METABOLISM OF SMOOTH MUSCLE

by

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I. INTRODUCTION

I. INTRODUCTION.

This work was undertaken to find out the relation between the mechanical response of smooth muscle and its metabolism. In the beginning of this work the spontaneous activity of the intestinal muscle was studied under different conditions, but later on it was found that under suitable conditions the cat's colon free from mucous coat could be electrically stimulated without injuring the tissue. This gave a very constant response which was favourable for the study of the effect of such conditions as asphyxia, iodo-acetic acid (I.A.A.) poisoning, etc. The observations made with this preparation are qualitatively similar to those made with spontaneously contracting muscle, but the former method presents far more accurate quantitative measurements of the effects of stimulants and depressants.

The chief object of the investigation was to determine the extent to which plain muscle was dependent on carbohydrate metabolism for the maintenance of its mechanical activity. The muscle was/

was studied under four sets of conditions, namely:

(a) Normal oxygenated muscle. In this case the muscle can oxidise both carbohydrate and non-carbohydrate material.

(b) Muscle poisoned with iodo-acetic acid. In this case the muscle can only oxidise non-carbohydrate material.

(c) Normal muscle deprived of oxygen. In this case the muscle can obtain energy by glycolysis and by phosphagen breakdown.

(d) Muscle poisoned with iodo-acetic acid and deprived of oxygen. The breakdown of phosphagen is the only known source of energy in this condition.

Relatively little work has been done on the metabolism of plain muscle and hence it is necessary to consider the outstanding facts established regarding the metabolism of other forms of muscle.

<u>Frog's skeletal muscle.</u>

An enormous amount of work has been done with this preparation, but most of this work has been done under very specialised conditions, namely on muscles suspended in nitrogen and stimulated frequently.

The/

The work of chief interest in relation to plain muscle is that which has been done on muscles suspended in Ringer's fluid and stimulated sufficiently infrequently to permit the escape of the products of metabolism.

Hill and Kupalava (1929) studied the frog's skeletal muscle and found that it could be stimulated in oxygenated Ringer's solution for many hours without showing signs of fatigue; while under anaerobic conditions the contractions ceased when either the carbohydrate store was exhausted or when the concentration of lactic acid in the muscle reached 0.3 per cent. They also found that glucose added could be used by muscle under anaerobic conditions.

Kerley (1931) estimated the total carbohydrate and the lactic acid contents in the frog's skeletal muscle. She found the resting total carbohydrate from 0.58 to 1.7 per cent. and the resting lactic acid from 0.008 to 0.055 per cent. After incubation for 3 hours at 37°C. in Ringer's solution she observed a loss of carbohydrate from 0.4 to 1.6 per cent. and a production of lactic acid from 0.25 to 1.0 per cent. These figures show that the frog's skeletal muscle possesses a large store of labile carbohydrate/

carbohydrate.

The work done on skeletal muscle stimulated rapidly in nitrogen led to the belief that the muscle used only carbohydrate as fuel. Recent work has, however, shown that this is incorrect. Ochoa (1930) reported a series of experiments on the oxygen consumption and the carbohydrate content of muscles stimulated at the rate of one stimulus per minute for 18-30 hours. He found that there was sufficient carbohydrate in the normal muscles to account for the calculated disappearance of carbohydrate. However, if the sugar content was reduced by insulin convulsions, the total energy developed was greater than could have been supplied by the complete oxidation of the available carbohydrate in the muscle. Under anaerobic conditions the lactic acid formed corresponded to the carbohydrate originally present.

Gemmill (1935) found that when frog's isolated sartorii were stimulated nine times a minute for periods of 3-6 hours in oxygenated Ringer's solution, the average utilisation of carbohydrate accounted for only 42 per cent. of the total energy exchange as obtained from the oxygen consumption. Therefore he concludes that other material than carbohydrate is/

is oxidised to supply energy for contraction of isolated frog's muscle under aerobic conditions.

<u>Cardiac Muscle.</u> Clark, Gaddie and Stewart (1931) found that the frog's heart under aerobic conditions used a mixed diet and could reduce the total carbohydrate from 1.4 per cent. to 0.73 per cent. in 24 hours; but it did not use glucose when this was added to the perfusing fluid. They also found that the frog's heart deprived of oxygen utilised carbohydrate by turning it to lactic acid. This process was arrested by acidity. They further observed that this behaviour depended on the buffers present in the perfusate. When the fluid volume was small and unbuffered the heart was arrested rapidly in about 20 minutes, while with a large amount of buffered fluid the heart continued to function anaerobically for hours and only became arrested when a considerable proportion of the total carbohydrate had been utilised. Glucose added to the fluid when the heart had ceased to beat anaerobically, could be utilised, as was shown by Freund and König (1927) and confirmed by Clark, Gaddie and Stewart (1932).

Clark, Eggleton and Eggleton (1932) showed that heart muscle poisoned with I.A.A. and provided with/

with oxygen can function for many hours. Such a muscle does probably not form lactic acid. It is uncertain whether it oxidises carbohydrates.

Smooth muscle. In general it may be said that my investigations show that the metabolic processes of smooth muscle show a closer resemblance to those of cardiac than of skeletal muscle although the recent work on skeletal muscle indicates that the difference between skeletal and cardiac muscle is not as great as was formerly supposed.

Scheme of Investigation.

The research work carried out by the author can be divided under the following headings:-

I. The Effects on the Spontaneous Mechanical Activity of Smooth Muscle of the Following Influences:-

(1) Asphyxia in absence of glucose.

(2) Asphyxia in presence of glucose and of other carbohydrates.

(3) Asphyxia in fluids at varying pH.

(4) Poisoning with I.A.A.
  (a) under aerobic conditions.
  (b) under anaerobic conditions.

(5) Poisoning with cyanide.

(6) Washing out of muscle by change of fluid.

II. /

II. The Effects on the Mechanical Activity of Electrically Stimulated Smooth Muscle of the Influences enumerated under I.

III. Determination of the Amounts of Total Carbohydrate and Lactic Acid under the Following Conditions:

(1) Resting or initial value.

(2) After aerobiosis and anaerobiosis at 37°C.

(3) In condition of "rigor".

(4) After aerobiosis and anaerobiosis in glucose-Ringer's solution.

(5) After I.A.A. poisoning and aerobiosis with and without glucose.

II. METHODS.

II. METHODS.

Removal of tissue.

The tissues used were rabbit's gut (mostly ileum) and cat's colon. The rabbits were killed by breaking the neck and cats were stunned by an electro-lethaler and were decapitated during unconsciousness. The effects of anaesthesia were thus avoided in the majority of cases. In a few experiments the tissue was removed under ether anaesthesia for comparative study. The tissues were kept after removal in alkaline Locke's fluid surrounded with ice in case of mechanical experiments and they were at once thrown in frozen Locke's solution in case of biochemical experiments.

Removal of mucous coat.

The object of the experiments was to study smooth muscle and not the glandular tissue and hence it was necessary to remove the mucous membrane. Rosenthal and Lasnitzki (1928) found that the oxygen consumption of the smooth muscle of the rabbit's colon was 2.64 c.c. per g. dry weight per hour (= 0.53 c.c. per g. wet weight per hour), while the/

the oxygen consumption of the colon mucosa was about four times as great. This result shows that in metabolic studies it is essential to use smooth muscle free from mucosa, since when both are present the results will express chiefly the changes occurring in the mucosa. The removal of mucous coat was done as follows:-

(a) Cat's colon: The mucous coat was snipped off with a sharp pair of scissors under ice-cold Locke's fluid.

(b) Rabbit's gut: A glass tube with a bore of the size of the gut's lumen and about four inches in length was selected and both its ends were rounded smooth.(See Fig. 1). The gut was introduced from one end and it easily slipped in and came out through the other end. A longitudinal slit was made in the gut wall at this end and then this portion could be easily turned over on the glass tubing with the lumen turned out. By gentle traction the gut slips over the glass tube very easily and with a sharp blade of safety razor the mucous coat could be scraped off under frozen Locke's solution. On pulling out the gut through the entrance-end of the tube the gut is again inverted. On lifting the piece of the gut against light the scraped portions could be easily distinguished from/

from unscraped portion. In this way the desired length of the scraped gut was taken. This method was found much quicker than the usual method of spreading the slit-opened gut on a piece of cork-board and then scraping it.

Mechanical Experiments.

A modified Trendelenburg's technique was employed for the gut of the rabbit in certain experiments in which the complete gut was used. In the case of strips of muscle these were suspended, as described by Burn and Dale (1922). The arrangement of bath and thermostat used is a modification of that described by Burn and Dale (1922). The two glass baths in which the tissues were suspended had a capacity of 25 c.c. each and a device for filling up and emptying these two baths was arranged to allow quick filling with fluid at the temperature of the outer jacket.

A cylindrical glass container of about 100 c.c. capacity, which lies submerged in the thermostat bath, always holds warm Locke's solution ready to be introduced into the tissue bath. This container is connected at the one end to the tissue bath and at/

at the other end to the reservoir for the Locke's fluid (kept at a high level to facilitate automatic filling of the container). In between the container and the tissue bath, there is a spring clamp with long handles; on releasing this clamp warm saline solution flows into the tissue bath. The bottom part of the tissue bath holds a T-tube, one end of it is connected to the cylindrical container and the other end to a rubber tubing going out of the thermostat bath. This outlet is controlled by a clamp and on releasing it the tissue bath can be emptied. This arrangement greatly facilitated the washing out of the tissues when passing from the effect of one substance to another, without interfering with the temperature factor. The bath was kept at 37°C. by an electrically controlled thermostat, which kept the temperature constant within 0.1°C.

Locke's Solution. All the experiments, except otherwise mentioned, were carried out in alkaline Locke's solution of the composition of: $NaCl$ = 0.9 p.c.; KCl = 0.042 p.c.; $CaCl$ = 0.024 p.c. and $NaHCO_3$ = 0.05 p.c.

Air or oxygen and nitrogen from different jets/

jets could be bubbled at required rate for aeration and anaeration.

The contractions were recorded by a frontal lever on a slow moving drum.

Biochemical Methods.

(a) Estimation of Total Carbohydrate.

The piece of smooth muscle was dried gently between the moist filter paper and the moist weight was determined on a torsion balance. The method of determining the total carbohydrate is the same as that employed by Clark et al (1931) in this laboratory for the frog's heart muscle. This method was described by Ochoa (1930). The details of the procedure are as follows:-

(1) About 350 mgm. of the muscle (on the average) free from mucous coat have been used for this determination.

(2) The weighed muscle is thrown into 5 c.c. of 6 p.c. sulphuric acid cooled overnight in a refrigerator, contained in a Jena tube (17 x 170 mm.).

(3) Small funnels are placed on the tubes to avoid evaporation, they are placed in a water bath where they are maintained at boiling point for 3 hours/

hours.

(4) After an hour of boiling the muscle is broken into pieces with a glass rod.

(5) The contents of each tube are transferred to 25 c.c. standard flask, the tubes being washed three times with distilled water, then the flasks are diluted to the mark.

(6) 20 c.c. are taken from the contents of these flasks and are put into Erlenmeyer flask of 150 c.c. capacity. 5 c.c. of mercuric reagent are added and the contents shaken.
(The mercuric reagent has the following composition: Neutral mercuric sulphate, 3 gm.; sulphuric acid 10 p.c. - to 100 c.c.).

(7) Sufficient barium carbonate is added to the flask to neutralise the contents. The end-point is determined with litmus paper. About 6 gm. of $BaCO_3$ added in three instalments of 2 gm. are sufficient to neutralise the contents.

(8) The fluid is shaken thoroughly and is allowed to stand for 20 minutes.

(9) The fluid is shaken again and then filtered into a 100 c.c. Erlenmeyer's flask. The filtration takes a long time and is continued until as much filtrate as possible is obtained.

(10)/

(10) Two drops of saturated solution of sodium sulphate are added and the flask is shaken until the cloudy portion of $BaSO_4$ is visible.

(11) A small quantity of powdered zinc dust is added to precipitate the mercury. The contents are then filtered after shaking for 15 minutes.

(12) The glucose in the filtrate is estimated by the method of Hagedorn and Jansen (1923a, 1923b). 2 c.c. of filtrate are used for this estimation.

On all occasions a blank estimation is made; no muscle is used in this but all other processes are carried out.

The total carbohydrate is expressed as amount of reducing dextrose.

Lactic Acid Estimation.

The method employed is a modification of Friedemann, Cotonio and Shaffer's method (1927), which has been used and described by Kerley (1931). The details of the procedure are as follows:-

(1) After preparing the tissue and on determining its weight as described previously, it is at once thrown into ice-cold tungstic acid in

a/

a cooled mortar. (The mortar and pestle, 2/3 N. H_2SO_4 and 10% sodium tungstate solution are all kept overnight in a refrigerator). Equal parts of the sodium tungstate solution and sulphuric acid are mixed freshly for each estimation. The tissue is pulverised quickly with silver sand.

(2) The content is made up to 25 c.c. in a measuring flask and is then filtered.

(3) To 20 c.c. of the filtrate, 4 c.c. $CuSO_4$ (7% solution) and 4 c.c. calcium hydroxide (10% solution) are added. The content is shaken up and allowed to stand for 30 minutes.

(4) The content is filtered and 10 c.c. of the filtrate is used for lactic acid estimation.

(5) 10 c.c. of filtrate is put into a micro-Kjeldahl flask and 2.5 c.c. of manganese sulphate solution are added. (10 p.c. $MnSO_4$ in 10 N. H_2SO_4). The contents are heated to boiling, and when boiling commences N/200 $KMnO_4$ solution is added in drops until oxidation is complete. This is indicated by the solution turning a deep brown colour. The distillation is continued 5 minutes longer. All the distillate is trapped in 20 c.c. of N/10 sodium sulphite ($NaHSO_3$) solution.

(6)/

(6) The excess of $NaHSO_3$ is titrated with N/10 iodine solution, starch being used as the indicator.

(7) Then the acetaldehyde-bisulphite compound is decomposed with 10 c.c. of saturated solution of sodium bicarbonate.

(8) The liberated $NaHSO_3$ is titrated with N/200 iodine solution.

(9) Control experiment is made each day with a standard solution of lithium lactate.
(Standard solution of lithium lactate = 0.0533 g. per 100 c.c. 1 c.c. contains 0.5 mgm. lactic acid).

III. EXPERIMENTS WITH SPONTANEOUSLY CONTRACTING GUT MUSCLE.

III. EXPERIMENTS WITH SPONTANEOUSLY CONTRACTING GUT MUSCLE.

Effect of Asphyxia on Smooth Muscle.

Literature.

Clark and Gross (1923) found that cessation of oxygen supply or substitution of nitrogen for oxygen caused an immediate loss of tonus in the isolated intestine but that the pendulum movements continued for a long time. Hoskin and Hunter (1924) found that intestinal segments of young adult rats when suspended in alkaline Ringer's solution at 37°C. remained inactive but that bubbling of oxygen stimulated them to activity. Mikuliez-Radecki and Lueg (1924) working with the uteri of rabbits and cats in vivo found that with asphyxia there were temporary increases in tonus and rhythmicity which were soon followed by diminution in both. Garry (1928) found that lack of oxygen caused in the great majority of cases a rapid fall in tone of excised surviving visceral muscle and the fine rhythmical movements exhibited by the smooth muscle persisted for a time in absence of oxygen, but that these also finally disappeared. Crisler, van Liere and Booher (1932) working/

working with dog's stomach in vivo found decreased movement with oxygen lack. Gross and Clark (1923) also found that asphyxia abolished the response of the gut to adrenaline and pilocarpine, and Schmitt and Nicoll (1933) have shown that cyanide, hydrogen sulphide and carbon monoxide inhibit the response of isolated intestine to a variety of drugs that normally cause tonic contraction.

Results obtained by author - Fig. 2 shows the effect of asphyxia on the spontaneous activity of gut. The rabbit's stomach (Fig. 2.A.) is paralysed more rapidly than is the rabbit's duodenum (Fig. 2.B) or the rabbit's ileum (Fig. 12), and the cat's colon (Fig. 2.C) is paralysed much more slowly. The essential effects are an immediate fall in tonus, followed by a continuous decrease in the amplitude of the contractions, which terminates in arrest. It is interesting to note the appearance of "gradient phenomena" (described by Alvarez) in the cessation of movements under oxygen lack. A summary of different "gradient phenomena" observed by different workers is shown in Table I.

Effect /

Effect of Carbohydrates and Non-carbohydrate Substances on Revival of Mechanical Activity under Oxygen-Lack.

Literature.

Skeletal Muscle - Hill and Kupalav (1929) observed that frog's skeletal muscle ceased to contract under oxygen lack either when its lactic acid concentration reached 0.3 per cent. or when its available carbohydrate store was exhausted. Under the latter condition, addition of glucose revived the power of contractions.

Cardiac Muscle - Freund and König (1927) observed that the anaerobically arrested frog's heart could be revived with addition of glucose. This was confirmed by Clark, Gaddie and Stewart (1932). Further Gaddie and Stewart (1934) tried revival of such arrested hearts with different carbohydrates, carbohydrate derivates and non-carbohydrate substances. They found that complete revival was obtained with glucose and mannose only.

Smooth Muscle - Rona and Neukirch (1912) tried the action of a great number of organic compounds upon the intestine. They found the great majority of them to be inactive or of small effect. Only two sugars, d-glucose and d-mannose, and the sodium salts of/

of a few aliphatic acids such as the acetate and pyruvate, stimulated the intestine when present in low concentrations.

The author has studied the effect of different carbohydrate and non-carbohydrate substances on the mechanical response of smooth muscle arrested by oxygen lack and has obtained the results described below.

(A) Glucose - Fig. 13 shows the revival of mechanical contractions on addition of glucose in nitrogen-Ringer's solution and Fig. 3 shows that if glucose is already present in the fluid, oxygen lack does not affect the contraction. The addition of 0.1 per cent. glucose to the asphyxiated gut produces a marked rise of tonus and very strong pendulum movements, which are irregular in character at first but grow more regular later on. This activity continues for about 4 hours in anoxaemic conditions. These experiments indicate clearly that the anaerobic arrest which occurs in about 30 minutes in absence of glucose must be due to the smooth muscle having a very small store of available carbohydrate.

(B) Other carbohydrates - Mannose was found to behave exactly similar to glucose. The other carbohydrates/

carbohydrates tested were (a) pentoses: arabinose, xylose; (b) hexoses: galactose, fructose; (c) disaccharides: maltose, lactose, sucrose. All of these gave negative results, which agrees with the observations made by Gaddie and Stewart (1934) on the asphyxiated frog's heart.

