in some instances choline and pilocarpine were used with identical results. The excitability remained

elevated for some time after the glucose had been washed out, and the contractions with acetylcholine were not only more powerful but also better maintained (Fig. 8). This change in excitability could be obtained with amounts of glucose which had scarcely any direct stimulating effect. The period of recovery varied in length in different preparations; increase

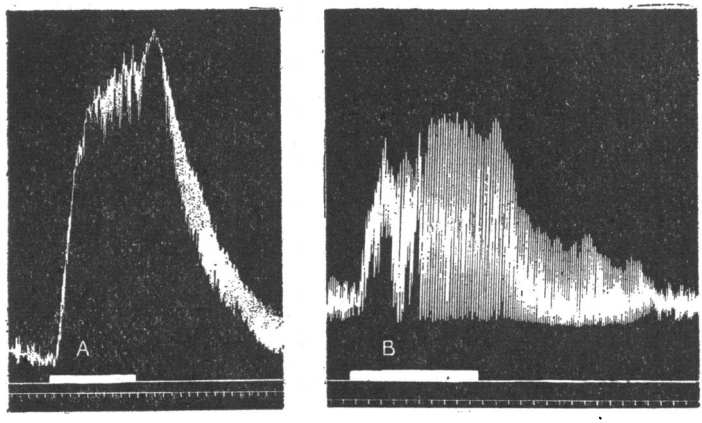

Fig. 6. Rabbit's intestine in 35 c.c. glucose-free Tyrode solution, lumen left open at both ends. Two preparations from different animals. A and B = 20 mg. glucose; latency at A 18, at B 15 sec. Time in 30 sec.

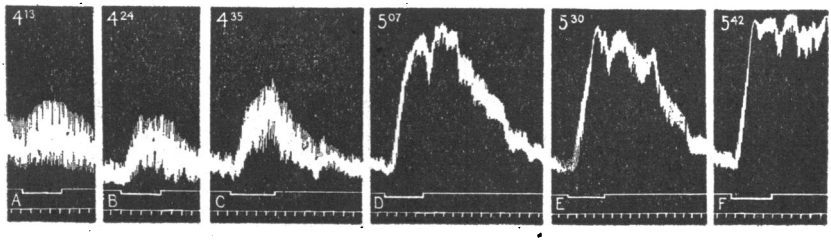

Fig. 7. Rabbit's intestine suspended at 3.50 p.m. in 35 c.c. glucose-free Tyrode solution; lumen left open at both ends. A–F = 10 mg. glucose; the latencies at C–F were 21, 21, 22 and 14 sec. On top of tracing, time when glucose was given. Time in 30 sec.

in the dose of glucose and in the time it was left in contact with the gut lengthened it, shortening the intervals between successive doses of acetylcholine and increasing their dosage shortened it.

Closing the lower end of the preparation did not affect the results, but when both ends were closed and the inside pressure raised to 2¼–3 cm. of water the stimulating effect of glucose diminished (Fig. 9 A, B) and, with repeated administration, it decreased further and sometimes dis-

appeared. In the experiment of Fig. 10 the longitudinal muscle had become insensitive to the stimulating effect of glucose (at C) following its repeated administration, but it was still effective in restoring the excitability of the muscle to acetylcholine. When in this condition glucose was given in the presence of a weak concentration of acetylcholine in the bath or vice versa, a strong and sustained contraction of the longitudinal muscle occurred. In Fig. 10, for instance, 5 μg. of acetylcholine (B) or 50 μg. glucose (C) when given separately, were ineffective, but when the acetylcholine was given with or after the glucose a strong contraction was obtained. Previous to the glucose even 100 μg. of acetylcholine (A) had no effect on the longitudinal muscle. The restoration of excitability without direct stimulation of the muscle by glucose therefore may have been due to the fact that the acetylcholine continuously liberated in the

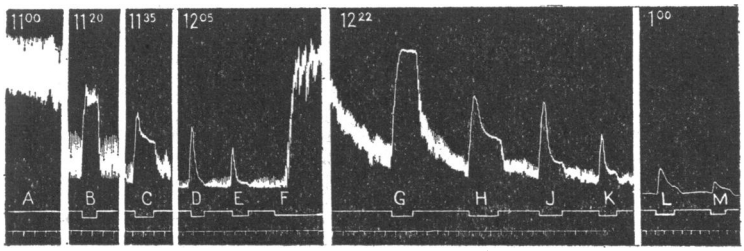

Fig. 8. Rabbit's intestine suspended at 11.00 a.m. in 14 c.c. glucose-free Tyrode solution. Lumen left open at both ends. B–E, G–M = 1 μg. acetylcholine chloride. F = 5 mg. glucose, latency 35 sec. Time in 30 sec.

intestinal wall did not reach a concentration sufficient for stimulation under these conditions. According to this assumption raising the concentration should bring back the stimulating action of glucose. This can be achieved by eserine. If given with a non-stimulating dose of glucose it causes after a latency of 20–50 sec. a slowly progressing but strong contraction of the longitudinal muscle, and its relaxation proceeds very gradually after washing out the eserine and glucose.

Effect on the circular muscle in the absence of glucose. The stimulating effect was small and irregular. The shortening of the gut was associated with a reduction in volume which was often so small (Fig. 9) as to be wholly accounted for by it. If the reduction was stronger it may have resulted from an increase in tone of the circular muscle. There was usually some increased activity after the glucose had been washed out and the longitudinal muscle had relaxed. A few rapid but strong contractions of the whole circular muscle sometimes occurred leading to lengthening of

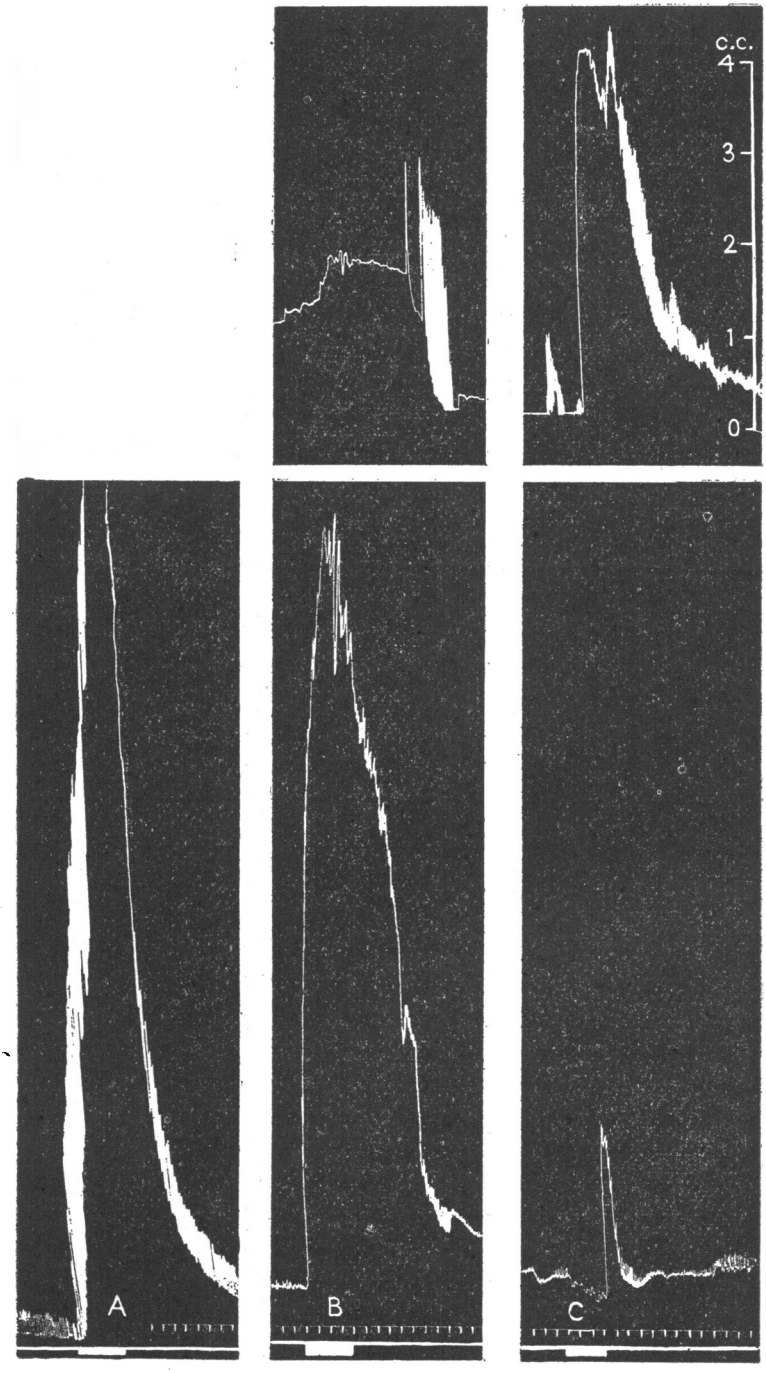

Fig. 9. Rabbit's intestine in 35 c.c. glucose-free Tyrode solution; lumen closed at lower end and at upper end after A. A and B = 75 mg. glucose, latencies 10 and 20 sec. C = 400 mg. sodium lactate. Time in 30 sec.

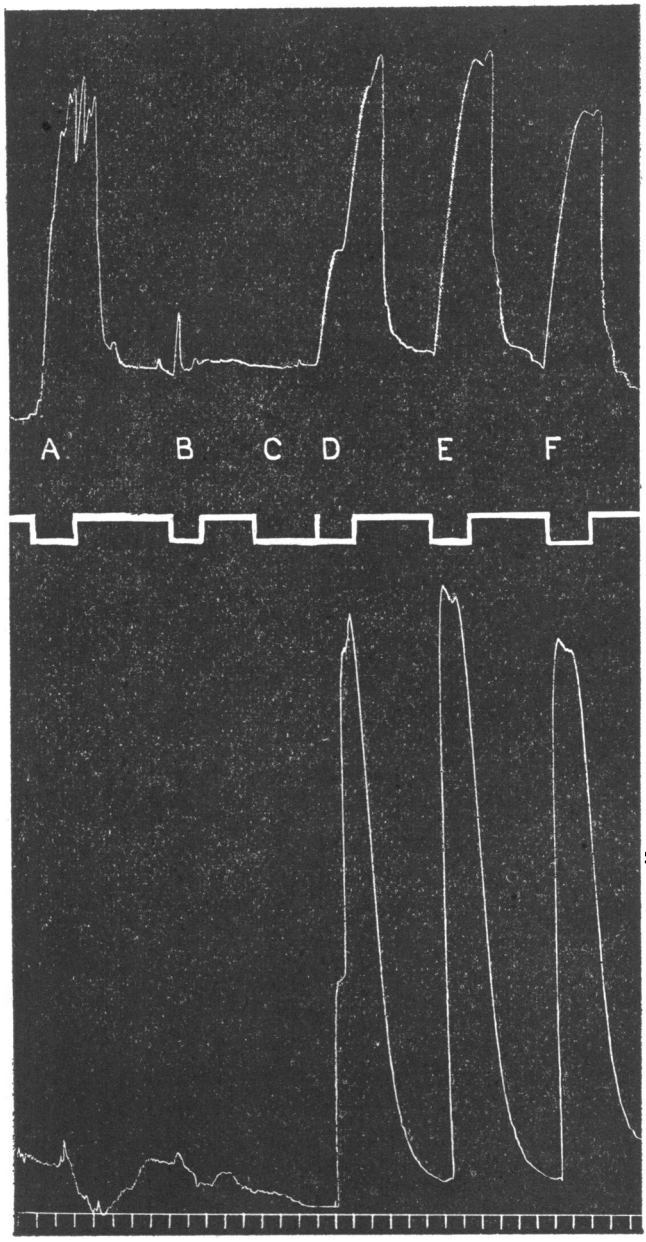

Fig. 10. Rabbit's intestine in 35 c.c. glucose-free Tyrode solution. A = 100 μg.; B, D, E and F = 5 μg. acetylcholine chloride. C = 50 mg. glucose for 3 min., D given after the second minute. Time in 30 sec.

the gut. There were occasionally isolated constrictions on different parts of the circular muscle, and in a few experiments regular rhythmic contractions of the whole layer at a frequency of 9–11 per min. occurred for 2 to 3 min. either before the shortening or after the relaxation of the longitudinal muscle had taken place (Fig. 9 B).

Effect in the presence of glucose. On a preparation suspended in Tyrode solution containing its normal 0·1% glucose the addition of enough glucose to double or treble its concentration in the bath caused no stimulation. It usually produced slight immediate inhibition in tone and activity. Further increase in the concentration of glucose caused greater inhibition which might be followed by slight stimulation of the muscle (Fig. 11 C). Direct observation of the preparation through the glass walls of the bath revealed the absence of contractions of the circular muscle. The effects on the longitudinal muscle were not due to an increase in tonicity of the bath fluid, since they occurred also when glucose was added in an appropriate volume of NaCl-free Tyrode solution in which the NaCl had been replaced by equimolecular amounts of glucose.

Other monosaccharides

The following sugars have been tested on the longitudinal muscle, no volume record being taken:

Mannose has a stimulating action, in the absence of glucose from the bath, and restores the excitability of the muscle to acetylcholine. The response increases with repeated administration. Mannose is about 25–30% as active as glucose. Stimulation starts after a longer latent period and reaches its maximum more slowly than after glucose. If kept in contact with the gut for 1–2 min. the effect reaches its maximum after the mannose has been washed out. In one experiment the latent period following 2·5 mg. of glucose was 27 sec., that following a similarly effective dose of 10 mg. of mannose was 37 sec. In another similar experiment the latent periods were 16 and 26 sec. respectively. As with glucose there was usually some immediate inhibition.

On preparations suspended in Tyrode solution containing glucose, mannose caused immediate inhibition, sometimes followed by some stimulation (Fig. 11 A) which was a little stronger than that caused by glucose in this condition.

Galactose had a weak stimulating action on preparations suspended in glucose-free Tyrode solution. The effect of 100 mg. corresponded to that of 2–3 mg. of glucose. Stimulation was often preceded by slight immediate inhibition, and after washing out the galactose the muscle

showed a period of increased excitability to acetylcholine. On preparations suspended in Tyrode solution containing glucose, galactose had an immediate inhibitory effect only.