(C) Trioses and their derivatives - Glyceric aldehyde and dioxyacetone as well as sodium pyruvate and sodium lactate did not revive the rhythmical contractions.

(D) Non-carbohydrates - Amino acids (alanine, glycine and leucine) and also sodium oleate were found ineffective.

Behaviour of Smooth Muscle with Locke's Solution of Different pH in Oxygen and Lack of Oxygen.

Literature. Many workers have observed the effect of change of pH of the perfusing fluid on the tone and mechanical contractions of the smooth muscle in presence of oxygen.

Moderate range of pH on tone. Relaxation of tone on the acid side of pH and increased tone on the alkali side of pH within moderate change have been noted by Hatai and Hammett (1920), Hammett (1922), Evans and Underhill (1923) and Gruber (1927). Small changes/

changes of pH have been investigated by Gaskell (1880) and Bayliss (1901), who found vaso-dilatation in the frog with weak lactic acid; Gaskell also observed that alkalies contracted the arteries. Young (1914) and Botazzi (1916-17) confirmed these results on mammalian intestine, the latter also found that alkalies caused an increase of tone.

Greater range of pH on tone. With greater range of pH the observations are different. Dixon (1902) found that weak lactic acid produced relaxation of the frog's stomach and while a certain increase in strength merely accelerated this effect, solution of 1/500 caused a rapid increase of tone with subsequent slow relaxation. Wild and Platt (1902) found that acidity caused vaso-constriction in the frog but that very weak acid sometimes caused a preliminary dilatation. Fardon (1908) obtained similar effects on the mammalian uterus and observed that alkali augmented the tonus. Fleisch (1918, 1921) found that slight and strong acidity caused smooth muscle to relax and contract respectively. Contraction of smooth muscle with acid has also been observed by Hooker (1912), Ishikawa (1914), and Fraenkel and Morita (1925). McSwiney and Newton (1927) working with moderate and large ranges/

ranges of pH found that moderate changes of pH to the acid side of pH 7.5 produced relaxation and to the alkaline side a contraction of smooth muscle. But with large changes of pH to the acid side caused a contraction (pH 5.9 to 2.1), and finally relaxation (beyond pH 2.1). To the alkaline side large changes (NaOH to 0.0175 N) caused contraction and still larger changes finally relaxation.

Effect of pH on smooth muscle in vivo. Barbour and Rapoport (1921) studied the effect of pH on the uterus of a bitch in vivo. They injected 5 c.c. of 1 per cent., 5 c.c. of 5 per cent. and 10 c.c. of 5 per cent. $NaHCO_3$ and observed relaxation of the uterus with temporary inhibition of rhythmic activity. 10 c.c. injection of 10 per cent. lactic acid was without any effect, but 10 c.c. of 5 per cent. HCl produced a marked contraction of the uterus.

pH on rhythmic contractions. Fardon (1908) found augmented tonus with diminution of spontaneous movements with alkali and finally death in contraction. Acid on the contrary lowered the tone and diminished the spontaneous contractions. Young (1914) found that 0.5 per cent. HCl abolished all movements. Evans and Underhill (1923) showed that the/

the effect of small increase in the pH was to augment the frequency of contractions while a decrease in pH caused a diminution in the rate. McSwiney and Newton (1928) confirmed these findings within moderate limits of pH change.

Experimental Results

The author studied the effects of different pH on the tonus and rhythmical movements of the rabbit's gut under oxaemic and anoxaemic conditions. No record about the effect of pH on smooth muscle under anaerobic conditions was found in the literature. Similar observations have however been made on the frog's heart by Clark, Eggleton and Eggleton (1932). They found that the effect of anoxaemia on the isolated frog's ventricle perfused with various Ringer's fluids of different pH was profoundly influenced by the pH. The heart could go on beating almost normally for about an hour in buffered alkaline fluid (pH 8.5) and afterwards the strength of beats began diminishing and grew very feeble after 90 minutes. In the buffered neutral fluid (pH 7.0) the strength of beats began diminishing after a few minutes and grew feeble in 60 minutes. In/

In the buffered acid fluid (pH 6.5) and unbuffered fluid, the strength of the beat began to diminish within 3 minutes, and after 20 minutes of oxygen lack the heart was very feeble.

The author made similar observations on the rabbit's ileum. Ringer's fluid of four different pH were used: (1) buffered alkaline fluid (pH 8.2) composed of NaCl 0.9%, KCl 0.042%, $CaCl_2$ (amorphous) 0.024% and $NaHCO_3$ 0.05%. Freshly prepared solution showed pH 8.2 while one kept overnight and air bubbled for some time showed pH 8.3 to 8.5; (2) Buffered acid fluid (pH 6.0) contained no $NaHCO_3$ but 0.02% N_2HPO_4 was used for buffering and N/10 HCl was added to bring down the pH to 6.0; (3) Buffered neutral fluid (pH 7.0) had the same composition as (2); here the reaction was brought down to pH 7.0; (4) Unbuffered fluid contained no buffering substance (pH 7.2).

Figures 12, 4, 5 and 6 show the effect of fluids at pH = 8.2, 6.2, 7.0 and 7.2 respectively. The tracings show good tonicity and quicker but small rhythmical contractions in the alkaline fluid (pH 8.2), while relaxed tonus and slow but bigger amplitude are seen in acid fluid (pH 6.2). This confirms/

confirms the findings of the former workers. The rate of depression of the activity produced by anaerobiosis was however almost identical in all four cases. This is a sharp contrast to the results obtained with the frog's heart (Clark et al 1932), for in this case asphyxia produced marked depression immediately in acid Ringer's fluid whilst in alkaline fluid the heart could maintain anaerobic activity for hours.

It was found however that if glucose were present the effect of pH on the rate of asphyxial arrest was well marked. In alkaline, glucose-containing fluid the gut can function anaerobically for several hours (Fig. 3), but in acid glucose-containing fluid anaerobiosis produced arrest as rapidly as in fluids without glucose (Fig. 7). Table II shows these effects.

These results indicate that the rabbit's gut resembles the frog's heart in that it can only obtain anaerobic energy by glycolysis in an alkaline reaction.

The difference in behaviour between the two tissues probably depends on the fact that the frog's heart has a considerable store of available carbohydrate whereas the rabbit's gut has a very small store/

store.

The anaerobic activity of skeletal or cardiac muscle in glucose-free fluid may be arrested not only by carbohydrate exhaustion but also by the accumulation of lactic acid produced by the glycolysis of the tissue carbohydrate. Skeletal muscle can neutralise considerable amounts of lactic acid and its activity ceases when the lactic acid concentration reaches 0.3 per cent. Accumulation of lactic acid to the concentration of 0.15 per cent. rapidly impairs the mechanical response of the frog's heart (Clark et al, 1932) and this happens anaerobically when the perfusate is limited. Intestinal muscle, however, does not possess sufficient carbohydrate for arrest by lactic acid production to occur in presence of glucose-free fluid. Measurements of the pH of the Locke's solution (glucose-free) at the moment when anaerobic arrest of the intestinal muscle occurred showed that this was never less than 7.8.

Activity /

Activity of I.A.A. Poisoned Smooth Muscle.

Literature.

Striated muscle. **Lundsgaard** (1930) found on direct stimulation in situ of I.A.A. poisoned frog's gastrocnemius that 110-115 single isometric contractions were carried out without production of lactic acid, but that after this the contractions grew weaker and the muscle passed into contracture and full rigor.

Frog's heart. Clark, Eggleton and Eggleton (1932) and Gaddie and Stewart (1934) examined the effect of I.A.A. on the frog's ventricle, in presence and absence of oxygen (vide page 50). The latter observers also found that the effect of I.A.A. could not be reversed by washing out the ventricle.

Results with smooth muscle.

Similar phenomena were observed with the gut. Addition of I.A.A. under aerobic conditions in concentration of 1:20,000 (Fig. 8) in the bathing fluid had no action on the contractions of the gut, but anoxaemia caused rapid arrest. The I.A.A. poisoned gut arrested by asphyxia does not revive on addition of glucose but is revived by aeration. The/

The effect of I.A.A. on the gut could not be reversed by washing out with Ringer's fluid. A comparative experiment (Fig. 9) on I.A.A. poisoned gut plus glucose in one of the baths and non-poisoned gut with glucose in the other showed arrest of contractions in the former and no effect on contractions in the latter when asphyxia was produced. When oxygen was introduced the poisoned gut was revived and continued to contract for a considerable period.

Activity of Cyanide-poisoned Muscle.

Literature.

Evans (1919) found that 0.5% NaCN produced instant death of gut muscle and in lower concentration this action was identical with that of oxygen lack. Concentrations of M/200 - M/500 produced an effect similar to that produced by asphyxia on isolated frog's and mammalian plain muscle and the action was reversible. The same author working with plain muscle (1923) found that cyanides depress, but apparently do not abolish the oxygen usage of the tissue. Further he suggested that there were two kinds/

kinds of respiratory process in plain muscle, one of which is inhibited by cyanide, while the other is not. Ets and Hemwall (1933) found practically complete inhibition of all rhythmic contraction in concentration of 1:100,000 NaCN on rabbit's intestine.

Experimental Results.

Fig. 10 shows the action of NaCN on the rhythmic activity and tonus of the rabbit's gut. A concentration of 1:5,000 was found to inhibit the rhythmicity and depression in tonus was marked. Concentrations lower than that caused some depression both in rhythmicity and tonicity but never completely abolished activity. At the concentration of 1:5000 the activity was reversible on washing (Fig. 11). Further(Figs. 10 and 11) glucose has the power to revive the activity in such cyanide poisoned gut. The result confirms the observation of the former worker (Evans), namely that cyanide prevents the utilisation of oxygen but does not interfere with anaerobic glycolysis.

Effect of Insulin on Utilisation of Glucose by Smooth Muscle.

Ets and Hemwall (1933) observed that when 5 units insulin and 0.25 per cent. glucose were present, the gut did not show diminution in amplitude of rhythmical contraction when 1:1,000,000 NaCN was added. When either insulin 5 units or glucose 0.25 per cent. alone was present, addition of same amount of NaCN showed decreased rate of amplitude and also depression in tone.

Fig. 7 shows that insulin does not improve the mechanical activity of the gut in 0.1 per cent. glucose - Locke's fluid and nitrogen. A similar result was found (Fig. 10) when the anoxaemia was induced by NaCN.

I was indeed unable to find any indication that the utilisation of glucose by plain muscle was improved by addition of insulin.

Effect /

Effect of Washing on Mechanical Activity

Frog's heart. Gaddie and Stewart (1934) observed that change of perfusion fluid washed out a part of some substance necessary for proper contraction of the frog's heart, so that changing the perfusion fluid two or three times definitely hastened the exhaustion. More frequent changing, however, had no further effect. Clark, Gaddie and Stewart (1931) found that the addition of soaps to the perfusion fluid caused a rise in oxygen consumption by the isolated frog's heart without any change in the R.Q. Gaddie and Stewart (1934) found that the addition of 0.1 p.c. sodium oleate as well as sodium linoleate caused temporary recovery to the exhausted heart. They further observed that this improvement was possible once only and could not be repeated many times as in the case of recoveries with glucose. They confirmed Clark's hypothesis (1913) that the soap restores some constituent of cell which is removed by perfusion.

Effect of washing of gut. A comparative observation on the power of revival of tonus and rhythmical activity of the rabbit's gut with and without washing and/

and influence of addition of glucose and oxygenation have been made. Figs. 12 and 13 show the normal behaviour of the gut and the partial revival in tonus and rhythmicity with either glucose or oxygen alone and the complete revival with both together. Figs. 14 and 15 show similar observations after washing out the perfusion fluid in which the gut was contracting. In these cases, though the tonus is regained to the original level the rhythmical characters do not return to the previous stage. It is interesting to note that freshly mounted bit of rabbit's duodenum (Fig. 16, A and B) showed rapid loss of tone and depressed rhythmicity in oxygenated Locke's solution and addition of graded amount of glucose produced graded revival in tone as well as rhythmicity.

The gut differs from the heart in that sodium oleate does not produce any beneficial effect on its mechanical activity after this has been impaired by washing. These experiments show that both glucose and oxygen are needed for the normal activity of gut muscle and that washing out of the perfusing fluid removes some substance which is essential for this normal activity.

Weiland (1912) working in Magnus' laboratory, investigated/

investigated the action of a saline solution in which a piece of intestine had been suspended for some time. He found this saline solution to have acquired the property of stimulating a normal piece of intestine (both its rhythm and tone). He further showed the presence of this substance both in muscle as well as in mucosa. This stimulating effect he observed could be antagonised by small doses of atropine.

Le Heux (1918) isolated the active constituent of this bio-dialysate in the form of pure crystalline choline.

Magnus (1930) in the Lane Medical Lectures for 1927 concludes that choline is in any case the chief constituent of the excito-motor action on intestine, if not the only one.

IV. RESULTS OBTAINED WITH ELECTRICALLY STIMULATED MUSCLE.

IV. RESULTS OBTAINED WITH ELECTRICALLY STIMULATED MUSCLE.

Introduction.

The experiments described in this thesis were commenced with strips of gut that were allowed to contract spontaneously. This method was found unsatisfactory for obtaining quantitative estimates of the amount of depression or stimulation produced by various agents. Experiments therefore were made to see whether a satisfactory method of electrical stimulation could be devised. This was discovered and used to check the results described in the previous chapter.

The results obtained with the stimulated muscle were qualitatively the same as those obtained with the spontaneously contracting muscle, but the greater accuracy of the former method permitted the clearer demonstration of certain important points.

Literature.

Evans (1925) observed the effect of stimulation on the lactic acid content in smooth muscle from the stomach of the tortoise and the frog and the bladder of the cat. In these experiments the muscle was suspended in a moist chamber containing air or oxygen/

oxygen and was stimulated for 1 to 2 seconds at intervals of 20 to 30 seconds with a galvanic current of sufficient strength to cause maximum contractions. The current required varied from 5 to 20 milliamperes.

Winton (1926) studied the influence of length on the response to an electrical stimulus of an unstriated muscle (the retractor penis of the dog). He used a special narrow chamber in which the muscle was suspended in Burn and Dale's solution. The electrodes used were spiral in shape and were heavily silver-chloride coated. Sometimes faradic but mostly direct currents were used. The muscle was suspended by means of threads and did not touch the electrodes. Winton found that relatively strong currents were required, and that the tissue could easily be injured by an unduly intense stimulus.

Eggleton (1934) measured the effect of stimulation on the phosphagen content of the retractor muscle of the foot of Mytilus edulis. The stimulus was applied through two nickel mesh electrodes at the upper and lower ends of the muscle chamber, the stimulus reaching the muscle through the sea water in which it was bathed. Mostly alternating/

alternating current (50 cycles) at 16 volts was used as stimulus. The amount of current passing between the electrodes was of the order of 60 milliamperes of which only a small fraction passed through the muscle itself. The stimulus either was applied continuously (for tetanising) or else was applied for 15 seconds once every minute.

Author's method of stimulating the gut muscle.

The author first tried stimulation of strips of cat's colon with D.C. current. A stimulus of 50 volts for 3 seconds every minute was found inadequate to produce good responses. On increasing the duration of stimulus to 6 seconds the amplitude of contraction was improved at first but began to fail after a short time. The voltage was increased to 70 and the response was found adequate for the purposes of the investigation. This high intensity of current was found however to produce injury to the muscle unduly. This was shown by abnormal behaviour and by irregular responses to the changed environments. Polarisation of the electrodes also was found to be a serious source of inconvenience with D.C. current at this high voltage.

Alternating/

Alternating current (50 cycles) at a low voltage of about 16 to 18 was therefore tried. This gave an adequate stimulus, did not injure the tissue nor did polarisation occur. The electrodes showed blackening after a day's use but could be cleaned easily. Observations on the tissue stimulated in this way could be carried on for over six hours, and at the end of such a period the tissue still showed no signs of injury.

In order to facilitate electrical stimulation it was necessary to use a narrow tube, and the volume of the bath was therefore reduced from 25 c.c. to 10 c.c. Smaller tubes were found difficult to use because the passage of gas bubbles tended to blow the fluid out of the tube when the latter was reduced to too small a diameter.

Electrodes of coiled silver wire were used. The arrangement of the electrodes and method of suspending the muscle strip is shown in Fig. 17. One of the electrodes passes through the narrow glass tube and comes out at the other end. Oxygen or nitrogen passes through the same way for oxygenation or nitrogenation of the environment. The muscle is tied to the bent part of the bottom end of the glass tube with a thread to avoid direct contact/

contact with the electrode and polarisation. The other end of the muscle is connected with a loaded lever to give isotonic contractions. The other electrode is wrapped round the upper part of the glass tube and dips in the top part of the perfusing fluid. Thus the muscle is stimulated by passage of current through the Locke's fluid in which oxygen or nitrogen is bubbled according to the nature of the observation.

The muscle was stimulated for 6 seconds every minute with an automatic make and break arrangement fixed on a revolving plate. It was found that muscle when stimulated would lift a considerable weight, and a weight of 5 gm. was hung on the recording lever at a distance from the pivot equal to the distance between the pivot and the attachment of the muscle. This arrangement had the advantage of abolishing effects due to slight changes in tonus. The current passing between the electrodes was measured and found to be of the order of 5 to 10 milliamperes, of which only a small fraction passed through the muscle.

In this series of experiments cat's colon free from mucous coat was used.

I./

I. Effect of Asphyxia.

A freshly mounted specimen in oxygenated Locke's solution shows rhythmical contractions, as well as response to the stimuli (Fig. 18). When nitrogen is replaced for oxygen, there is slight initial rise in tonus and amplitude of contraction followed by a good fall in tonus and greatly depressed spontaneous rhythmicity. Then there is gradual loss of tone and spontaneous rhythm ceases altogether after some time. Response to the stimuli continues for some time longer but the amplitude goes on diminishing till after some time the muscle is not stimulated at all. On reverting to oxygen the response to the stimulus is again shown by a sudden rise in tonus and good amplitude of contraction. It takes about half an hour to exhaust completely the muscle for the first time. Fig. 19 shows the typical effect of asphyxia for the third and fourth time. Subsequent asphyxia was always found to produce arrest much more rapidly than does the first asphyxia. The reaction of the perfusion fluid at the point of first or successive asphyxial arrest was never found to be below pH 7.8.

The augmentation of the rate of onset of asphyxia/

asphyxia when this is repeated confirms the conclusion that the gut has only a small store of carbohydrate which is available as a supply of anaerobic energy and this is depleted by the first asphyxia (vide Biochemical Experiments).