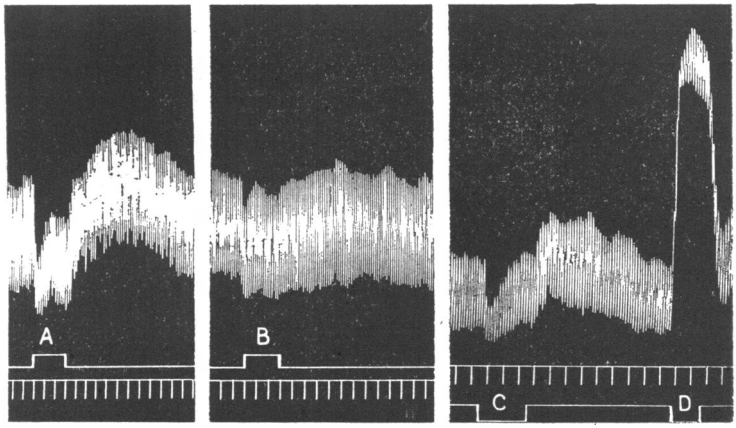

Fig. 11. Rabbit's intestine in 14 c.c. Tyrode solution; lumen left open at both ends. A = 100 mg. mannose; B = 100 mg. lactose; C = 100 mg. glucose; D = 1 μg. acetylcholine chloride. Time in 30 sec.

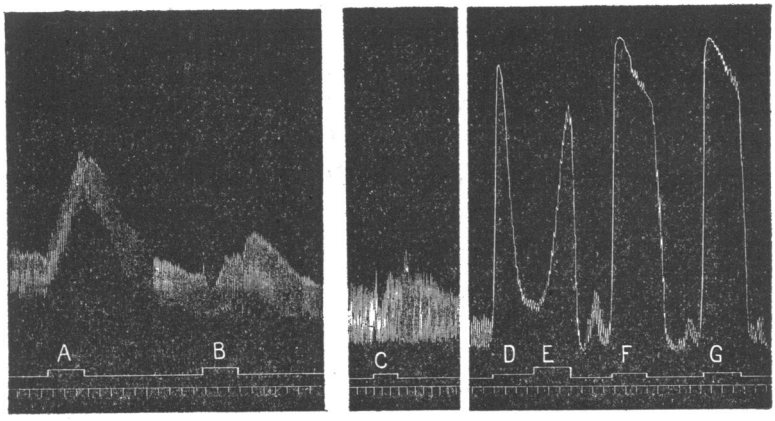

Fig. 12. Rabbit's intestine in 14 c.c. glucose-free Tyrode solution; lumen left open at both ends. A = 5 mg. glucose; B, C and E = 100 mg. laevulose; D, F and G = 10 μg. acetylcholine chloride. From C to G a new piece of intestine from same animal. E added without washing out the acetylcholine. Time in 30 sec.

Laevulose had a slight stimulative action on preparations suspended in glucose-free Tyrode solution followed by a period of increased excitability to acetylcholine. In the experiment of Fig. 12 the effect of 5 mg.

of glucose (A) was compared with that of 100 mg. of laevulose (B). During the latent period there was some inhibition. The muscle which had been unable to maintain the contraction caused by acetylcholine regained this property through the addition of laevulose to the bath. The contraction at D was maintained for 15 sec. only, although the acetylcholine was not washed out. 100 mg. of laevulose, which alone had a very slight stimulating effect (C), caused, in the presence of the acetylcholine, a strong contraction after a latency of about 20 sec. (E). The excitability remained elevated during the next minutes (F and G).

On preparations suspended in Tyrode solution containing glucose laevulose caused immediate inhibition which was not followed by stimulation.

Disaccharides

Maltose and *lactose* had no stimulating action if added in doses up to 100 mg. to a 14 c.c. bath containing no glucose. The addition of 200 mg. of maltose produced slight inhibition followed by slight stimulation which was less than that produced by 2 mg. of glucose and might have been due to glucose in the preparation as impurity. Lactose had no effect in restoring the excitability of the muscle to acetylcholine. No corresponding experiments were carried out with maltose. If tested in the presence of glucose, lactose caused slight immediate inhibition (Fig. 11 B).

Sodium pyruvate

On preparations suspended in glucose-free Tyrode solution sodium pyruvate has a strong stimulating effect on the longitudinal muscle and restores its excitability to acetylcholine. On the circular muscle stimulation is weak and irregular. The effect on the longitudinal muscle differs from that of glucose in that the latency is shorter and relaxation after washing out the bath more rapid. In all other details the effect resembles that of glucose. In the experiment of Fig. 13 the latencies of two doses of 1 mg. of pyruvate (A and F) were 8 and 9 sec., whereas those of equipotential doses of glucose (B and E) were 20 and 16 sec. respectively. Compared weight for weight sodium pyruvate was 10–30 times as active as glucose.

In the presence of glucose, pyruvate had on the longitudinal muscle a slight stimulating effect which was a little stronger than that of glucose.

Sodium lactate

Effect in the presence of glucose. Lactate had a stimulating action on both muscle layers. In a preparation suspended in such a way as to leave both ends open the contraction of the circular muscle sometimes caused

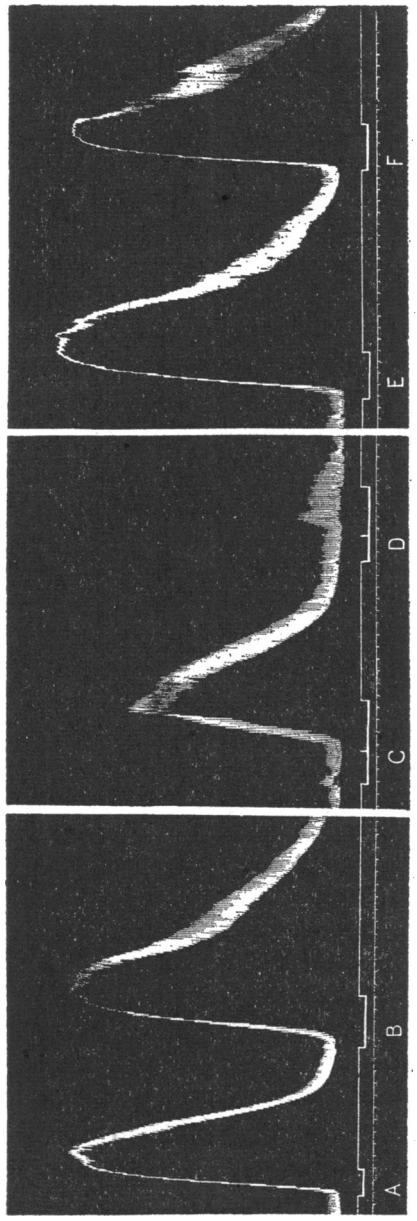

Fig. 13. Rabbit's intestine in 14 c.c. glucose-free Tyrode solution; lumen left open at both ends. A and F = 1 mg. sodium pyruvate; B and E = 15 mg. glucose; C and D = 5 mg. phloridzin for 3 min. followed after the first minute by 1 mg. sodium pyruvate (C) and 15 mg. glucose (D). Time in 30 sec.

expulsion of great amounts of excreted juice through the lower end producing frothing and interference with the action of lactate. It was therefore necessary even when the effect on the longitudinal muscle alone was recorded to tie the lower caecal end over the glass cannula connected with the rubber tube to the aspirator (see 'Method'). The contents of the gut then emptied into the cannula.

The doses of sodium lactate necessary to produce strong stimulating actions were so great as to increase the tonicity of the solution. For instance, the addition to a 45 c.c. bath of 150 mg. of sodium lactate, which is equimolecular to 79 mg. of NaCl, represented an increase in salt concentration of about 20%. Miss M. Vogt [1942], who had found that such changes in salt concentration caused strong stimulating effects on both muscle layers, drew our attention to the fact that responses to lactate injections without correction of the salt factor might be accounted for partly by an increase in salt concentration. In the following experiments lactate, therefore, was added to the bath in the appropriate volume of NaCl-free Tyrode solution, the NaCl being replaced by equimolecular amounts of sodium lactate.

The stimulating effect on the longitudinal muscle of increasing doses of lactate is seen in Fig. 14. Stimulation started after a latency of a few seconds. The addition to the 45 c.c. bath of 40 mg. of sodium lactate or less had no longer a stimulating effect. After closing the upper end of the preparation and raising the inside pressure of the gut to $2\frac{1}{2}$ cm. of water the shortening effect of large doses of lactate was somewhat diminished. In some experiments the shortening was interrupted by strong contractions of the circular muscle layer causing lengthening of the gut beyond its original level. In the experiment of Fig. 14, at D, the lengthening occurred before washing out the lactate and was associated with a strong contraction of the circular muscle. At B the gut volume is recorded simultaneously; the sudden lengthening is associated with a great reduction in volume due to contraction of the whole circular muscle layer.

Effect in the absence of glucose. Whereas the absence of glucose did not appear to modify the stimulating effect of lactate on the circular muscle that on the longitudinal one was reduced or even abolished. In the experiment of Fig. 15 the small effect obtained with 300 mg. of lactate (at B) is contrasted with the strong stimulation of the longitudinal muscle by glucose. The lactate did not appear to modify the response of the muscle to subsequent administration of acetylcholine, but the response to glucose became reduced for some time. This is seen by a comparison of the responses to glucose at A, C and D in Fig. 15. After successive

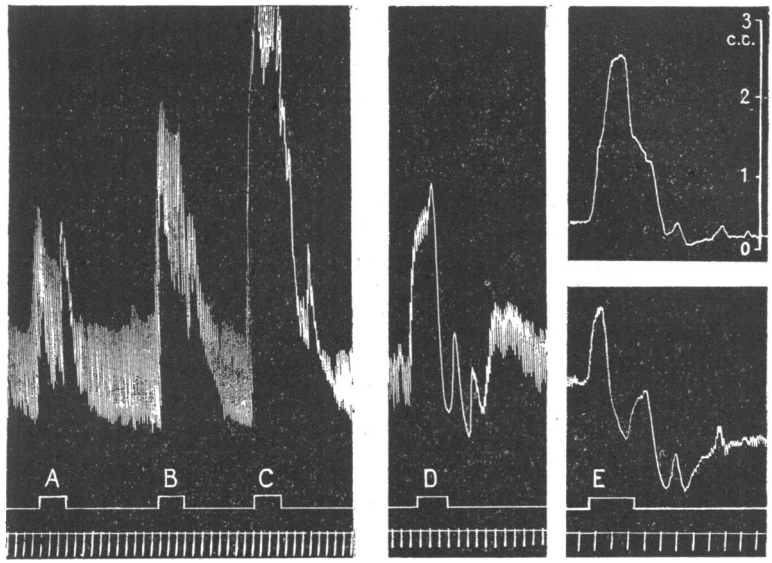

Fig. 14. Two preparations of rabbit's intestine in 45 c.c. Tyrode solution. Lower lumen closed; upper lumen closed after D. A = 75 mg., B, D = 150 mg. and C, E = 300 mg. sodium lactate. D and E the second preparation. Time in 30 sec.

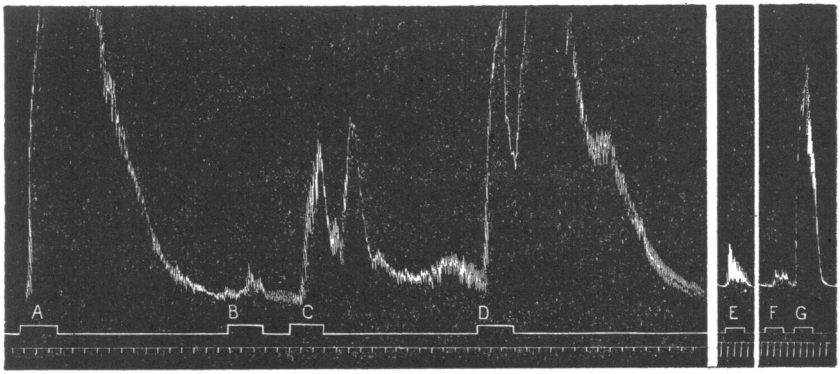

Fig. 15. Two preparations of rabbit's intestine in 45 c.c. Tyrode solution. Lower lumen closed. A, C, D and F = 50 mg. glucose; latency at A 18, at C 42 and at D 34 sec. B = 300 mg., E and G = 150 mg. sodium lactate. E, F and G the second preparation. Time in 30 sec.

administration of large doses of lactate the longitudinal muscle sometimes showed a period of complete insensitivity to the stimulating action of glucose, its sole effect then being an increase in excitability to stimulating substances such as acetylcholine. In this condition lactate also regained its stimulating action on the longitudinal muscle. This is shown by the difference in the response to lactate at E and G in experiment of Fig. 15. The stimulating action of glucose which, previous to the lactate, had been pronounced was nearly abolished when given at F after three successive doses of lactate. Sometimes lactate in the course of the experiment acquired a stimulating action on the longitudinal muscle without any addition of glucose to the bath. Having for six or seven injections stimulated the circular muscle only, the lactate from the next injection onwards suddenly stimulated strongly both muscle layers.

On fresh preparations the stimulating effect of lactate on the circular muscle usually consisted of more or less regular rhythmic contractions of the whole wall at a frequency of about seven per minute. These could be observed through the glass wall of the bath and were responsible for the rhythmic changes seen in the volume record of Fig. 16. In preparations which had been suspended for a long time and had become tired the effect of lactate on the circular muscle resulted often in a more sustained tonic contraction which lasted until the lactate had been washed out.

Phloridzin

Glucose. Phloridzin inhibits the stimulating action of glucose on the longitudinal muscle (Fig. 13 D), but it appears to have no direct action on the gut itself. Therefore on a relaxed preparation suspended in glucose-free Tyrode solution it has no effect or only slightly inhibits the rhythmic contractions. On such a preparation it has also no influence on the response to acetylcholine. When the phloridzin has been washed out complete recovery of the stimulating action of glucose ensues (Fig. 13 E). The inhibition of the stimulating action by phloridzin is responsible for the depression produced on preparations suspended in glucose containing Tyrode solution. In Fig. 17 A and B the phloridzin depresses mainly the tone, and at C the rhythmic movements. The recovery between the two administrations of 30 mg. of phloridzin at A and B resembles the stimulating action which glucose produces in the experiment of Fig. 6 A, and the inhibition of the rhythmic movements at C represents a reversal of the stimulating effect of glucose such as shown in Fig. 6 B.