II. Effect of carbohydrate and non-carbohydrate substances on the revival of activity in oxygen lack.

All the substances used were pure "Analytical Reagents" and methyl-glyoxal was prepared by myself. Dr Gaddie kindly supervised this work.

(a) Effect of glucose and mannose.

Maclean and Smidley (1912) found that the heart of the dog and rabbit, in presence of oxygen, utilised glucose and mannose fairly rapidly. Pomothy (1933) found glucose first and mannose next in order of the hexose group to be removed by the isolated cat's heart from the perfusion fluid in oxygen. Gaddie and Stewart (1934) observed equal mechanical revival of the arrested frog's heart in nitrogen with glucose as well as mannose.

Fig. 20(A) is a typical record of the gradual exhaustion of the muscle in alkaline Locke's fluid in/

in an atmosphere of nitrogen and of recovery on addition of glucose. The recovery on addition of glucose is a little delayed; generally it takes about 5 to 8 minutes to show the first response. Again as is seen in Fig. 20, the revived response depends on the amount of glucose added; 0.02 per cent. concentration can restart the response to the stimulus and 0.2 per cent. shows the maximum response. No further improvement is seen on further addition of higher concentration of glucose.

When recovery is complete, changing of the perfusion fluid leads to a rapid re-exhaustion of the muscle which can then be revived again by glucose or mannose. This process of alternate exhaustion and recovery can be repeated many times on the same piece of muscle. Fig. 20(B) shows the same piece of muscle exhausted after washing and the effect of mannose on revival. Here again this hexose behaves exactly like glucose. At the end of this comparative Fig. 20, it is seen that augmentation produced by mannose could not be further increased by glucose.

(b) /

(b) Other carbohydrates.

Maclean and Smedley (1912) found that the hearts of the dog and rabbit, in presence of oxygen, in addition to glucose and mannose could utilise galactose to a slight extent, but maltose, fructose and xylose, not at all. The negative result with fructose was also obtained by Steinberg (1927) using rabbit's heart muscle, and by Ashford (1933) with brain. Working with embryonic tissues under anaerobic conditions, Dickens and Greville (1932) found that some were able to convert only glucose to lactic acid, while others were able to convert fructose as well. Pomothy (1933) found some removal of fructose and still less of galactose by the isolated cat's heart from the perfusion fluid in oxygen. Gaddie and Stewart (1934) observed no revival of arrested frog's heart in nitrogen with fructose, galactose or other carbohydrates.

Experimental Results.

Fig. 21.E. shows very negligible revival of smooth muscle with fructose and Fig. 21.D. no revival with galactose. Maltose also gave negative results (Fig. 22.C).

(c) Non-carbohydrate substances.

(i) Protein - Amino acids are capable of acting as source of energy under aerobic conditions in the frog's heart, has been observed by Clark, Gaddie and Stewart (1931). Freund and König (1927) observed restoration of activity of the exhausted anaerobic heart with glycine and alanine. This conclusion was investigated by Gaddie and Stewart (1934) who obtained completely negative results. The survival of I.A.A. poisoned smooth muscle under aerobic conditions indicates that it can utilise non-carbohydrate sources of energy. Alanine and glycine were therefore tested on smooth muscle exhausted in nitrogen. The results, however, were completely negative (Fig. 22(A).)

(ii) Lipoids.- Clark, Gaddie and Stewart (1931) and Gaddie and Stewart (1934) studied the effect of lipoids (vide p. 35) and found that they did not act as a source of energy for the frog's heart.

Experimental Results - Fig. 22(B) shows the effect of sodium oleate on smooth muscle arrested in nitrogen and it was completely unable to restore the activity.

(d) /

(d) Metabolic derivatives.

Gaddie and Stewart (1934) investigated the effect of intermediate products of carbohydrate derivatives on the frog's heart. Two trioses, dihydroxyacetone and glyceric aldehyde, were tried on the frog's exhausted ventricle. The former was found without effect, but the latter revived the ventricle to a certain extend in low concentration and produced toxic effects in higher concentration.

Other breakdown products, namely methylglyoxal and sodium pyruvate were also tested. Methylglyoxal produced a sustained recovery of the heart but sodium pyruvate was without any effect.

On Smooth Muscle - In anaerobiosis sodium lactate and pyruvate (Fig. 22(B) did not produce recovery of the activity of smooth muscle. Methylglyoxal produced partial recovery (Fig. 23(A) in low concentrations, but higher concentrations proved toxic (Fig. 23(B) and if muscle was allowed to remain for a long time in contact with it the condition became irreversible with glucose or oxygen (Fig.23(B).

The substances tested under this section are summarised in Table III. The results given in Table III show glucose and mannose are utilised equally rapidly by the gut muscle, that fructose is/

is utilised to a small extent, but that the gut muscle cannot utilise any of the other carbohydrates tested. The result with possible breakdown products of carbohydrate show that gut can utilise glyceric-aldehyde and methyl-glyoxal in very low concentrations, but in higher concentration, these substances produce spasmodic contracture and injure the muscle.

III. Action of Iodo-Acetic Acid.

Clark, Eggleton and Eggleton (1932) made detailed observation of I.A.A. in different concentrations on frog's heart anaerobically and aerobically. They have observed that this drug is not a mere general protoplasmic poison, but it exerts a highly specific action on the process or structure of the heart which is only required during anaerobic activity. A concentration of 1:5,000 does not affect mechanical response under adequate aeration, but on complete removal of oxygen the heart is arrested after about 20 beats. This action is reversible within limited concentration of the poison. Gaddie and Stewart (1934) confirmed this observation and have observed that I.A.A., 1:20,000 concentration/

concentration in perfusion fluid, has little or no effect for several hours but when oxygen is excluded the heart ceases to contract after about 7 minutes. Re-admission of oxygen to the paralysed heart (together with massage if contraction has entirely ceased) causes a complete revival and this process of alternate stoppage by I.A.A. in absence of oxygen and revival by oxygen can be repeated many times.

On Smooth Muscle - This poison behaves exactly in the same manner as it does with the frog's heart; the only difference noted is that smooth muscle can survive higher concentrations of I.A.A. than can the heart. Fig. 24 shows the effect of I.A.A. 1:10,000, where cessation of activity in nitrogen and its revival in oxygen is well marked. This alternate cessation in nitrogen and revival in oxygen could be repeated many times. But if this concentration is left in the perfusing fluid (Fig. 25) or allowed to act on the muscle for about 15 minutes (Fig. 26), then on reverting to oxygen, no revival is seen. The muscle, like the frog's heart, when asphyxiated passed gradually into tonic contracture and then some relaxation. Fig. 26 well illustrates the quick cessation of all activity when total stoppage of oxygen/

oxygen is brought about suddenly. (The drum was accelerated to note the exact time of cessation in oxygen lack). The lowest time for complete cessation of activity in anaerobiosis is about 3 minutes. This suggests that this muscle must contain very little phosphagen, like the frog's heart, where also the cessation is equally quick and phosphagen P content is about 1 γ - 2 γ per average ventricle. Zanghi (1930) has estimated the phosphagen P value of smooth muscle from the gut and bladder of the avians and mammals and his normal average figure is 10-14 mgm. per 100 gm. tissue (probably with mucous coat).

Eggleton (1934) found phosphagen P (= arginine phosphoric acid) value in freshly dissected retractor muscle of Mytilus edulis to be about 11.5 mg. per 100 gm. and muscle aerated for a few hours showed an average value of 24 mgm. per 100 gm. tissue. She has observed that as in skeletal muscle appreciable quantities of phosphagen are present when the muscle is so fatigued that it fails to respond mechanically to further stimulation. (It appears that mobilisable phosphagen is very limited). But in smooth muscle as in the skeletal and cardiac muscle/

muscle, there is complete absence of phosphagen when the muscle is dead, i.e. incapable of recovery.

Arguing from the point of limited availability of phosphagen in smooth muscle the quick cessation of activity in the I.A.A. poisoned muscle can be explained by the hypothesis that the mechanism of action is the same here as it is in the frog's heart. This agrees with the finding of Eggleton and Eggleton (1929) that the phosphagen content of mammalian plain muscle is only 2.3 to 5.1 mgm. of phosphagen P per 100 gm.

When a muscle poisoned with I.A.A. is under aerobic conditions, glucose produces no beneficial effect, nor does sodium lactate (Fig. 27). When, however, the activity of the I.A.A. poisoned muscle is decreasing, then addition of sodium pyruvate can improve the mechanical response (Fig. 28).

At this stage the action of various substances was tested on the I.A.A. poisoned gut both in presence of oxygen and during asphyxia; the results obtained are summarised in Table IV.

In presence of oxygen glucose did not produce any beneficial action nor did sodium lactate (Fig. 27).

In/

In presence of oxygen sodium pyruvate (Fig. 28) and methyl-glyoxal (Fig. 29) produced a slight increase in the amplitude of contractions but in the absence of oxygen these substances produced no certain effect.

The action of methyl-glyoxal on the I.A.A. poisoned gut is of interest because Barrensheen et al (1931) showed that methyl-glyoxal accumulated in skeletal muscle poisoned with I.A.A. and Ledebur (1933) pointed out that in skeletal muscle methyl-glyoxal produced a rigor similar to that produced by iodoacetic acid. In the case of normal asphyxiated gut methyl-glyoxal in low concentration (0.01 p.c.) produced a beneficial action and in larger concentrations (0.05 p.c.) it produced a tonic contraction which might or might not be reversible (Fig. 23, A and B). In the I.A.A. poisoned gut it produced increased contractions and rise of tonus when oxygen was present, but when the gut was asphyxiated it caused immediate rigor (Fig. 29). These results support the possibility that the rigor which occurs in asphyxiated I.A.A. poisoned gut muscle may be associated with the accumulation of methyl-glyoxal. Goldenberg et al (1935) have however been unable to demonstrate any such accumulation/

accumulation in animals in vivo.

Gaddie and Stewart (1934) have shown that the sodium-iodo-acetate poisoned frog's ventricle when arrested in nitrogen could be revived with glutathione, but the recovery was never complete. They suggest that iodo-acetic acid has other effects than that of inactivating co-glyoxalase and these other effects are irreversible by any means yet discovered.

In the case of gut muscle the author was unable to detect any beneficial action produced by addition of glutathione to I.A.A. poisoned and asphyxiated muscle.

Effect of Vitamin B_1 Concentrate on the Utilisation of Lactates.

Various authors have produced evidence that the utilisation of lactates by nervous tissue depends on the presence of vitamin B_1.

Gavrilescu, Meiklejohn, Passmore and Peters (1932) found increased uptake of oxygen in vitro in the presence of lactate upon addition of vitamin B_1 concentrate in case of avitaminous pigeon's brain.

Peters and Thompson (1934) found accumulation of/

of pyruvic acid in the avitaminous tissue and its disappearance in presence of vitamin B_1.

Birch and Harris (1934) have found that lactic acid accumulates in the brain in vitamin B_1 deficiency. Influence of lactic acid in producing convulsion in vitamin B_1 deficiency has been observed by Kinnersley and Peters (1930).

Furthermore in the case of heart muscle, Evans et al (1934) have shown that the heart muscle can oxidise lactates, whilst other workers have shown that the normal cardiac function is impaired by vitamin B_1 lack.

Harris (1934) found that vitamin B_1 was needed for normal functioning of the heart muscle and was concerned in the removal and oxidation of lactic acid (a substance of the nature of co-enzyme for lactic acid dehydrogenase).

Birch and Mann (1934) found that bradycardia produced in vitamin B_1 deficient rats could be removed with vitamin B_1.

Lack of vitamin B_1 is well known to cause derangement of gut function and therefore the author made experiments to determine whether the vitamin B_1 influenced the oxidation of lactates by the gut.

Results /

Results with Smooth Muscle.

Normal (not avitaminous) cat's colon was used. The strips were mucous free and were stimulated in alkaline Locke's fluid without glucose. Effect of added sodium lactate and vitamin B_1 concentrate was studied on the change produced on rhythmic contractions and tonus of the muscle. The concentrate of vitamin B_1 was supplied by Dr Todd and contained 400 doses of vitamin B_1 per gram. On each occasion a fresh solution was prepared in alkaline Locke's fluid and the acidity was neutralised by addition of N/10 NaOH or sodium bicarbonate solution.

The gut was exhausted in nitrogen and then revived in oxygen and washed out for a few times. Addition of 0.1 per cent. sodium lactate and vitamin B_1 did not show any beneficial effect but addition of glucose showed its beneficial action. This experiment was repeated on freshly removed as well as gut removed 24-48 hours previously. In no case did sodium lactate plus vitamin B_1 produce a beneficial effect.

V. RESULTS OF BIOCHEMICAL EXPERIMENTS.

V. RESULTS OF BIOCHEMICAL EXPERIMENTS.

Introduction.

The experiments described in this section were undertaken for the purpose of analysing the sources of energy utilised in the contraction process of smooth muscle. This subject has been investigated from the point of view of carbohydrate metabolism. Firstly, the total carbohydrate and lactic acid contents in fresh muscle were determined; then the changes produced in these constituents by the effect of aerobiosis, anaerobiosis and "rigor" were studied. The power of smooth muscle to utilise added glucose was also determined, and finally the effect of I.A.A. on the power of smooth muscle to utilise both its own available carbohydrate store and added glucose, were investigated.

The plain muscle studied was that of the rabbit's ileum and cat's colon. Rosenthal and Lasnitzki (1928) found that the oxygen consumption of the rabbit's colon mucosa was about four times as great as that of the muscle alone.(vide pp.10 and 11).

The/

The mucosa was therefore removed in Locke's solution from the muscle at 0°C. The inevitable injury produced by separating the muscle from mucous membrane gave higher figures for lactic acid content than those obtained by other workers. Since the purpose of these estimates was to serve as controls for experiments made with surviving strips, it was not possible to use more drastic methods (such as freezing with carbon dioxide snow) to arrest lactic acid production during isolation of the muscle. The variation in the values obtained was considerable, but fortunately it was possible always to make control estimations in all the experiments described in this section of the thesis and thus to eliminate the effect of individual variation.

Literature.

Soluble carbohydrate, glycogen and lactic acid contents of the smooth muscle have been estimated by various workers.

Saiki (1908) found that there was very little glycogen in the frog's stomach and bladder and that the lactic acid content in fresh specimens was about 0.06 per cent.

Evans (1925) investigated the soluble carbo-

hydrate/

hydrate, glycogen and lactic acid content of smooth muscle from different animals and also in different tissues from the same animal, and observed a great disproportion in the minute glycogen content of the muscle and heavy lactic acid formation. In a later communication Evans (1926) stated that his figures for glycogen in the intestine were too low.

Rosenthal and Lasnitzki (1928) studied the lactic acid formation in the rabbit's stomach muscle and colon muscle. Their results show the following values for lactic acid production (g. per 100 g. wet weight per hour): rabbit's stomach muscle, anaerobic (a) in glucose free Ringer's fluid 0.04, (b) in Ringer's fluid with 0.2 p.c. glucose 0.6; rabbit's colon muscle in glucose Ringer's fluid, (a) aerobic 0.031, (b) anaerobic 0.49.

Haarmann (1932) who used dog's gut and human uterine muscle, found that under anaerobic conditions there was little formation of lactic acid in the absence of glucose, but that when glucose was added there was a large formation.

Horne and Magee (1933) found 0.008 to 0.025 per cent. glycogen in the gut (muscle plus mucosa) of the rabbit.

I. /

I. Lactic Acid Content of Fresh Gut Muscle.

Literature.

Evans (1925) estimated the values of lactic acid in smooth muscle from different animals under various conditions. He used chiefly the zinc lactate method. He found the resting lactic acid values in cold blooded animals were lower than in mammals. Again the value varied from animal to animal and also from tissue to tissue of the same animal. Even the range of variation in the same tissue of one animal was very large. His figures for the mammals are shown in Table V.

Experimental Results.

The results obtained by the author with fresh muscle are shown in Tables VI and VII. The average value obtained with the cat's colon is 0.117 per cent. and the range of variation is from 0.074 to 0.175 per cent.; while the average value with the rabbit's ileum is 0.173 per cent. with a range of variation from 0.073 to 0.300 per cent. These figures are higher than those obtained by other workers, but this is probably due to the inevitable injury produced by separating the muscle from the mucous membrane.

II /

II. Total Carbohydrate Content of Fresh Gut Muscle.

Literature.

Evans (1925) found the total carbohydrate content of the tortoise stomach to be 0.51 per cent. He found in the retractor penis of dogs 0.06 per cent. soluble carbohydrate and 0.2 per cent. lactic acid and the latter figure rose to 0.3 per cent. in rigor. In the fresh dog's intestine he found 0.005 per cent. glycogen and 0.05 per cent. lactic acid and the latter figure rose to 0.08 per cent. In a later communication Evans (1926) stated that his figure for glycogen in the intestine was too low. He could not establish any relation between lactic acid formation and glycogen or soluble carbohydrate loss in smooth muscle.

Haarmann (1932) working with minced muscle under standard anaerobic conditions, concluded that the glycogen was the principal source of lactic acid formation in the normal skeletal muscle, while glucose was the chief source in the heart muscle and the sole precursor of lactic acid in smooth muscle.

Experimental Results.

In view of these observations the total carbohydrate contents of cat's colon and rabbit's ileum/

ileum, free from mucous coat were determined. The results obtained by the author are shown in Tables VIII and IX.

The average value with cat's colon was 0.684 per cent. and the range of variation was from 0.490 to 0.947 per cent.; while the average value with rabbit's ileum was 0.595 per cent. and the range of variation was from 0.418 to 0.943 per cent. These results agree fairly well with the results obtained by the previous workers.

Total Carbohydrate Loss and Lactic Acid Production under Aerobiosis.

Smooth muscle from the rabbit's ileum and cat's colon (free from mucous coat) were kept in aerated alkaline Locke's solution at 37°C.(without glucose) for one hour in most experiments and for two hours in a few experiments. In these experiments the baths containing the tissues were replaced with long test-tubes with a wide bore in order to avoid loss of fluid due to spurting and frothing. The test-tubes were fitted with rubber corks and glass tubes for bubbling oxygen or nitrogen. The arrangements are shown in Fig. 30. In/

In this way the loss of perfusing fluid was found to be negligible on the completion of the experiments. Control estimations in the fresh muscle were made with each experiment. A few experiments were made with electrically stimulated muscle. The values for carbohydrate loss are shown in Tables X and XI and those for lactic acid production in Tables XII and XIII. These tables show a carbohydrate loss from 0.16 to 0.22 per cent. and a lactic acid production from 0.015 to 0.052 per cent. in oxygen in one hour.

These results show that plain muscle can oxidise carbohydrates.

Carbohydrate Loss and Lactic Acid Formation in Anaerobiosis and 'Rigor'.

Evans (1925) investigated the formation of lactic acid in anaerobiosis and under conditions which in striated muscle will cause rigor mortis. He produced anaerobiosis either by immersing the tissue in Ringer's solution containing N/1000 NaCN or by exposing the tissue without immersion in fluid to a continuous current of moist commercial nitrogen. He observed rapid lactic acid accumulation during anaerobiosis and found that a maximum/

maximum was reached at the end of 8 hours' survival at room temperature. He also produced rigor by heating to 70°C. or by $CHCl_3$ and observed a rise in lactic acid content of smooth muscle under these conditions.

Rosenthal and Lasnitzki (1928) determined the lactic acid content in smooth muscle from rabbit's stomach by Warburg's manometric method and found very low lactic acid production under anaerobic conditions with glucose-free Ringer's fluid, namely 0.04 per cent. (wet weight per hour).

Experimental Results.

The author produced anaerobiosis by suspending the tissue at 37°C. in nitrogenated Ringer's solution for one hour (a similar duration to the oxygenation experiments). Rigor was produced by keeping the mucous free pieces of gut in Locke's solution (without glucose) at room temperature for 24 hours. Tables XIV, XV, XVI and XVII show the values of lactic acid formation and carbohydrate usage observed under these conditions.

These tables show a lactic acid production of 0.14 per cent. in anaerobiosis and 0.1 to 0.19 per cent. under rigor. Thus the maximum lactic acid formation is 0.2 per cent. The figures for carbohydrate/

carbohydrate utilisation show 0.21 to 0.24 per cent. in anaerobiosis and 0.26 per cent. in rigor condition. These results indicate that the amount of carbohydrate in smooth muscle that can be glycolysed is about 0.25 per cent.

This explains satisfactorily the facts observed by Evans (1925) who found a lactic acid formation in smooth muscle up to 0.3 per cent. but could not establish any relation between the glycogen content and lactic acid formation.

The Utilisation of Tissue Carbohydrate.

The object of the experiments described under carbohydrate loss and lactic acid production under aerobiosis, anaerobiosis and rigor, was to determine how much of its own carbohydrate the muscle could convert into lactic acid. The result of outstanding importance is that although the total carbohydrate contents of the gut muscle amounted in some cases to as much as 0.760 per cent., yet under no conditions was it possible to cause a loss of more than 0.26 per cent. The amount of carbohydrate in the gut available for conversion into lactic acid is/

is therefore about 0.25 per cent.

The gut suspended for 1 hour in oxygenated Locke's solution utilised, however, from 0.16 to 0.22 per cent. carbohydrate. There appears therefore to be a small quantity of labile carbohydrate in the gut and this is fairly rapidly exhausted even under aerobic conditions.

This conclusion was confirmed by the following experiment. Two pieces of cat's colon were suspended in oxygenated Ringer's fluid and were removed after 1 hour and after 3 hours respectively. The results are shown in Table XVIII. The following average values were obtained in three experiments. The total carbohydrate content (in g. per 100 g. muscle) was:- after one hour 0.437; after 3 hours 0.426. The lactic acid excreted (g. per 100 g. muscle) was:- during the first hour 0.100 and during the next 2 hours 0.039. Since the fluid was changed at the end of the first hour the cessation of carbohydrate breakdown was not due to the accumulation of lactic acid. These results indicated that most of the carbohydrate breakdown and lactic acid excretion occurs during the first hour of the isolation.

The lactic acid content found in the control strips of the cat's colon (about 0.12 per cent.) is/

is three times that found by Evans (1925) in the dog's intestine. It is probable therefore that between 0.05 per cent. and 0.10 per cent. of lactic acid is formed during the manipulation of the muscle.

The figures suggest that in the muscle in situ there is about 0.35 per cent. of labile carbohydrate, that about 0.10 per cent. of this is changed to lactic acid during manipulation and that another 0.25 per cent. is broken down during the first hour of isolation irrespective of whether the conditions are aerobic or anaerobic.

Tables XI and XII show a considerable variation in the amount of lactic acid formed under aerobic conditions. This suggested that a variable portion of the tissue received an inadequate oxygen supply even in oxygenated fluid. The oxygen consumption of the tissue may be assumed to be about 0.5 per cent. per gram per hour (Rosenthal and Lasnitzki, 1928) which equals 0.008 c.c. per gram per minute. The thickness of typical pieces of colon as estimated from their weight and area was found to be 0.14 cm. Warburg (1923) found the thickness of tissue (d) which will receive an adequate oxygen supply when suspended in fluid saturated with oxygen, and using a quantity (A) of oxygen per gram per minute, was given by the following formula

d /

$d = \sqrt{8D/A}$, where D is Krogh's constant for oxygen diffusion which at 37°C. is about 1.7×10^{-5}.

In this case $d = \sqrt{\frac{8 \times 1.7 \times 10^{-5}}{0.008}} = 0.13$ cm.

Since the average thickness of the tissue was about 0.14 cm. it seems probable that when oxygen was perfused some pieces got an oxygen supply adequate to prevent lactic acid formation, whilst others slightly thicker got insufficient oxygen.

It has been pointed out that under anaerobic conditions the gut can only convert about 0.15 per cent. or 1.5 mgm. per gram of its carbohydrate to lactic acid. The production of 1.5 mgm. lactic acid from glycogen provides energy equivalent to about 0.4 cals. The gut under aerobic conditions uses about 0.5 c.c. oxygen per gram per hour and this would suffice to oxidise about 0.7 mgm. carbohydrate. The oxidation of this amount is equivalent to an energy release of about 3 cals. Hence the glycolysis of the available carbohydrate of the gut is only adequate to supply an amount of energy equal to that released under aerobic conditions in $\frac{60 \times 0.4}{3} = 8$ min.

This/

This is a remarkable contrast to the skeletal muscle and cardiac muscle of the frog, where the tissue carbohydrate available for utilisation is only exhausted after some hours of anaerobic activity. The figures also show that under aerobic conditions nearly the whole of the carbohydrate utilisation occurs during the first hour, and since an isolated gut can continue to function in glucose-free Locke's fluid for several hours it is evident that it must be able to oxidise other material in addition to the carbohydrate.

Utilisation of Added Glucose.

Literature.

Skeletal muscle. Hill and Kupalav (1929) found that the frog's sartorius muscle produced anaerobically more lactic acid with Ringer's solution containing 0.1 p.c. glucose than it did in Ringer's solution without glucose. They further observed that the mechanical activity could be revived with addition of glucose when it ceased due to exhaustion of its intrinsic carbohydrate in anaerobiosis.

Frog's /

Frog's Heart. Freund and König (1927) found that the frog's heart perfused with oxygen free alkaline Ringer's fluid were arrested in 2 to 3 hours, but that when glucose was present the hearts maintained good contractions for at least 6 hours. These conclusions have been confirmed by Clark, Gaddie and Stewart (1932) and they also observed that a heart arrested by lack of oxygen could be revived by glucose. They also have shown that when glucose is present in the perfusion fluid in anaerobiosis, the heart obtains energy by converting the added glucose into lactic acid.

Results with smooth muscle. Observations on the mechanical activity of the gut when glucose is present in the perfusion fluid in anaerobic (Fig. 13) and aerobic (Figs. 12 and 16) conditions show that addition of glucose revives the activity of gut muscle after this has been arrested by asphyxia and helps to maintain the normal rhythm and tonicity of the gut under aerobic conditions. In this way the gut differs from the heart muscle under aerobic conditions, which does not utilise added glucose but shows a parallel behaviour under anaerobic conditions. The formation of lactic acid by cat's colon/

colon muscle in 0.1 per cent. glucose Locke's solution under aerobic and anaerobic conditions was next estimated. The results are shown in Table XIX.

The lactic acid formation in oxygen, air and nitrogen is 0.255, 0.330 and 0.616 per cent. respectively in 3 hours. Glucose is found to be converted into lactic acid under both oxaemic and anoxaemic conditions, which supports the conclusion drawn from the effect of glucose on the mechanical response of the gut under aerobic and anaerobic conditions.

The substitution of air for nitrogen reduced the lactic acid production to one half, but even when oxygen was perfused there was still a considerable lactic acid production. Rosenthal and Lasnitzki (1928) found that the lactic acid production per hour of the rabbit's colon muscle in presence of glucose was 0.03 per cent. in aerobiosis and 0.49 per cent. in anaerobiosis. My figures (0.255 per cent. in oxygen and 0.0616 per cent. in nitrogen) show a much smaller difference, and the probable reason for this is that the aerobic lactic acid production is unduly high owing to the thickness/

thickness of the tissue.

The essential fact shown by my figures is that large quantities of lactic acid are produced by the gut muscle in presence of glucose, and hence the failure of the gut to produce similar quantities in absence of glucose is due to exhaustion of the available carbohydrate and not to the accumulation of lactic acid preventing further production.

Utilisation of Lactate by Smooth Muscle.

Literature.

Dog's heart. Evans, Hsu and Kosaka (1934) found a very heavy formation of lactic acid by the lungs from the blood sugar and observed its consumption by the beating heart of the heart-lung preparation in dogs.

In cats. Grant (1933) observed the formation of liver glycogen in cats with all organs intact after infusion of ammonium lactate in the superior mesentric vein.

Frog's skeletal muscle. Mayerhof and Boyland (1931) found that aerobic muscles poisoned with I.A.A. had a R.Q. of 0.7 which was restored to normal value of 0.95 by addition of sodium d-lactate.

Mawson/

Mawson (1933) had observed that added lactate is used by the oxygenated I.A.A. poisoned sartorius for the synthesis of phosphagen. This was shown by the comparison of amount of phosphagen in muscle without added lactate and with added lactate. He also found that the muscle without lactate becomes non-irritable and passes into rigor quicker than that with added lactate.

The same observer (1932) found that neither by the minced muscle nor by muscle in intact limb of frog could lactic acid be removed anaerobically in the presence of I.A.A.

Smooth muscle. Evans (1925) found the lactic acid content of smooth muscle after survival for 20 hours in nitrogen and a subsequent 24 hours in air, was less than the content of muscle which had been kept for 6½ hours in nitrogen. He concluded that lactates produced during anaerobic conditions were oxidised under the subsequent aerobic conditions.

Experimental Results.

The author made a few experiments to determine whether the gut muscle metabolised lactate in presence of oxygen. Colon strips were suspended for 3 hours in glucose-free Locke's fluid containing 0.004/

0.004 per cent. sodium lactate and oxygen was passed. The results are shown in Table XX. Three experiments were made and the amount of lactic acid recovered from the muscle and fluid in excess of the lactate originally present was 0.20 per cent. of the muscle weight. Control experiments showed that oxygenation for 3 hours of lactate solutions of the same strength did not cause any loss of lactate. This result indicates that there is no extensive oxidation of lactates by the gut muscle, but the experiment does not prove that no oxidation occurs, because it has already been shown that even when oxygen is perfused the gut may produce a certain amount of lactic acid.

Action of I.A.A. on Smooth Muscle.

Literature.

Skeletal muscle. The fundamental work of Lundsgaard(1930) showed that skeletal muscle poisoned with iodo-acetic acid did not produce lactic acid though it could perform a considerable amount of work.

Mawson (1932) made comparative estimations of the lactic acid formation in skeletal muscles of the frog in the resting state both with I.A.A. poisoned and normal muscle under anaerobic conditions. He observed/

observed no production of lactic acid in the poisoned muscle while the non-poisoned muscle showed a heavy production.

Kerley (1931) estimated the lactic acid content in I.A.A. poisoned muscle of the frog in the different conditions of (a) fatigue in hydrogen, (b) anaerobiosis in hydrogen and (c) aerobiosis. She found that the lactic acid content showed only a very small average increase.

Heart muscle. Clark, Gaddie and Stewart (1932) studied the survival of anaerobic frog's heart and the lactic acid formation in I.A.A. poisoned and non-poisoned hearts under different conditions. They observed a quick arrest of the mechanical activity and no formation of lactic acid in the case of the poisoned heart.

Results with smooth muscle. Experiments in which the mechanical response of gut muscle was measured (vide Mechanical Experiments) showed that when this was poisoned with sodium-iodo-acetate (S.I.A.) (1:10,000) it continued to contract in apparently normal manner as long as oxygen was supplied, but that asphyxia caused arrest in a few minutes. The rapidity of arrest made it impracticable/

impracticable to study the lactic acid production of S.I.A. poisoned muscle during asphyxia, and this was therefore studied in muscle suspended in oxygenated Locke's fluid containing glucose, a condition under which normal gut muscle produces a considerable amount of lactic acid. S.I.A. (1:10,000) reduced the lactic acid production to less than one-quarter of the value obtained with the normal muscle both when the muscle contracted spontaneously and when it was stimulated electrically (Table XXI).

It was thought possible that the failure of 0.01 per cent. S.I.A. to abolish completely the lactic acid production might be due to a large initial lactic acid production during the period of poisoning. The effect of 1:10,000 (N/2080) sodium iodo-acetate on carbohydrate breakdown and lactic acid production was studied in glucose-free Locke's solution. The results are shown in Tables XXII and XXIII. They show that S.I.A. does produce a slight increase in the carbohydrate breakdown in the first 10 minutes of its action, but that it produces no corresponding increase in lactic acid production. The lactic acid production during the first two hours is, however, only 30 per cent. less in the S.I.A. poisoned muscle than in the normal muscle/

muscle than in the normal muscle. The results in Tables XXI, XXII and XXIII show therefore that S.I.A. has a powerful action in reducing the glycolysis of sugar in the fluid surrounding the muscle but has a less marked action on the glycolysis of the carbohydrate contained in the muscle. The most probable reason for this result is that S.I.A. penetrates muscles slowly.

Gaffar (1935) has shown that at 40°C. N/1000 S.I.A. takes about 20 minutes to reduce the lactic acid formation of frog's skeletal muscle to one-half normal. His results are in accordance with those of Meyerhof and Boyland (1931) who found that I.A.A. took 30 minutes to penetrate fully the sartorius of _Rana_ _temporaria_, whilst Lohmann(1931) found that I.A.A. took nearly an hour to inhibit completely the lactic acid formation of the muscle pulp.

VI. DISCUSSION.

VI. DISCUSSION.

1. Anaerobic Activity.

The results of the biochemical analyses suggest that most of the lactic acid found in the gut muscle prepared in the manner described in the thesis is formed during manipulation after isolation. Hence the true value of the reducing substances in the fresh gut muscle is probably the sum of the amounts found of reducing substances and lactic acid. In the case of cat's colon the true resting value for reducing substances is probably about 0.9 per cent.; from 0.10 to 0.15 per cent. undergoes glycolysis during isolation and a further 0.25 per cent. is readily glycolysed, but the remaining 0.5 per cent. is not glycolysed even after prolonged exposure to anaerobic conditions. The gut muscle when isolated contains therefore only about 0.25 per cent. of carbohydrate that is available for the supply of energy, and under anaerobic conditions this supply is only adequate to support the normal activity of the gut for from 5 to 15 minutes.

Experiments on the mechanical response of the gut muscle during asphyxia at first suggested that its behaviour was quite unlike that of the frog's /

frog's heart. The gut was arrested rapidly both in acid and in alkaline Ringer's fluid, whereas the frog's heart can maintain a prolonged anaerobic activity in alkaline fluid. The biochemical analyses explain these differences, since they show that unlike the heart, the gut muscle possesses no large store of carbohydrate available for production of anaerobic energy by glycolysis.

Gut muscle resembles the frog's heart in that it can maintain its activity by glycolysing glucose added to the perfusion fluid.

2. Aerobic Condition.

Another difference between the two tissues is that under aerobic conditions the frog's heart does not oxidise or glycolyse glucose present in the perfusion fluid, whereas the gut muscle usually glycolyses a considerable amount of glucose even under aerobic conditions. This suggests that the latter muscle does not obtain an adequate supply of oxygen throughout its thickness. The application of Warburg's formula confirms this conclusion. The difference observed can therefore be explained by/

by the fact that the gut muscle is too thick to obtain an adequate oxygen supply throughout its substance even when oxygen is supplied freely.

Apart from these two important differences the metabolism of mammalian gut muscle shows a very close resemblance to that of frog's heart. The frog's heart under aerobic conditions utilises a mixed diet of carbohydrate and non-carbohydrate material. This must also be true of the mammalian gut muscle for the following reasons:

(a) The gut muscle under aerobic conditions exhausts nearly the whole of its available carbohydrate in an hour, but it can continue regular activity for many hours without the addition of glucose.

(b) Iodo-acetic acid abolishes glycolysis and yet does not markedly depress the activity of gut muscle provided that this is supplied with oxygen (Figs. 9 and 24).

The activity of the gut muscle is however maintained best when it is supplied with both glucose and oxygen (Figs. 12, 13 and 16). Therefore the optimum condition for the activity of the gut muscle appears to be when it is utilising both carbohydrate/

carbohydrate and non-carbohydrate sources of energy.

3. Influence of S.I.A.

The results shown in Tables XXI, XXII and XXIII suggest that S.I.A. acts immediately on the surface of the muscle and inhibits glycolysis of sugar present in the fluid, but it takes the greater part of an hour to abolish all glycolysis in the interior of the muscle. This hypothesis is difficult to prove, because in the unpoisoned muscle very little glycolysis of muscle carbohydrate occurs after first hour of isolation, and it is difficult to determine whether this small glycolysis is further reduced by S.I.A.

The mechanical activity of the gut muscle when poisoned with S.I.A. and deprived of oxygen is arrested in about 3 minutes. The sources of available anaerobic energy other than carbohydrate are, therefore, very small. This agrees with the finding of Eggleton and Eggleton (1929) that the phosphagen content of mammalian plain muscle is only 2.3 to 5.1 mg. of phosphagen P. per 100 g., whilst Eggleton (1934) has shown that the arginine phosphate of Mytilus muscle is reduced in asphyxia. It/

It is reasonable therefore to assume that the short anaerobic activity in the S.I.A. poisoned gut muscle measures the time required to exhaust its small store of phosphagen. The effect of S.I.A. on gut muscle is in all respects similar to its action upon the frog's heart.

4. Influence of pH.

It is of interest to note that the effect of acidity on the gut muscle is similar to its effect on the heart. An acidity of pH 6.0 does not markedly affect the activity of the gut muscle as long as this is supplied with oxygen, but when the oxygen supply is cut off the gut is rapidly arrested, presumably because the acidity inhibits glycolysis (Fig. 7).

5. Various Carbohydrate and Breakdown Products.

The power of mammalian gut muscle to utilise various carbohydrates and breakdown products of carbohydrate appears to be identical with the powers of the frog's heart (cf. Table III).