Sodium pyruvate. Its stimulating action is also inhibited by phloridzin but less than that of glucose. Fig. 13 shows the effect of 5 mg. of phloridzin

on equipotential doses of glucose and pyruvate. The response to glucose is almost abolished (D), whereas pyruvate is still highly active (C). However, the contraction is smaller, starts after a longer latency and

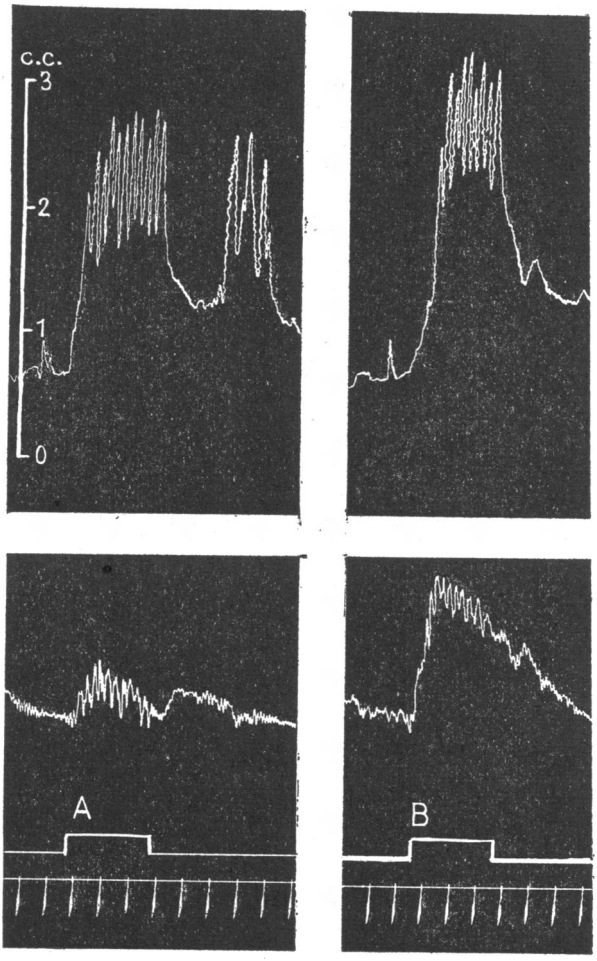

Fig. 16. Rabbit's intestine in 45 c.c. glucose-free Tyrode solution. A = 150 mg., B = 300 mg. sodium lactate. Time in 30 sec.

proceeds more slowly than that produced by pyruvate without phloridzin (A and F). Larger doses of phloridzin completely inhibit the action of pyruvate.

Sodium lactate. Phloridzin does not affect the strong stimulating action on the circular muscle and does not inhibit the stimulation of the

longitudinal muscle when observed in the absence of glucose from the bath.

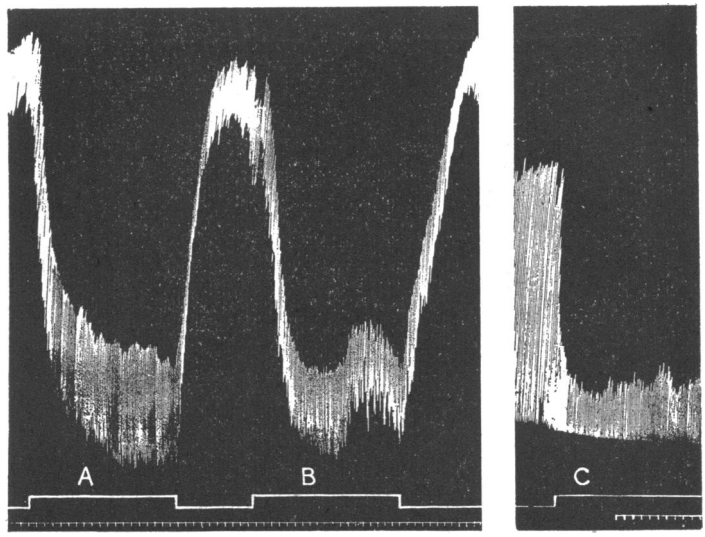

Fig. 17. Rabbit's intestine in 35 c.c. Tyrode solution; lumen left open at both ends. A and B = 30 mg., C = 20 mg. phloridzin. C, preparation from different rabbit. Time in 30 sec.

Atropine

Effect in the absence of glucose. In the beginning of an experiment, while the longitudinal muscle is slowly relaxing, atropine (10–50 μg. to a 45 c.c. bath) may cause rapid relaxation. At later stages the inhibitory effect of atropine is slight and evanescent and may even be absent. Rhythmic contractions of the longitudinal muscle if present continue although added acetylcholine has no stimulating effect.

Glucose. During the stimulating action of glucose atropine causes rapid and sometimes complete relaxation of the longitudinal muscle, the spontaneous activity of which may cease (Fig. 18). After a few minutes rhythmic activity and tone reappear even when the atropine remains in the bath and although the muscle remains insensitive to added acetylcholine. Atropine thus produces a trough-like depression on the tracing (Fig. 18 B). When atropine is given before glucose the stimulation is delayed and diminished but not abolished, even though added acetylcholine is ineffective.

Acetylcholine and muscarine. Small doses of atropine abolish the

stimulating action of acetylcholine and muscarine not only on the longitudinal but also on the circular muscle (Fig. 2).

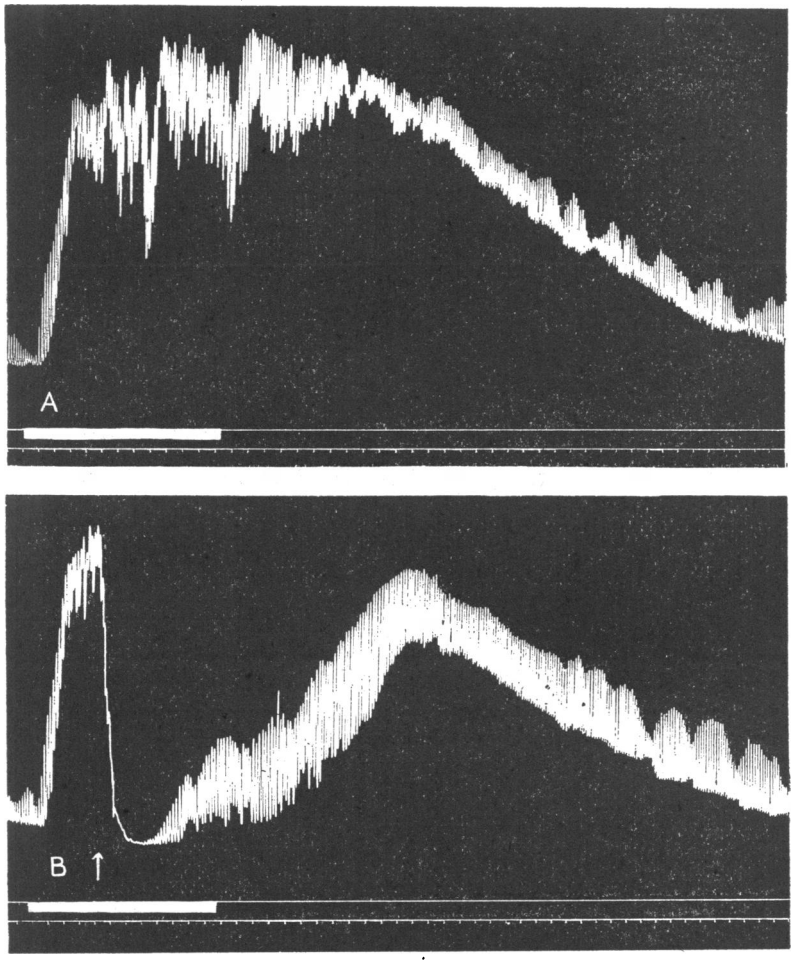

Fig. 18. Rabbit's intestine in 35 c.c. glucose-free Tyrode solution; both lumen left open. A and B = 20 mg. glucose for 6 min. At the arrow 50 μg. atropine until end of tracing. Time in 30 sec.

The suspended cooled preparation of the intestine

A preparation suspended in glucose-free Tyrode solution after having been kept in such a solution at −1° C. for 24–72 hr. reacts differently from a freshly suspended preparation. Apart from the fact that the

spontaneous activity is weak or even absent it responds differently to acetylcholine, eserine and glucose. Roughly speaking the responses resemble those obtained on fresh preparations suspended in Tyrode solution containing glucose.

The sensitivity to acetylcholine is of the same order as that of fresh preparations, but it is difficult to render the muscle inexcitable by repeated administration of acetylcholine. The muscle remains contracted as long as the acetylcholine is in contact with the gut (Fig. 19 A, D); even after large doses contraction is at least partially retained (Fig. 19 E, H), and when they are given at short intervals no intensification of the state of

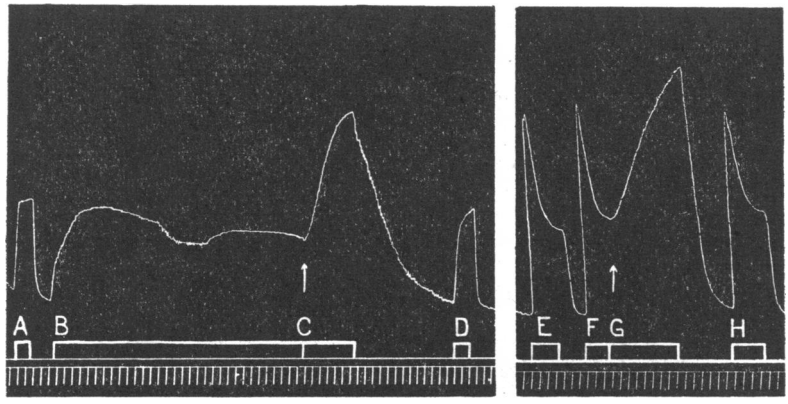

Fig. 19. Rabbit's intestine in 14 c.c. glucose-free Tyrode solution: cooled for over 72 hr.; both lumen left open. A and D = 2 μg.; E, F and H = 5 μg. acetylcholine chloride. B = 10 μg. eserine sulphate. C = 30 mg. glucose without washing out eserine. G = 30 mg. glucose without washing out acetylcholine. Time in 30 sec.

decreased excitability is brought about. Only when successive doses are given without washing out the previous ones relaxation of the muscle may occur. The response to eserine also shows the difficulty of rendering the longitudinal muscle inexcitable despite the absence of glucose from the bath. The longitudinal muscle of the fresh preparation, in this condition, does not contract at all to eserine or contraction is maintained for a few seconds only. On the cooled preparation eserine causes a sustained contraction (Fig. 19 B), although the effect is weaker and more delayed than that of a similar dose on a fresh preparation in the presence of glucose.

On the cooled preparation glucose had no stimulating action, large doses causing some immediate inhibition of tone. Cooling a preparation for 4–5 hr. did not abolish the stimulating effect but reduced it. After

cooling for 12 hr. or longer stimulation was no longer obtained. But even on such a preparation it became possible to demonstrate the effect if the glucose was given during an eserine or acetylcholine contraction. In the experiment of Fig. 19 the cooled gut had been suspended for over 2 hr. in glucose-free Tyrode solution. During this period glucose, given in doses of 25–30 mg., never caused contraction although the muscle responded well to 0·2 μg. of acetylcholine. At B 10 μg. of eserine were given. After 16 min., during which the muscle remained contracted, 25 mg. of glucose were given (C) without washing out the eserine; a further contraction ensued starting after a latency of about 20 sec. and continuing until the glucose and eserine were washed out. At G is seen the effect of glucose during an acetylcholine contraction. In this experiment glucose did not increase the excitability of the muscle to subsequent doses of acetylcholine. In others excitability increased, but the effect was small and of short duration.

The cooled preparation in its reactions to eserine and acetylcholine did not behave like a muscle deficient of glucose, and in that case no stimulating action of glucose might be expected. Even a fresh preparation has to be suspended for some time in glucose-free solution before glucose exerts its stimulating effect. The powerful rhythmic contractions during this period apparently lead to the gradual depletion of the energy stores of the longitudinal muscle. It seemed possible that this condition was never really attained in the cooled preparation where there is only weak rhythmic activity. To test this possibility we tried to deplete a fresh preparation of its energy stores before cooling it. In the experiment of Fig. 20 a piece of intestine was suspended in glucose-free Tyrode solution (first column of the figure). After about 1 hr., 50 mg. of glucose were added for 1 min. (A). The strong contraction which raised the lever beyond the upper border of the tracing started after a latency of 25 sec. When the muscle had again relaxed the preparation was placed in a glucose-free Tyrode solution at $-1°$ C. and kept there for over 24 hr. when it was re-examined. Acetylcholine did not produce sustained contractions of the longitudinal muscle and the responses decreased with repeated administration. Glucose had retained its stimulating action although the response was altered. There was a latency of several minutes during which slight inhibition occurred, the contraction proceeded more gradually and lasted much longer. The contraction following the first dose of glucose again had raised the lever beyond the upper border of the tracing, but the response diminished with subsequent administration of glucose, the effect of the third dose being shown at B. The prolongation

of the response is no new phenomenon. Even on a fresh preparation the stimulating action of glucose in time becomes more prolonged, particularly after repeated administration. The long latency may be explained by the fact that the amounts of acetylcholine and choline liberated from a cooled preparation are relatively small, since the latency was at once shortened when the glucose was given in the presence of a weak concentration of acetylcholine or eserine. In the experiment of Fig. 20 the contractions to 1 μg. of acetylcholine were not maintained and that at D was weaker than that at C. Without washing out the acetylcholine 50 mg. of glucose were added at E; the ensuing quick contraction started after a latency of 15 sec. The glucose and acetylcholine were washed out after 1 min.,

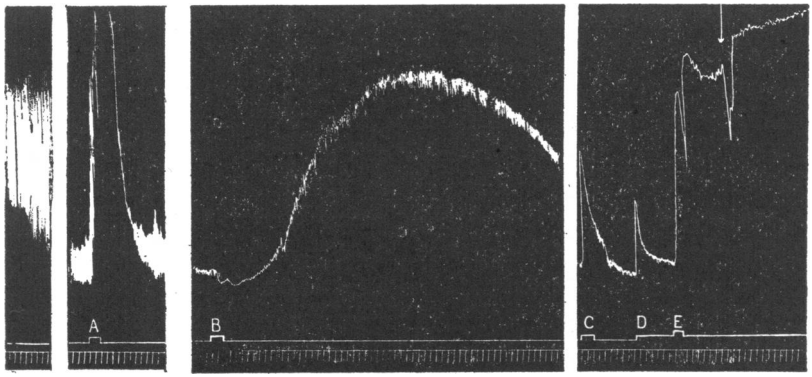

Fig. 20. Rabbit's intestine in 60 c.c. glucose-free Tyrode solution; cooled after A for over 24 hr. A, B and E = 50 mg. glucose for 90 sec. C and D = 1 μg. acetylcholine chloride; the acetylcholine at D till end of tracing. At the arrow, washing. Time in 30 sec.

causing evanescent relaxation until fresh acetylcholine was added to the bath. It was more than 40 min. before the muscle had relaxed again. During the whole time the acetylcholine concentration in the bath was kept constant. Eserine had a weak stimulating action on this preparation, and glucose given during its presence caused a strong contraction of the longitudinal muscle after a latency of 15 sec.

Perfusion of the intestine

In the beginning of the perfusion the gut exhibited some tone and rhythmic activity, but after a time it relaxed completely and showed no movements. This stage was reached earlier when the perfusion fluid contained no glucose. At the beginning the greater part of the fluid was collected from the venous cannula, but as perfusion continued the leakage

from the lumen increased and sometimes amounted to more than half of the outflowing fluid.