The power of methyl-glyoxal and glyceric aldehyde to restore partial activity of gut muscle in/

in nitrogen suggests the probability of these being intermediate products in the path of glycolysis.

Sodium pyruvate produces a definite beneficial effect on I.A.A. poisoned gut muscle (Fig. 28). This suggests that I.A.A. stops the process of glycolysis before this stage. Haarmann (1932) found that lactic acid could be produced by muscle poisoned with monobromacetic acid on addition of pyruvate.

6. Tone and Rhythmicity.

The tone and rhythmicity of the isolated gut are maintained when both glucose and oxygen are available (Figs. 12, 13 and 16). Gradual depletion of carbohydrate store even in oxygen produces gradual loss of the tone and of the amplitude of rhythmic contractions in the freshly removed gut (Fig. 16). After the gut has been washed for some time it can no longer be stimulated by glucose, although this may cause some increase in tonus. (Figs. 14 and 15). These observations suggest that for normal maintenance of tone and rhythmicity both glucose and oxygen and probably choline (Magnus 1930) are essential.

VI. SUMMARY.

SUMMARY.

1. Isolated gut muscle contains only about 0.25 per cent. of carbohydrate available for glycolysis.

2. Isolated gut muscle in presence of oxygen oxidises about 1 mg. carbohydrate per gram per hour.

3. Isolated gut muscle in presence of glucose produces considerable quantities of lactic acid both under aerobic and anaerobic conditions. The deeper portions of the muscle probably do not obtain an adequate oxygen supply even in oxygenated fluid. Under anaerobic conditions about 2 mg. glucose per gram per hour are glycolysed.

4. Sodium-iodo-acetate (1:10,000) inhibits glycolysis of glucose in Locke's fluid in contact with the gut muscle.

5. Periodic electrical stimulation increases the glycolysis by about 12 per cent.

6. Asphyxial arrest of the mechanical movements of gut muscle is not due to accumulation of acid but to exhaustion of its labile carbohydrate store.

7./

7. The mechanical experiments confirm the biochemical findings that the gut muscle has only a small reserve of available carbohydrate.

8. The gut muscle probably utilises a mixed diet of carbohydrate and non-carbohydrate material in aerobiosis.

9. The activity of the gut muscle is maintained best when it is supplied with both glucose and oxygen.

10. I.A.A. poisoned gut muscle has a very limited activity under anaerobiosis; this suggests a small phosphagen content.

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VIII. TABLES.

Table I.

"Gradient Phenomena" in the Gut.

	Observer	Phenomena	Upper part	Lower part
1	Alvarez (1928)	Movement activity	Quicker	Slower
2	Evans (1923)	Oxygen usage	More	Less
3	Evans (1925)	Resting Lactic Acid Content	More	Less
4	Horne and Magee (1933)	Glycogen Content	Low	High
5	Author (1935)	Asphyxial Arrest of Mechanical Activity	Quick	Delayed

Table II.

Time in minutes until asphyxial arrest is produced by nitrogen in the isolated rabbit's ileum contracting spontaneously.

Reaction of the perfusing fluid	Glucose-free Locke's solution	Glucose containing Locke's solution
pH = 8.2	5 - 10	several hours
pH = 6.2	5 - 10	3 - 4

Table III.

Beneficial action of carbohydrates, etc. on (a) the asphyxiated gut and (b) the asphyxiated and exhausted frog's heart.

Substance	Asphyxiated cat's colon	Asphyxiated and exhausted frog's heart. (Gaddie and Stewart, 1934)
Polysaccharides:		
Starch	-	-
Disaccharides:		
Maltose	-	-
Lactose	-	-
Sucrose	-	-
Monosaccharides:		
Glucose	+++	+++
Mannose	+++	+++
Fructose	+	-
Galactose	-	-
Pentoses:		
Arabinose	-	-
Xylose	-	-
Possible breakdown products of carbohydrates:		
Dihydroxyacetone	-	-
Glyceric aldehyde	++*	++*
Methyl glyoxal	++*	++
Pyruvate	-	-

Various/

Table III (contd.)

Substance	Asphyxiated cat's colon	Asphyxiated and exhausted frog's heart. (Gaddie and Stewart, 1934)
Various substances:		
Aminoacids:		
Glycine and alanine	–	–
Lipoids:		
Sodium oleate	–	–

+++ = complete revival
++ = partial revival or temporary revival
\+ = feeble and short revival
– = no revival
* = poisoning in higher concentrations

Table IV.

Action of different substances on I.A.A. poisoned and normal cat's gut under aerobic and anaerobic conditions.

Substances tested and concentration in p.c.	Normal gut		I.A.A. poisoned gut.	
	Aerobiosis	Anaerobiosis	Aerobiosis	Anaerobiosis
Glucose(0.1 p.c.)	+++	+++	–	–
Methylglyoxal(*) (0.01 p.c.)	+	+	+	– (†)
Glyceric aldehyde (*) (0.01 p.c.)	+	+	+	– (†)
Sodium pyruvate(0.1 p.c.)	++	–	+	–
Sodium lactate(0.1 p.c.)	–	–	–	–

+++ = good beneficial effect.

++ / + = moderate and slight beneficial effect for short time.

– = no effect.

* = poisons in higher concentration

† = sudden contracture.

Table V.

Resting lactic acid content in per cent.
(Evans, 1925).

Tissues	Cat	Dog	Bullock
1. Small intestine	-	0.042 (0.012-0.076	-
2. Intestine	0.178	-	-
3. Uterus	0.083 (0.024-0.141)	-	-
4. Oesophagus	0.05	-	-
5. Bladder	0.214	-	-
6. Retractor penis	-	-	0.139 (0.072-0.27)

Table VI.

Lactic acid content of freshly isolated cat's colon.

No.of expt.	Weight of muscle used.	Lactic acid in gm. per 100 gm. muscle
1	0.874	0.119
2	1.218	0.104
3	1.346	0.135
4	0.718	0.156
5	0.756	0.088
6	0.768	0.137
7	0.382	0.153
8	0.492	0.148
9	0.206	0.175
10	0.312	0.154
11	0.516	0.074
12	0.352	0.131
13	0.430	0.081
14	0.452	0.077
15	0.664	0.080
16	0.630	0.111
17	0.508	0.087
18	0.812	0.108
19	0.612	0.107
	Mean =	0.117 per cent.

Range -

0.074 to 0.175 p.c.

Table VII.

Lactic acid content of freshly isolated rabbit's ileum.

No. of expt.	Weight of muscle used.	Lactic acid p.c. per 100 gm. muscle.
1	0.572	0.093
2	0.452	0.125
3	0.622	0.071
4	0.382	0.308
5	0.337	0.250
6	0.277	0.120
7	0.356	0.306
8	0.396	0.274
9	0.310	0.148
10	0.472	0.073
11	0.522	0.143
	Mean =	0.173 per cent.

Range 0.073 - 0.300 p.c.

Table VIII.

Total carbohydrate content of freshly isolated cat's colon.

No. of expt.	Weight of muscle used.	Total carbohydrate in gm. per 100 gm. muscle.
1	0.430	0.684
2	0.337	0.676
3	0.271	0.639
4	0.287	0.643
5	0.262	0.661
6	0.304	0.641
7	0.190	0.682
8	0.250	0.512
9	0.290	0.902
10	0.202	0.947
11	0.232	0.747
12	0.232	0.781
13	0.332	0.660
14	0.262	0.638
15	0.462	0.490
16	0.390	0.641
	Mean =	0.684 per cent.

Range -

0.490 - 0.947 p.c.

Table IX.

Total carbohydrate content of freshly isolated rabbit's ileum.

No. of expt.	Weight of muscle used.	Total carbohydrate in gm. per 100 gm. muscle.
1	0.188	0.623
2	0.212	0.427
3	0.392	0.418
4	0.212	0.943
5	0.300	0.625
6	0.272	0.625
7	0.167	0.505
8	0.132	0.532
9	0.152	0.678
10	0.270	0.580
	Mean =	0.595

Range -

0.418 to 0.943 p.c.

Table X.

Utilisation of carbohydrate by cat's colon in oxygenated Locke's fluid without glucose at 37°C.

No. of expts.	Control Muscle		Experimental Muscle	
	Muscle weight in gm.	Total carbohydrate in g. per 100 g. muscle	Muscle wt. in g.	Total carbohydrate in g. per 100 g. muscle.
	(A) Oxygenation for 1 hour			
1	0.190	0.682	0.162	0.684
2	0.250	0.512	0.192	0.526
3	0.290	0.901	0.300	0.609
4	0.202	0.947	0.188	0.349
	Mean =	0.760		0.542
	(B) Oxygenation for 2 hours			
1	0.322	0.607	0.326	0.373
2	0.332	0.660	0.252	0.440
3	0.262	0.638	0.272	0.470
4	0.462	0.490	0.646	0.435
5	0.390	0.641	0.298	0.608
	Mean =	0.607		0.465

(A) Carbohydrate loss in 1 hour = 0.218 p.c.

(B) do. 2 hours= 0.142 p.c.

Table XI.

Utilisation of carbohydrate by rabbit's ileum in oxygenated Locke's fluid without glucose at 37°C. in 1 hour.

No.of expt.	Control Muscle		Experimental Muscle	
	Muscle wt. in gm.	Total carbohydrate in g. per 100 g. muscle	Muscle wt. in gm.	Total carbohydrate in g. per 100 g. muscle.
1	0.212	0.427	0.242	0.383
2	0.392	0.418	0.262	0.322
3	0.212	0.943	0.132	0.627
4	0.300	0.625	0.252	0.281
5	0.272	0.625	0.242	0.625
6	0.167	0.505	0.172	0.445
7	0.132	0.532	0.122	0.461
8	0.152	0.678	0.188	0.464
9	0.270	0.580	0.148	0.427
	Mean =	0.592		0.437

Carbohydrate loss in 1 hour = 0.155 p.c.

Table XII.

Lactic acid formation by cat's colon in oxygenated Locke's fluid without glucose at 37°C.

Lactic acid production (A) = 0.052 p.c.
do. (B) = 0.057 p.c.
do. (C) = 0.176 p.c.

No. of expts.	Control muscle		Experimental muscle.		Perfusion fluid		Total lactic acid in g. per 100 g. muscle.
	Muscle wt. in gm.	Lactic acid in g. per 100 g. muscle	Muscle wt. in gm.	Lactic acid in g. per 100 g. muscle	Lactic acid in mg. per 100c.c. fluid	Lactic acid in g. per 100 g. muscle	
		(A) Oxygenation for 1 hour					
1	0.382	0.153	0.542	0.080	1.7	0.085	0.165
2	0.492	0.148	0.502	0.071	2.0	0.100	0.171
3	0.206	0.175	0.300	0.132	1.0	0.083	0.215
4	0.452	0.077	0.596	0.060	2.4	0.100	0.160
5	0.664	0.080	0.576	0.060	2.1	0.090	0.150
6	0.630	0.111	0.532	0.082	2.2	0.109	0.191
	Mean =	0.123		0.097		0.078	0.175
		(B) Oxygenated for 1 hour and stimulated electrically					
1	0.452	0.077	0.920	0.044	2.1	0.066	0.110
2	0.664	0.080	0.696	0.037	2.1	0.075	0.112
3	0.630	0.111	0.482	0.109	2.1	0.109	0.218
	Mean =	0.089		0.063		0.083	0.146
		(C) Oxygenated for 2 hours.					
1	0.312	0.154	0.340	0.173	2.9	0.201	0.374
2	0.516	0.074	0.650	0.151	4.0	0.153	0.304
3	0.352	0.131	0.622	0.050	2.3	0.092	0.142
4	0.430	0.081	0.486	0.143	3.5	0.180	0.323
	Mean =	0.110		0.130		0.156	0.286

Table XIII.

Lactic acid formation by rabbit's ileum in oxygenated Locke's fluid without glucose at 37°C. in 1 hour.

No. of expt.	Control muscle		Experimental muscle		Perfusion fluid.		Total lactic acid in g. per 100 g. muscle after experiment.
	Muscle wt. in g.	Lactic acid in g. per 100 g. muscle	Wt. of muscle in g.	Lactic acid in g. per 100 g. muscle	Lactic acid in mg. per 100c.c. fluid	Lactic acid in g. per 100 g. muscle	
1	0.452	0.125	0.552	0.094	2.1	0.095	0.189
2	0.622	0.071	0.522	0.050	1.7	0.081	0.131
3	0.382	0.308	0.377	0.168	2.0	0.131	0.299
4	0.337	0.250	0.432	0.092	1.2	0.070	0.162
5	0.277	0.120	0.212	0.159	1.0	0.119	0.278
6	0.356	0.306	0.432	0.148	2.5	0.145	0.293
7	0.396	0.274	0.398	0.153	1.5	0.094	0.247
	Mean	0.213		0.123		0.105	0.228

Lactic acid production = 0.015 per cent.

Table XIV.

Total carbohydrate value under anaerobic conditions.

No. of expt.	Control Muscle		Experimental Muscle	
	Muscle wt. in g.	Total carbo-hydrate in g. per 100 g. muscle	Muscle wt. in g.	Total carbo-hydrate in g. per 100 g. muscle
Nitrogenation for 1 hour at 37°C. in Ringer's solution (without glucose)				
(a) Cat's Colon				
1	0.190	0.682	0.160	0.488
2	0.250	0.512	0.200	0.398
3	0.290	0.901	0.242	0.761
4	0.202	0.947	0.152	0.524
	Mean =	0.760		0.542
(b) Rabbit's Ileum.				
1	0.212	0.427	0.242	0.200
2	0.392	0.418	0.200	0.250
3	0.212	0.943	0.147	0.375
4	0.300	0.625	0.267	0.390
5	0.212	0.625	0.222	0.344
6	0.167	0.505	0.200	0.277
7	0.132	0.532	0.100	0.390
8	0.152	0.678	0.114	0.561
9	0.270	0.580	0.222	0.369
	Mean =	0.592		0.348

Carbohydrate utilised in anaerobiosis:

Cat's colon = 0.218 per cent.
Rabbit's ileum = 0.244 per cent.

Table XV.

Lactic acid value under anaerobic condition.

No.of expts.	Control Muscle		Experimental muscle.		Perfusion fluid.		
	Muscle wt.in g.	Lactic acid in g. per 100 g. muscle	Muscle wt.in g.	Lactic acid in g. per 100 g. muscle	Lactic acid in mg. per 100c.c.	Lactic acid in g. per 100 g. muscle	Total lactic acid in g. per 100 g. muscle (after expt.)
Nitrogenation for 1 hour at 37°C. in Ringer's solution. (without glucose)							
(a) Cat's Colon.							
1	0.382	0.153	0.382	0.097	3.1	0.193	0.290
2	0.492	0.148	0.417	0.085	3.5	0.208	0.295
3	0.206	0.175	0.256	0.096	2.2	0.211	0.307
	Mean =	0.158		0.092		0.204	0.296
(b) Rabbit's Ileum.							
1	0.452	0.125	0.252	0.136	1.5	0.148	0.284
2	0.622	0.071	0.492	0.089	3.2	0.163	0.252
3	0.382	0.308	0.367	0.302	3.2	0.218	0.520
4	0.337	0.250	0.257	0.307	2.2	0.211	0.518
5	0.277	0.120	0.292	0.143	1.5	0.129	0.272
6	0.356	0.306	0.356	0.175	2.0	0.143	0.318
7	0.396	0.274	0.422	0.130	2.5	0.148	0.278
	Mean =	0.213		0.183		0.165	0.348

Lactic acid formed:

Cat's colon = 0.138 per cent.

Rabbit's ileum = 0.135 per cent.

Table XVI.

Total carbohydrate value under 'rigor' condition.

No. of expts.	Control Muscle		Experimental Muscle	
	Muscle wt. in g.	Total carbohydrate in g. per 100 g. muscle	Muscle wt. in g.	Total carbohydrate in g. per 100 g. muscle
Kept at room temperature for 24 hours in Ringer's solution (without glucose)				
	(a) Cat's Colon.			
1	0.190	0.682	0.167	0.561
2	0.250	0.512	0.250	0.400
3	0.290	0.901	0.302	0.515
4	0.202	0.947	0.122	0.537
	Mean =	0.760		0.503
	(b) Rabbit's Ileum.			
1	0.167	0.505	0.220	0.100
2	0.132	0.532	0.147	0.382
3	0.152	0.678	0.220	0.398
4	0.270	0.580	0.210	0.386
	Mean =	0.573		0.316

Carbohydrate utilised:

Cat's colon = 0.257

Rabbit's ileum = 0.257

Table XVII.

Lactic acid value under 'rigor' condition.

No. of expts.	Control Muscle		Experimental Muscle		Perfusion Fluid		Total lactic acid in g. per 100 g. muscle (after experiment)
	Muscle wt. in g.	Lactic acid in g. per 100 g. muscle	Muscle wt. in g.	Lactic acid in g. per 100 g. muscle	Lactic acid in mg. per 100c.c. fluid	Lactic acid in g. per 100 g. muscle	
	Kept at room temperature for 24 hours in Ringer's solution (without glucose)						
	(a) Cat's Colon.						
1	0.382	0.153	0.452	0.096	4.2	0.233	0.329
2	0.492	0.148	0.410	0.087	4.1	0.250	0.337
3	0.206	0.175	0.307	0.116	3.2	0.250	0.366
	Mean =	0.158		0.099		0.244	0.344
	(b) Rabbit's Ileum.						
1	0.277	0.120	0.232	0.142	1.5	0.161	0.303
2	0.356	0.306	0.270	0.087	3.0	0.277	0.364
3	0.396	0.274	0.302	0.111	2.5	0.208	0.319
	Mean =	0.233		0.113		0.215	0.328

Lactic acid formed:

Cat's colon = 0.186

Rabbit's ileum = 0.095

Table XVIII.

Cat's Colon - showing carbohydrate loss and lactic acid production in 1 hour in O_2 at 37°C. and during subsequent 2 hours.

Column I = Total carbohydrate in the muscle after 1 hour.

Ia = Lactic acid in the fluid round I after 1 hour.

II = Total carbohydrate in the muscle after 3 hours in O_2. After 1 hour the fluid round it (IIa) was changed and in fresh fluid (IIb) O_2 was continued for the next 2 hours.

IIa = Lactic acid in the fluid round II after 1 hour.

IIb = Lactic acid in the fresh fluid round II after next 2 hours.