The effluent from perfusions with eserinized solutions caused contraction of the rectus muscle of the frog whether glucose had been added to or was absent from the perfusion fluid. Usually the stimulating effect was strongest in the early samples, decreasing after 30–60 min. of perfusion, at first quickly and then more and more slowly. The contraction of the frog rectus resulted in part from acetylcholine, in part from choline and from an unidentified substance or substances. Destruction of acetylcholine by NaOH abolished only part of the stimulating action, and the fluid then assayed on the rectus muscle against choline yielded a higher choline value than the choline equivalent obtained by acetylation of the sample. For instance, in Exp. 3 of Table 1 the perfusate collected during 1 hr. perfusion contained 4·5 μg. of acetylcholine. After destruction of the acetylcholine by NaOH the perfusate was assayed against choline on the rectus muscle. If the alkali-resistant stimulating principle had been wholly choline the perfusate would have contained 11·9 mg., but the choline equivalent obtained by acetylation was 2·1 mg. The unaccounted part must therefore have been due to the presence in the fluid of an unidentified principle, which need not have had a stimulating action but may have sensitized the muscle to choline. The discrepancy between the choline estimates obtained by acetylation and by direct assay of the perfusate was usually greatest in samples which showed strong frothing when air was bubbled through them.

Two series of experiments were carried out, the results of which showed that glucose apparently did not influence the output of choline and acetylcholine from the intestine. Perfusions were started without glucose. After 1¼–2 hr., when the output had come to a relatively steady level, 50–200 mg. of glucose (or of pyruvate) were injected into the arterial cannula or the perfusion fluid was changed over to one containing 0·1% of glucose. This caused a powerful stimulation of the gut which continued for several minutes and then disappeared even if the perfusion was continued with glucose. The change over to glucose containing perfusion fluid did not increase the output of stimulating substances, and if their concentrations had been declining they continued to do so. The injections of glucose or pyruvate sometimes produced a slight evanescent increase, but in other experiments the opposite effect was obtained. Similar slight changes were obtained after an injection of a few c.c. of glucose-free solution.

In other experiments the intestines were perfused either with or

without glucose, perfusate was collected during 1 hr. perfusion and assayed for acetylcholine, choline and the unknown stimulating principle. The latter was expressed as choline and was obtained by subtracting the choline equivalent obtained by acetylation from the value obtained by assaying the alkali-resistant effect of the sample against choline on the frog rectus. The collection of the effluent was started 40 min. after tying in the arterial cannula. In the first experiments the stimulating effect appeared to be more pronounced when glucose was absent from the perfusion fluid. Later experiments did not confirm this result and showed that there were great individual variations. Table 1 gives the results

TABLE 1. Output of acetylcholine, etc., during one hour's perfusion from 100 cm. small intestine

Exp.	Perfusate contains	Equivalent of acetylcholine chloride in μg.			Equivalent of choline chloride in mg. by acetylation			Unidentified substance expressed in mg. of choline chloride			Length of perfused piece in cm.
		a	*b*	(*a*+*b*)	*a*	*b*	(*a*+*b*)	*a*	*b*	(*a*+*b*)	
1	Glucose, eserine	0·5	1·0	1·5	0·7	0·8	1·5	0·1	0	0·1	97
2	Glucose, eserine	1·5	2·0	3·5	0·5	1·0	1·5	0·3	0·5	0·8	138
3	Glucose, eserine	0·5	4·0	4·5	0·4	1·7	2·1	3·6	6·2	9·8	103
4	Glucose, no eserine	—	—	—	0·6	1·0	1·6	1·8	1·8	3·6	75
5	No glucose, eserine	0·9	0·9	1·8	0·8	1·0	1·8	3·9	2·6	6·5	105
6	No glucose, eserine	1·8	3·7	5·5	0·7	0·7	1·4	1·1	1·5	2·6	94
7	No glucose, eserine	2·2	3·0	5·2	1·0	1·9	2·9	1·1	2·4	3·5	108
8	No glucose, no eserine	—	—	—	0·4	1·8	2·2	0·9	2·8	3·7	96

obtained from eight experiments. The perfusate collected from the venous cannula and from the lumen were assayed separately. The results for the venous perfusate are given in the columns *a*, those of the fluid from the lumen in the columns *b*. The results are calculated for 100 cm. length of intestine, the actual length of the perfused part being given in the last column.

DISCUSSION

Increased synthesis and liberation of acetylcholine or choline is not the cause of the stimulating action of glucose on the longitudinal muscle. If that were so any increase in the concentration of acetylcholine around the muscle should have a similar effect, but acetylcholine added to the bath was unable to produce sustained contraction, and large doses given

at short intervals rendered the longitudinal muscle inexcitable. Similarly, if the acetylcholine which is continuously liberated from the intestine was allowed to accumulate by using eserine to prevent its destruction no contraction or only an evanescent one occurred on the longitudinal muscle. The circular muscle, however, contracted, a fact which may be taken as evidence that accumulation of acetylcholine took place in this condition. Direct evidence for our conclusion was provided by the perfusion experiments. Glucose, despite its stimulating effect, caused no increase in the output of choline or acetylcholine, and the continuous output of these substances was of the same order when perfusion was carried out with a solution free of glucose or containing it. Since increased synthesis could have a stimulating effect only if the substances were also liberated in increased amounts the results exclude an increase in liberation as well as in synthesis. The observations on cooled preparations appeared at first more difficult to interpret. The failure of glucose to stimulate the intestine after it had lost the ability to synthesize acetylcholine suggested a close connexion between the two mechanisms. The failure, however, could be attributed to the fact that the energy stores of the longitudinal muscle did not become depleted in a cooled preparation in which the muscle exhibited only weak rhythmic activity. If such a depletion was brought about before the cooling, glucose retained its stimulating effect.

Our results are in agreement with the concept that the normal tone and rhythmic activity are due to the inherent property of the longitudinal muscle but are greatly enhanced by and partly dependent upon the continuous release of choline and acetylcholine. Glucose stimulates the muscle by supplying the chemical energy necessary for the restoration of the normal activity and tone and by making it sensitive to the released acetylcholine and choline. Neither of these factors alone would explain all our observations. The lack of a strict parallelism between sensitivity of the longitudinal muscle to acetylcholine and its tonic and rhythmic activity shows that increased excitability is not the sole cause of the stimulating effect of glucose. The first effect is in fact an increase in excitability without change in activity or tone, since doses of glucose too small to have a stimulating effect were able nevertheless greatly to increase the sensitivity of the muscle to acetylcholine. The effect of phloridzin also stresses the role of glucose as energy source for the muscle fibre. It completely suppressed the stimulating action of glucose but did not influence the acetylcholine contraction of a preparation suspended in glucose-free Tyrode solution. The effects of atropine are best explained

if both factors are taken into account. The immediate relaxation of tone and diminution or cessation of rhythmic activity it produces when given at the height of a strong stimulating effect of glucose may result from a sudden abolition of the effect of the continuously liberated choline and acetylcholine. The reappearance of tone and activity, despite the fact that the atropine remains in contact with the gut and that added acetylcholine remains ineffective, could be explained as due to the inherent property of the muscle fibre for which the glucose supplies the necessary energy. Experiments of Neukirch & Rona [1912] on the rabbit's heart can be explained only on the assumption that glucose provides the necessary chemical energy. Glucose stimulates the perfused heart although it is inhibited by choline and acetylcholine. It is unlikely that the mechanism for the stimulating action differs fundamentally from that of the intestine.