No.of expts.	Total carbohydrate in per cent.		Lactic acid in g. per 100 g. muscle		
	I	II	Ia	IIa	IIb
1	0.415	0.410	0.095	0.097	0.027
2	0.484	0.480	0.115	0.110	0.058
3	0.412	0.390	0.096	0.095	0.033
Mean =	0.437	0.426	0.102	0.100	0.039

Total carbohydrate content:-

after one hour = 0.437 per cent.
after three hours = 0.426 per cent.

Lactic acid excreted:-

during the first hour = 0.100 per cent.
during next two hours = 0.039 per cent.

Table XIX.

Lactic acid production with 0.1 per cent. glucose-Ringer's solution under different conditions (control value of lactic acid 0.135 per cent.)

Cat's Colon.

No. of expts.	Muscle wt. in g.	Muscle lactic acid in g. per 100 g. muscle	Perfusion fluid Lactic acid in mg. per 100 c.c. fluid	Perfusion fluid Lactic acid in g. per 100 g. muscle	Total lactic acid in g. per 100 g. muscle	Area
O_2 for 3 hours at 37°C.						
1	0.484	0.119	6.09	0.315	0.434	290 sq.mm.
2	0.482	0.116	6.50	0.344	0.470	277 "
3	0.450	0.062	5.0	0.277	0.339	335 "
4	0.502	0.062	5.2	0.237	0.319	424 "
	Total 1.918	Mean= 0.090		Mean= 0.300	Mean= 0.390	Mean = 3.31 sq.cm.
Air for 3 hours at 37°C.						
1	0.864	0.180	13.6	0.301	0.481	
2	1.510	0.115	14.0	0.233	0.348	
3	0.772	0.145	10.0	0.324	0.466	
4	0.496	0.106	9.1	0.459	0.565	
	Mean =	0.111		0.354	0.465	
N_2 for 3 hours at 37°C.						
1	1.142	0.153	24.0	0.529	0.682	
2	0.516	0.161	12.6	0.610	0.771	
3	0.468	0.165	11.5	0.614	0.779	
4	0.554	0.110	14.7	0.665	0.775	
	Mean =	0.147		0.604	0.752	

Lactic acid production in 3 hours in:
Oxygen = 0.255 per cent.
Air = 0.330 "
Nitrogen = 0.616 "

Table XX.

Effect of sodium lactate on smooth muscle.

Control.

No Muscle.

To alkaline Ringer's solution with 0.1 per cent. glucose 4 mg. sodium lactate (= 3.2 lactic acid) per 100 c.c. was added and kept at 37°C. with O_2 bubbling.

Duration of oxygenation ~~in min.~~	Initial equivalent amount of lactic acid in mgm. per 100 c.c.	Amount of lactic acid after experiment in mg. per 100 c.c. fluid	
10 min.	3.2	3.1	Complete recovery of added lactate
3 hours	3.2	3.0	

Cat's Colon.

4 mgm. per 100 c.c. sodium lactate was added. Oxygenation for 3 hours in Ringer's solution (without glucose).

No. of expt.	Muscle wt. in g.	Muscle lactic acid in g. per 100 g. muscle	Lactic acid in fluid		Total lactic acid in g. per 100 g. muscle
			in mg. per 100 c.c. fluid	in g. per 100 g. muscle (after deduction of added amt.	
1	0.966	0.100	8.8	0.145	0.245
2	0.620	0.090	5.6	0.089	0.179
3	0.648	0.097	5.5	0.088	0.185
					0.203

Table XXI.

Cat's colon strip poisoned with sodium-iodo-acetate (S.I.A.) and suspended in oxygenated Locke's fluid containing 0.1 per cent. glucose.

Conc. of S.I.A. per cent.	Duration of expt. in hrs.	No.of expts.	Lactic acid content (g. per 100 g.muscle) (a) Control	(b) Experimental muscle and fluid	(c) Increase
(A) Spontaneous Contractions					
0	3	5	0.153	0.463	+ 0.310
0.01	3	3	0.152	0.229	+ 0.077
0.03	3	3	0.226	0.283	+ 0.055
(B) Electrical Stimulation					
0	2	3	0.100	0.566	+ 0.463
0.01	2	3	0.100	0.150	+ 0.050

Table XXII.

Carbohydrate breakdown by cat's colon strips normal and poisoned with S.I.A.(N/2080) in oxygenated Ringer's fluid without glucose. (in g. per 100 g. muscle)

Control muscle	Normal Muscle Oxygenation for		S.I.A. poisoned muscle Oxygenation for		
	10 min.	2 hours	10 min.	2 hours	
0.607	0.523	0.373	0.418	0.405	
0.660	0.620	0.440	0.482	0.402	
0.638	0.448	0.470	0.562	0.392	
0.490	0.500	0.435	0.501	0.432	
0.641	0.691	0.608	0.554	0.488	
0.607	0.556	0.465	0.503	0.424	= Mean
	0.051	0.142	0.104	0.183	= Breakdown

Table XXIII.

Lactic acid production by cat's colon strip normal and poisoned with S.I.A.(N/2080) in oxygenated Ringer's fluid without glucose (in g. per 100 g. muscle)

Control muscle	Normal Muscle Oxygenated for		S.I.A. poisoned muscle oxygenated for		
	10 min.	2 hours	10 min.	2 hours	
0.154	0.150	0.374	0.151	0.316	
0.074	0.106	0.304	0.097	0.224	
0.131	0.131	0.142	0.122	0.141	
0.081	0.137	0.323	0.150	0.257	
0.110	0.131	0.286	0.130	0.234	Mean
	0.021	0.176	0.020	0.124	Production

IX. FIGURES.

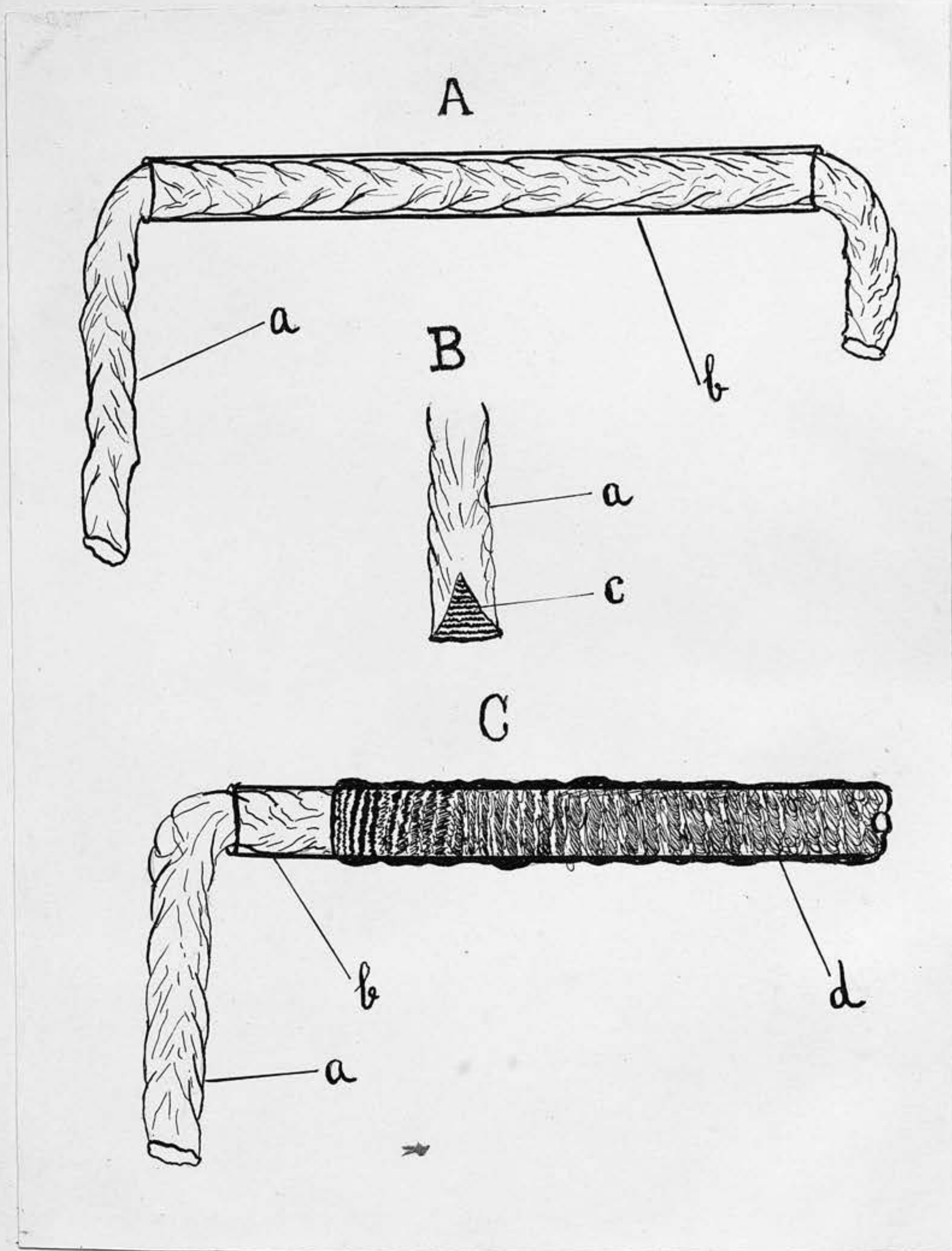


Fig. 1. Eversion of rabbit's gut.
A = Gut slipped in the glass tube.
B = Longitudinal slit at one end of the gut.
C = Lumen of the gut everted over the tube.
a = gut; b = glass tube.
c = longitudinal slit; d = mucosa of the gut.

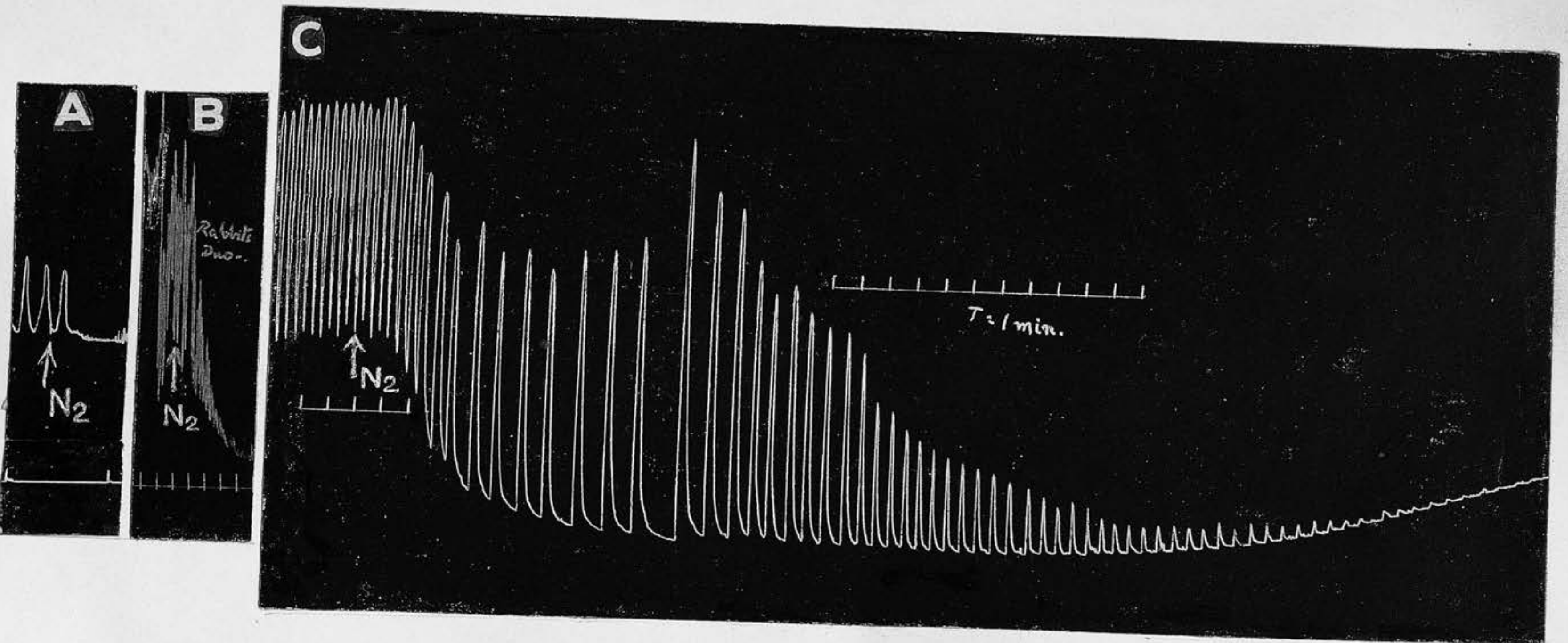


Fig. 2. Asphyxial arrest of spontaneous activity of gut. A = rabbit's stomach, B = Rabbit's duodenum, C = Cat's colon. (Time = 1 min.)

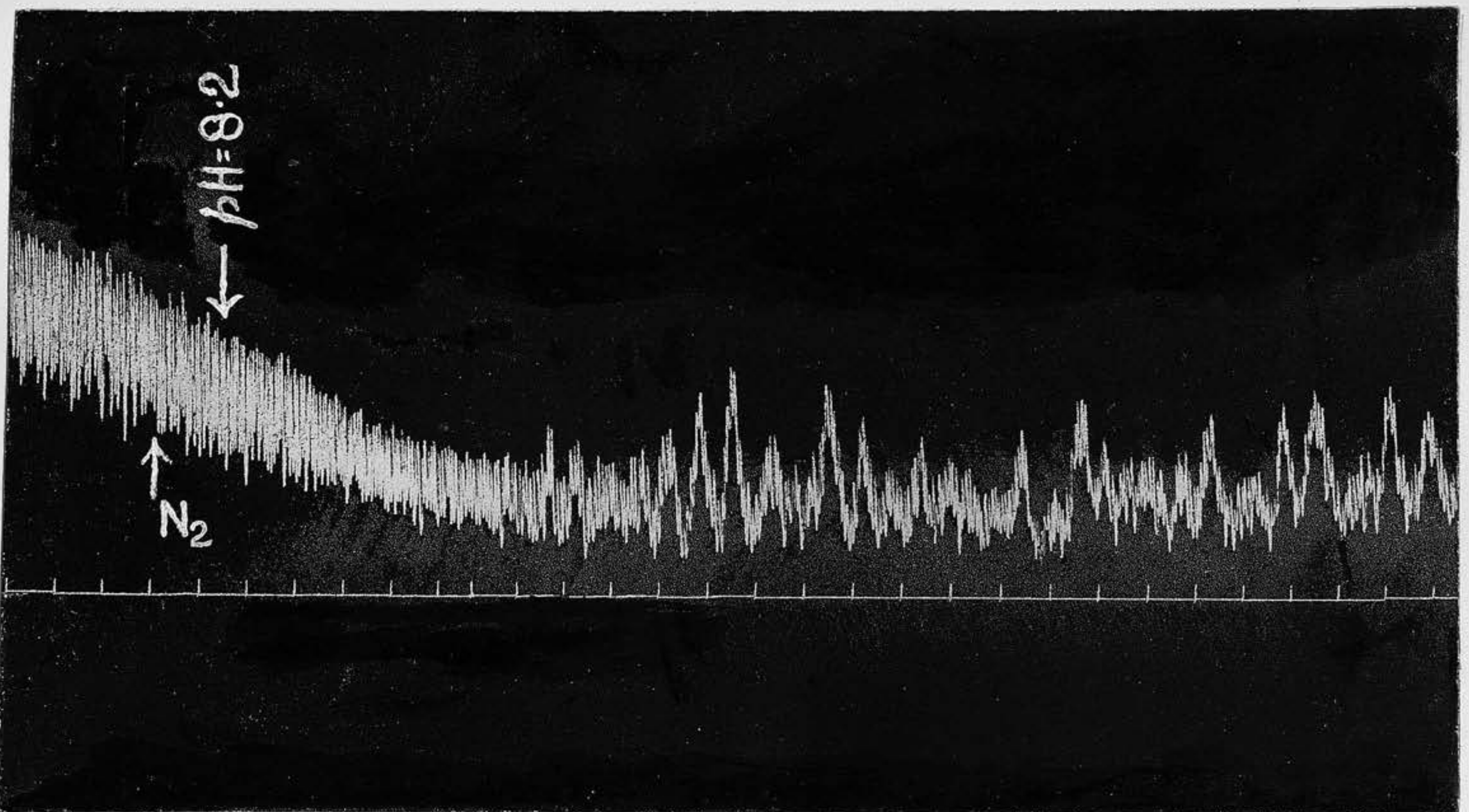


Fig. 3. Effect of asphyxia on spontaneous activity of rabbit's ileum in 0.1 per cent. glucose alkaline Locke's fluid (pH = 8.2). (Time = 1 min.)

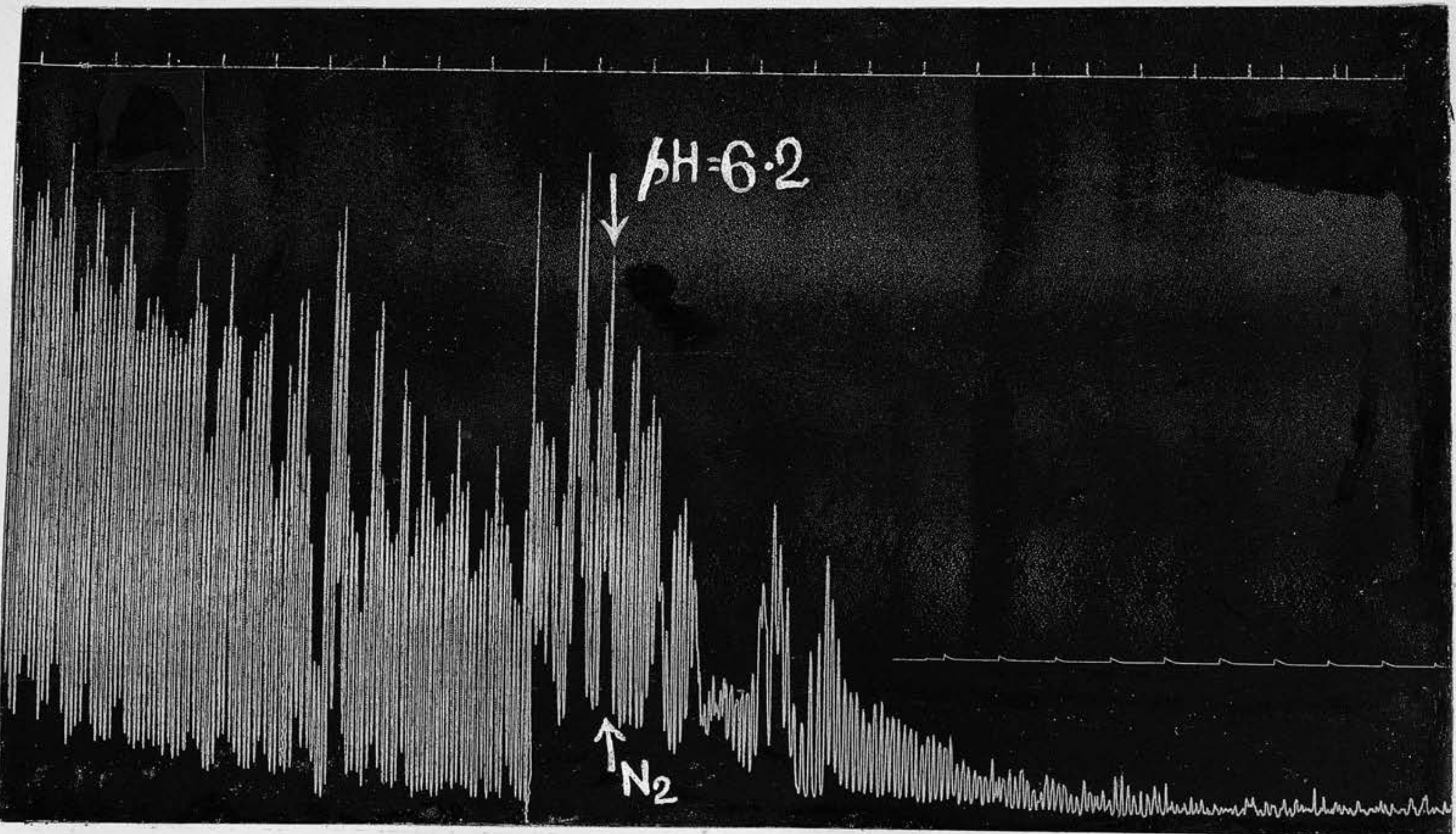


Fig. 4. Effect of asphyxia on spontaneous activity of rabbit's ileum in acid fluid. (pH = 6.2). Time = 1 min.

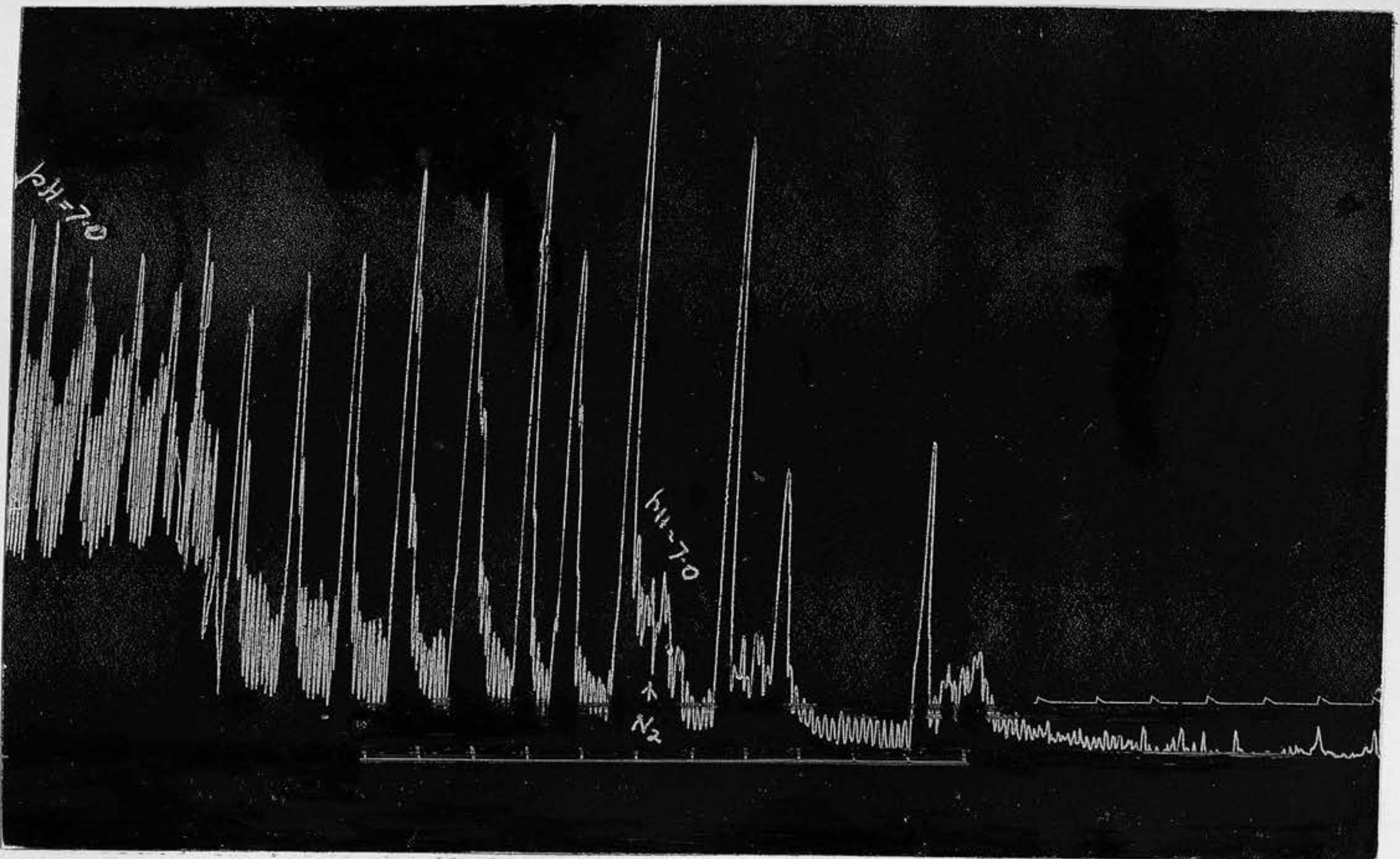


Fig. 5. Effect of asphyxia on spontaneous activity of rabbit's ileum in buffered fluid (pH = 7.0). Time = 1 min.

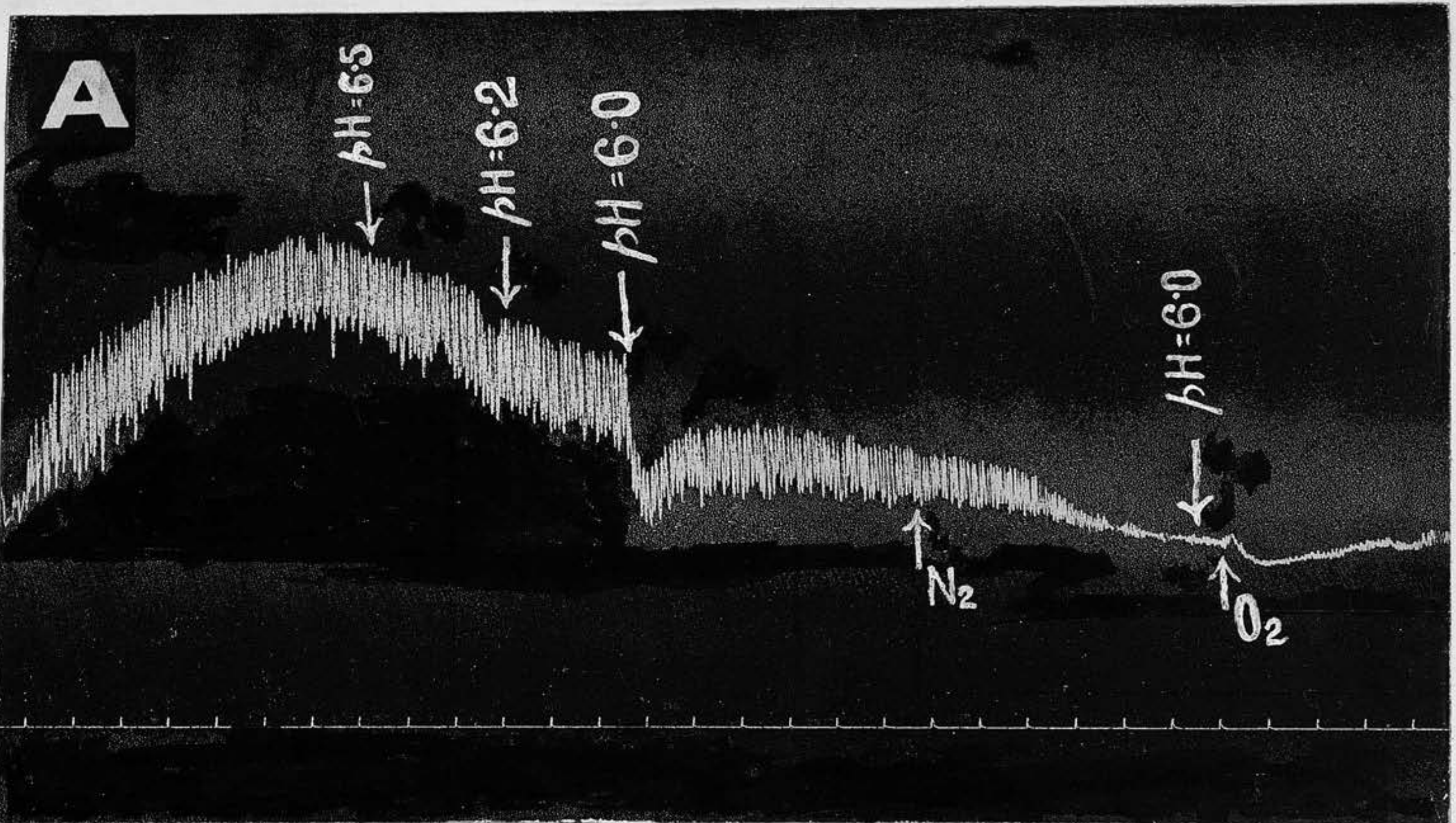


Fig. 7. Effect of asphyxia on spontaneous activity of rabbit's ileum in 0.1 per cent. glucose acid Locke's fluid (pH = 6.0). Time = 1 min.

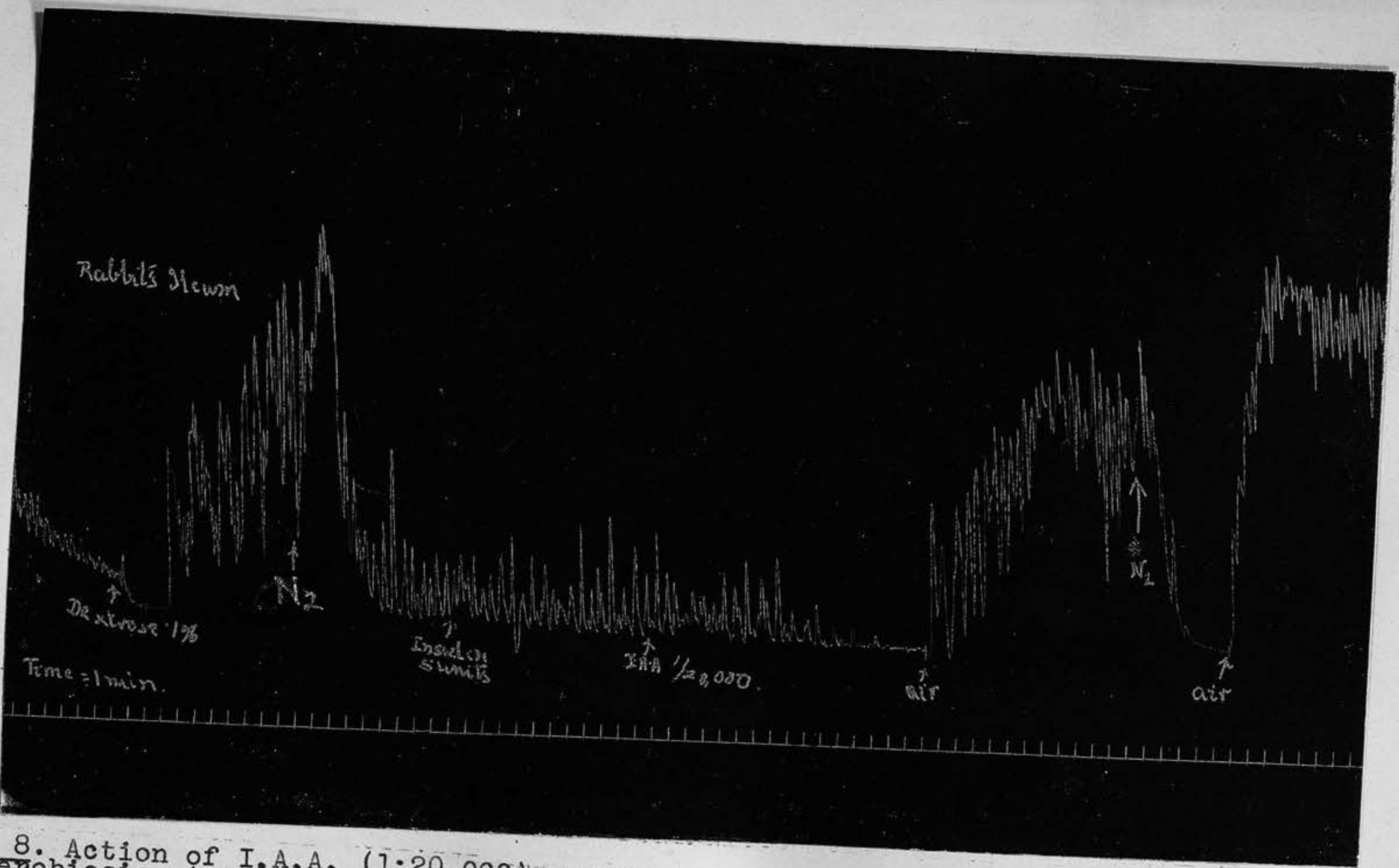


Fig. 8. Action of I.A.A. (1:20,000) on spontaneous activity of rabbit's ileum. Anaerobiosis stops the activity and re-oxygenation revives it. (Time = 1 min.)

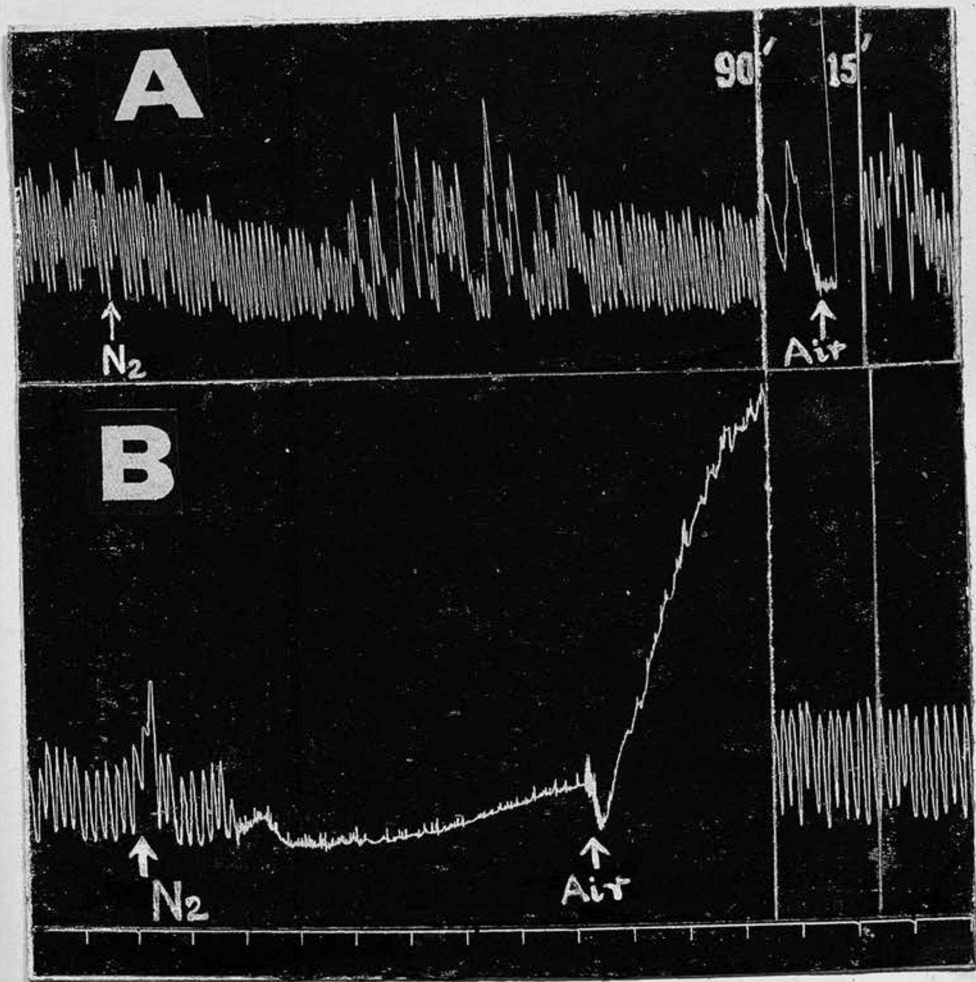


Fig. 9. Effect of asphyxia on spontaneous movements of rabbit's ileum in 0.1 per cent. glucose - Ringer's fluid, A = without I.A.A.
do. do. B = with I.A.A. (1:10,000)
Time marked in figures indicates stoppage of drum.
(Time = 1 min.)