On the other hand, some observations clearly show that under certain conditions glucose stimulates the intestine only if sufficient acetylcholine is present. On cooled preparations it was often necessary to add acetylcholine or eserine to the bath in order to demonstrate the stimulating effect of glucose or the effect could be enhanced and accelerated by this procedure. On fresh preparations suspended in such a way as to record volume changes the stimulating effect of glucose sometimes disappeared with repeated administration. In such a condition it reappeared when the glucose was given with eserine or acetylcholine, although these substances alone had no stimulating effect on the longitudinal muscle.

The increase in the response to repeated administration of glucose may be due to the fact that the energy stores in the muscles are used up gradually, or more likely to the fact that the muscle becomes gradually more sensitive to the released choline and acetylcholine. This assumption is in agreement with observations on changes in sensitivity often seen on smooth muscle preparations. A gradual increase in tone of the longitudinal muscle which we have sometimes observed on preparations suspended in glucose-containing Tyrode solution could be explained similarly.

The conception that glucose exerts a stimulating action when a muscle has been more or less depleted of its energy stores provides a plausible explanation for the lack of a strong stimulating action on the circular muscle, which, unlike the longitudinal, exhibits little spontaneous activity. The muscle may therefore retain sufficient energy reserves for a long time, even in the absence of glucose from the bath, being comparable in this respect to the longitudinal muscle of a cooled preparation.

The stimulating action of the various sugars and of pyruvate must be

explained in the same way as that of glucose, since in its presence their stimulating action was only slight. The fact that phloridzin inhibits pyruvate, although less than glucose, cannot be explained on the known action of phloridzin. The inhibition of the action of glucose can be attributed to inhibition of the first phosphorylation stage; there is no evidence that phloridzin has a similar action on the oxidation of pyruvate. Unlike the various sugars and pyruvate, lactate appears to be unable to replace glucose for the functioning of the longitudinal muscle, or it has this property only to a slight extent. Its stimulating effect on the longitudinal muscle must be regarded as a direct effect. Otherwise it would not be more pronounced when glucose is present in the suspension fluid and so often disappear when, in the absence of glucose, the muscle becomes more or less depleted of its energy stores. The strong stimulating action which lactate exerts on the circular muscle must also be regarded as a direct effect. If we compare the possible role of lactate in the metabolism of the active longitudinal muscle of the rabbit's intestine with that of the heart muscle a striking difference is evident. The heart not only readily oxidizes lactate but actually prefers it to glucose [Evans, Grande & Hsu, 1935].

There are some differences and parallelisms between the actions of the various sugars and related substances on the longitudinal muscle of the intestine and in their ability to promote synthesis of acetylcholine in brain tissue or in a perfused sympathetic ganglion where the synthesis resulted in restoration of conductivity across the ganglionic synapse. On the intestine galactose was 2–3% and mannose 25–30% as active as glucose, whereas the respective figures on the ganglion were 5 and 60%. Laevulose had no effect in restoring conductivity in the ganglion, it had a doubtful effect in accelerating synthesis of acetylcholine in brain tissue, and a slight stimulating effect on the longitudinal muscle of the intestine. Lactose and maltose did not stimulate the intestine nor did they restore conductivity in the ganglion or accelerated synthesis of acetylcholine in brain tissue. On the other hand, pyruvate, which on the intestine was many times more effective than glucose, was less than half as active on the ganglion. The greatest disagreement was observed with regard to lactate. Its activity in accelerating synthesis of acetylcholine appeared to be equal to that of pyruvate in the ganglion and brain tissue, whereas it was unable to replace glucose in the intestine. Different sugars and allied substances appear, therefore, to vary in their ability to replace glucose in different physiological processes.

According to Kahlson & MacIntosh there are definite differences in

the latencies of the restorative effects of pyruvate, glucose and galactose, that of pyruvate being shorter and that of galactose longer than that of glucose. Similar differences occurred in the latencies of the stimulating effects of these substances on the intestine suggesting identical metabolic events for the substances in both processes. Kahlson & MacIntosh have suggested that glucose may act after conversion into pyruvate and galactose after conversion into glucose.

Summary

1. Preparations of the isolated small intestine of the rabbit were suspended in Tyrode solution, with and without glucose, and the reactions of the longitudinal and circular muscle were studied and recorded separately. The following observations were made:

(*a*) *Acetylcholine*, *muscarine* and *eserine* stimulate both the longitudinal and circular muscle. The effect on the longitudinal muscle disappears in glucose-free solution, whereas that on the circular muscle persists and is then the sole response to be obtained.

(*b*) *Glucose* and *pyruvate*. In glucose-free Tyrode solution the longitudinal muscle relaxes, its rhythmic activity diminishes or disappears and acetylcholine no longer causes sustained contractions. Following repeated administration of acetylcholine at short intervals the muscle may become insensitive to it and to other stimulating substances. In this condition glucose and pyruvate greatly stimulate the muscle, and the effect is followed by a period of increased excitability of the muscle. On the circular muscle the stimulating effect of glucose and of pyruvate is small and irregular. Glucose and pyruvate added to a preparation suspended in Tyrode solution containing glucose cause slight inhibition followed by slight stimulation of the longitudinal muscle.

(*c*) *Laevulose* had a slight stimulating effect on the longitudinal muscle, that of *galactose* was 3–4% and that of *mannose* 25–30% that of glucose. Large doses of these sugars produced slight immediate inhibition which was best seen in the presence of glucose. *Maltose* and *lactose* had no stimulating action.

(*d*) *Lactate* differs in its action from glucose or pyruvate in the following ways: (1) it stimulates mainly the circular muscle, (2) this effect is not influenced by glucose, and (3) stimulation of the longitudinal muscle is more pronounced in the presence of glucose than in its absence. In this condition it renders the longitudinal muscle less sensitive to the stimulating action of glucose.

(*e*) *Phloridzin* inhibits the stimulating action of glucose and to a

smaller extent that of pyruvate. In the absence of glucose it has no effect on the muscle and does not influence its responses to lactate or acetylcholine.

(*f*) *Atropine* given during the action of glucose may cause complete relaxation of the longitudinal muscle and cessation of its rhythmic activity. The effect soon disappears even if the atropine is left in the bath and the muscle insensitive to added acetylcholine. Small doses of atropine abolish the stimulating effect of acetylcholine and of muscarine on the longitudinal as well as on the circular muscle.

2. The stimulation of the longitudinal muscle by glucose is not due to increased synthesis of acetylcholine or choline in the intestinal wall. It is due to the fact that glucose supplies the chemical energy necessary for the tonic and rhythmic activity of the muscle and makes it excitable to the acetylcholine and choline continuously released in the intestinal wall. This conclusion is based (1) on the response of the muscle to acetylcholine in the absence of glucose, (2) on the fact that glucose does not influence the release of acetylcholine and choline from the perfused gut, and (3) on the observation that a cooled preparation which has lost its property of synthesizing acetylcholine is stimulated by glucose provided its reserves of chemical energy have been depleted before the cooling. The relative inability of glucose to stimulate the circular muscle may be explained by the fact that this muscle lacks powerful spontaneous activity and therefore does not become depleted of its energy stores in the absence of glucose. Pyruvate, mannose and galactose can replace glucose in its action on the intestine and their stimulating effects must be explained in the same way as that of glucose. This does not apply to the stimulating actions of lactate which must be regarded as direct effects.

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