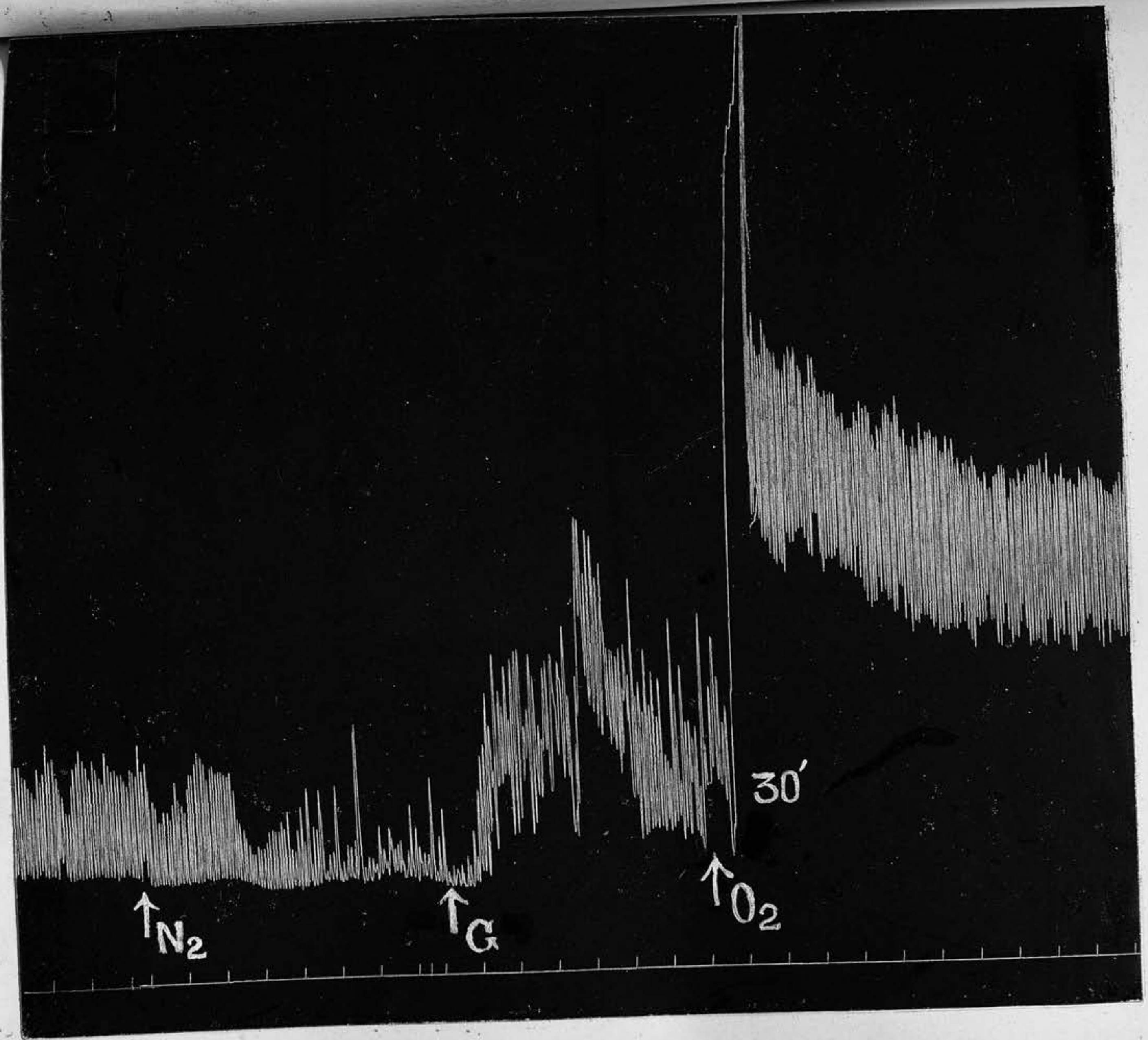


Fig. 13. Effect of glucose (0.1 p.c.) and then oxygenation on revival of spontaneous movements and tonus of rabbit's ileum. Time marked in figure indicates stoppage of drum. (Time = 1 min.)

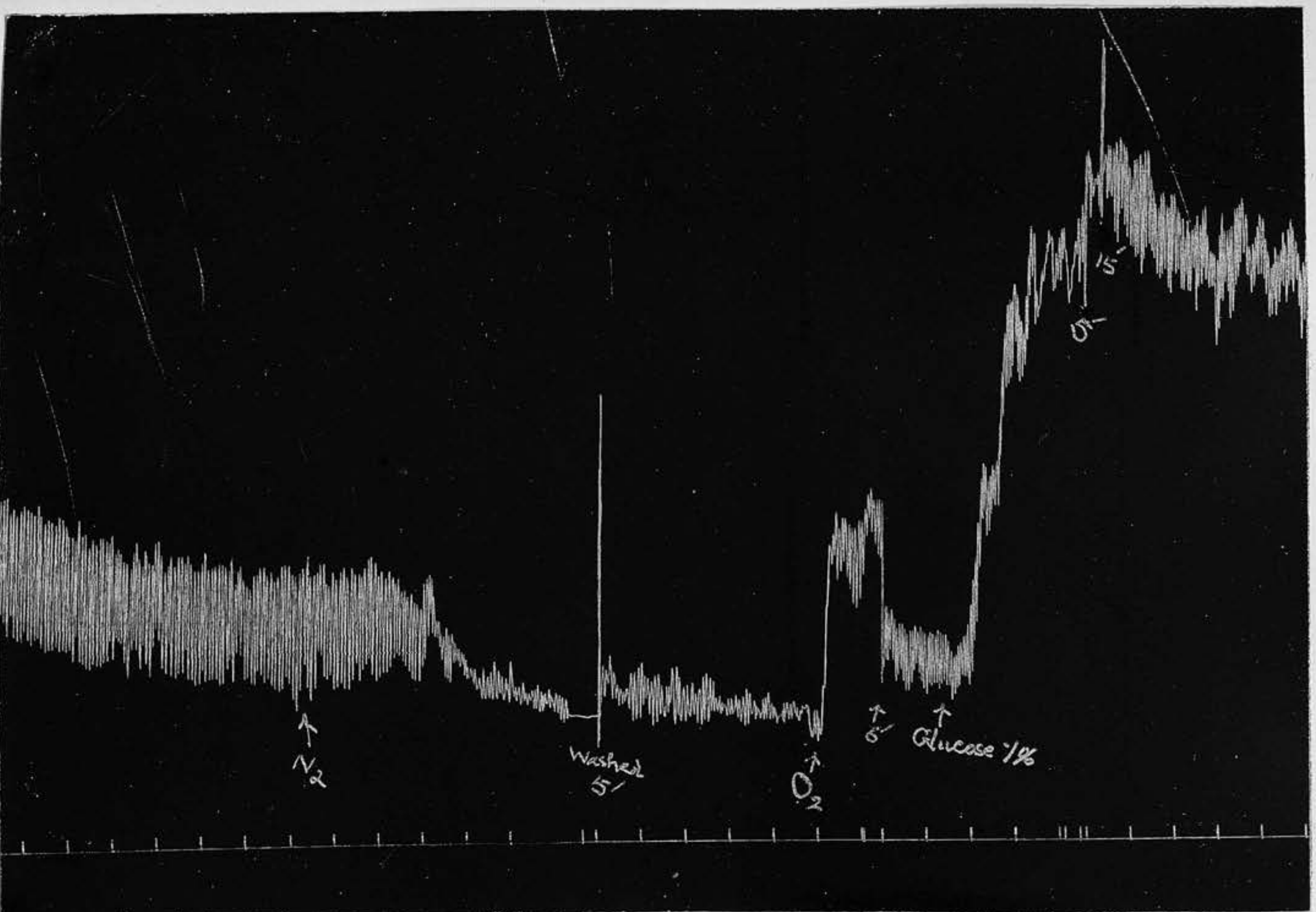


Fig. 14. Effect of washing out of the asphyxially arrested rabbit's ileum and revival with oxygenation first and then with 0.1 per cent. glucose. Time in figures indicates stoppage of drum. (Time = 1 min.)

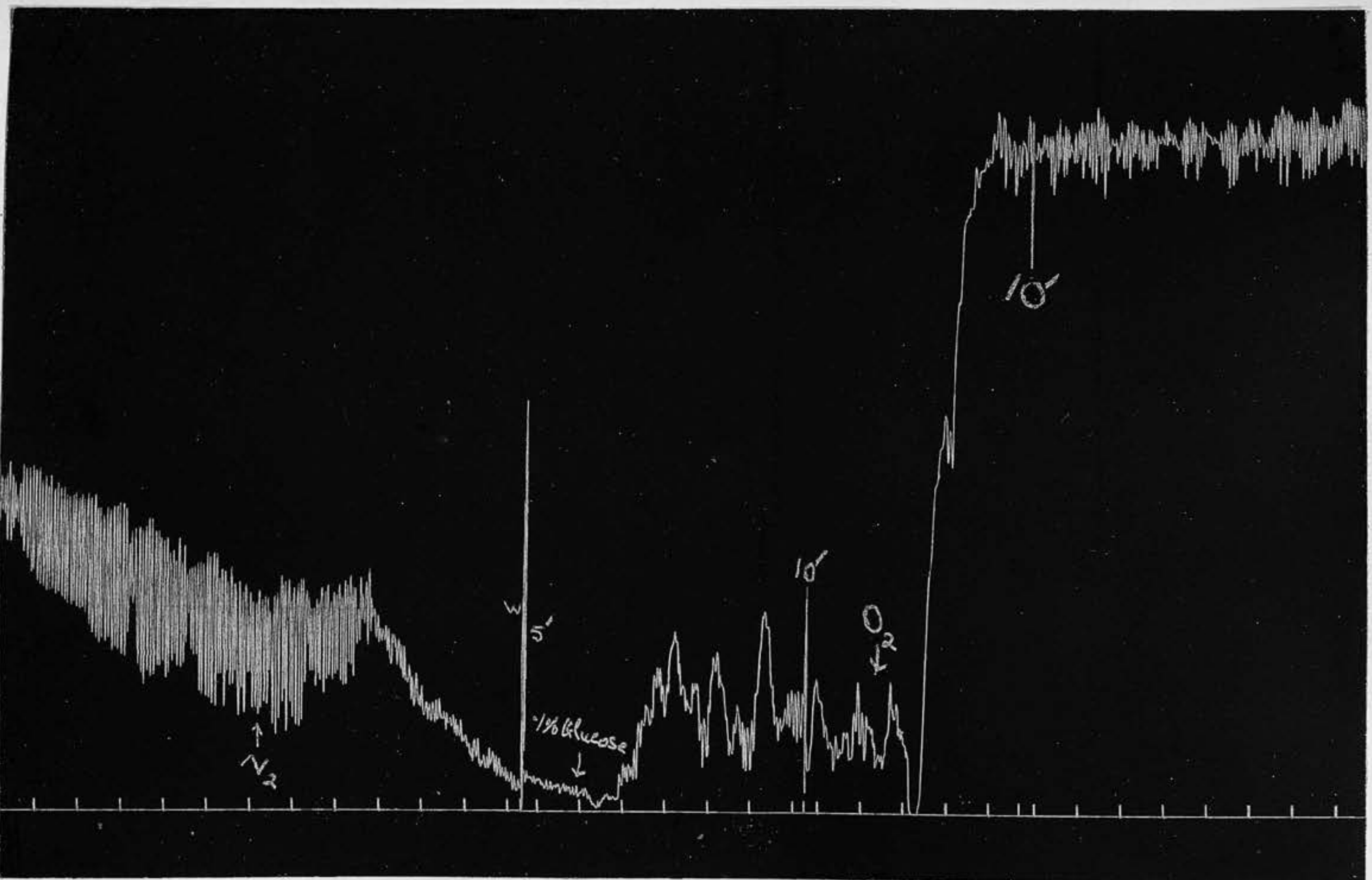


Fig. 15. Effect of washing out of the asphyxially arrested rabbit's ileum and revival with 0.1 per cent. glucose first and then with oxygenation. Time in figures indicates stoppage of drum. (Time = 1 min.)

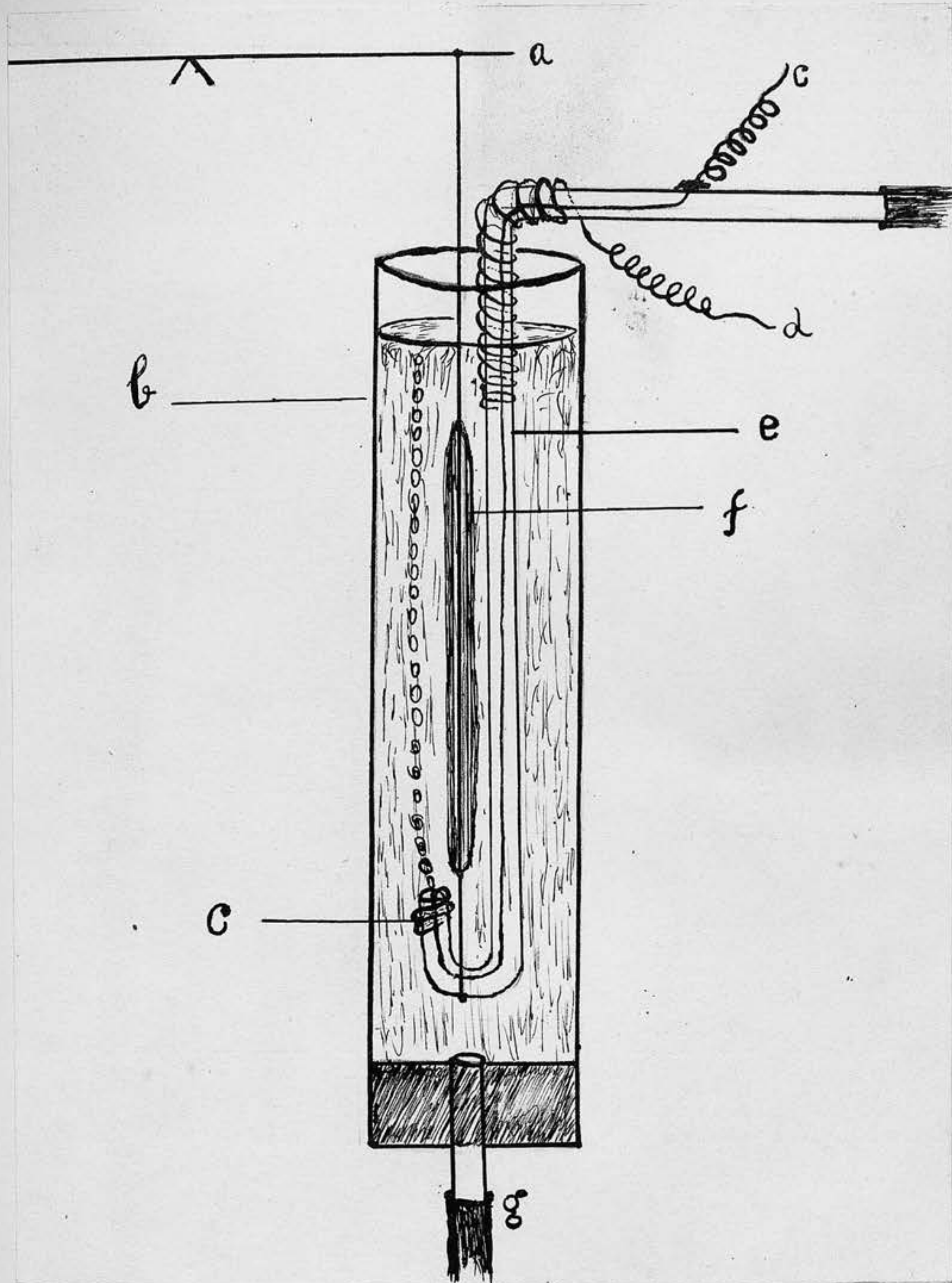


Fig. 17. Scheme of stimulating the smooth muscle.
a = lever; b = bath; c and d = electrodes.
e = Glass tube through which electrode c is passing to the bottom end of the muscle and also oxygen or nitrogen is bubbled through it.
f = Strip of smooth muscle.
g = Outlet and inlet for perfusing fluid.

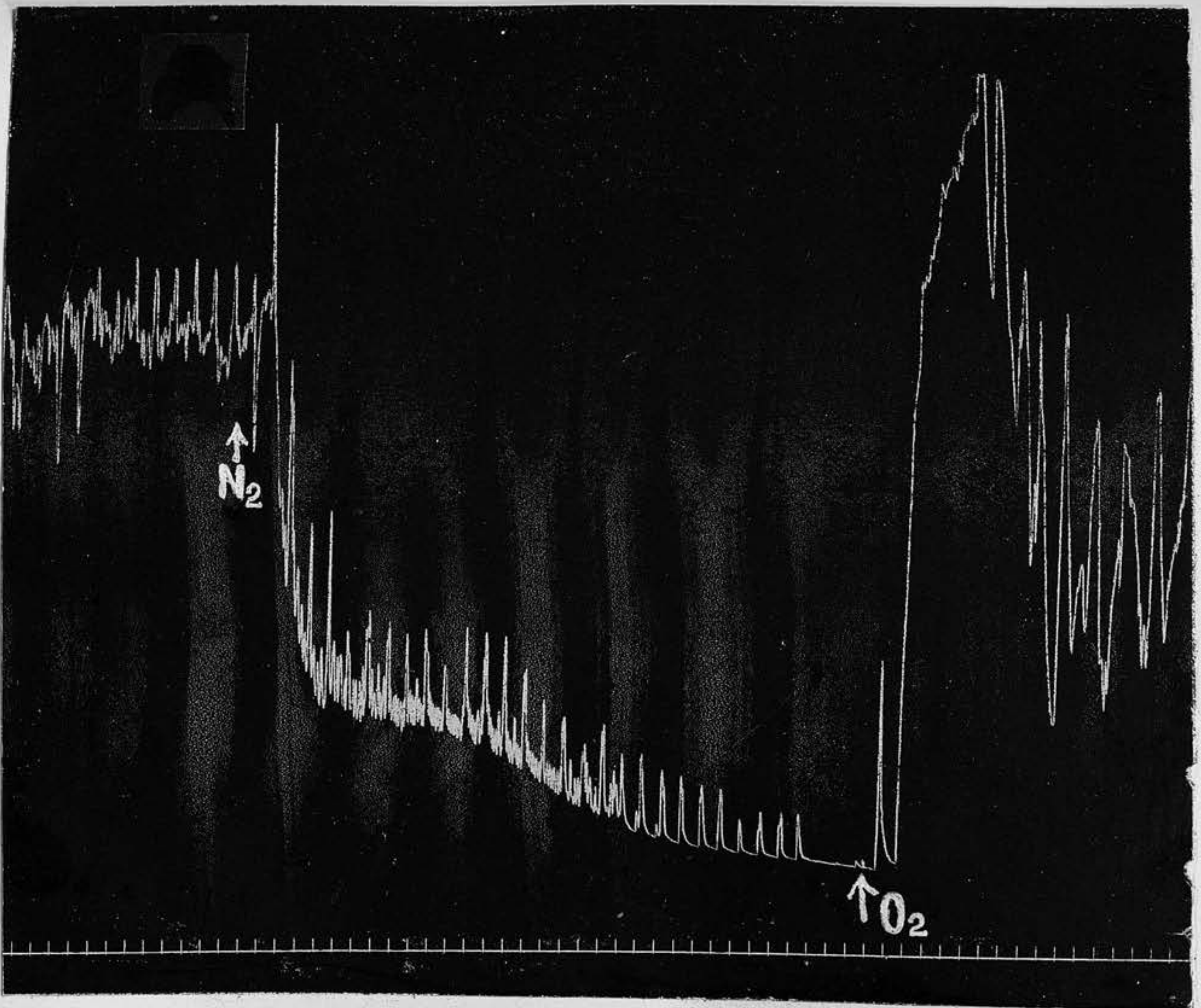
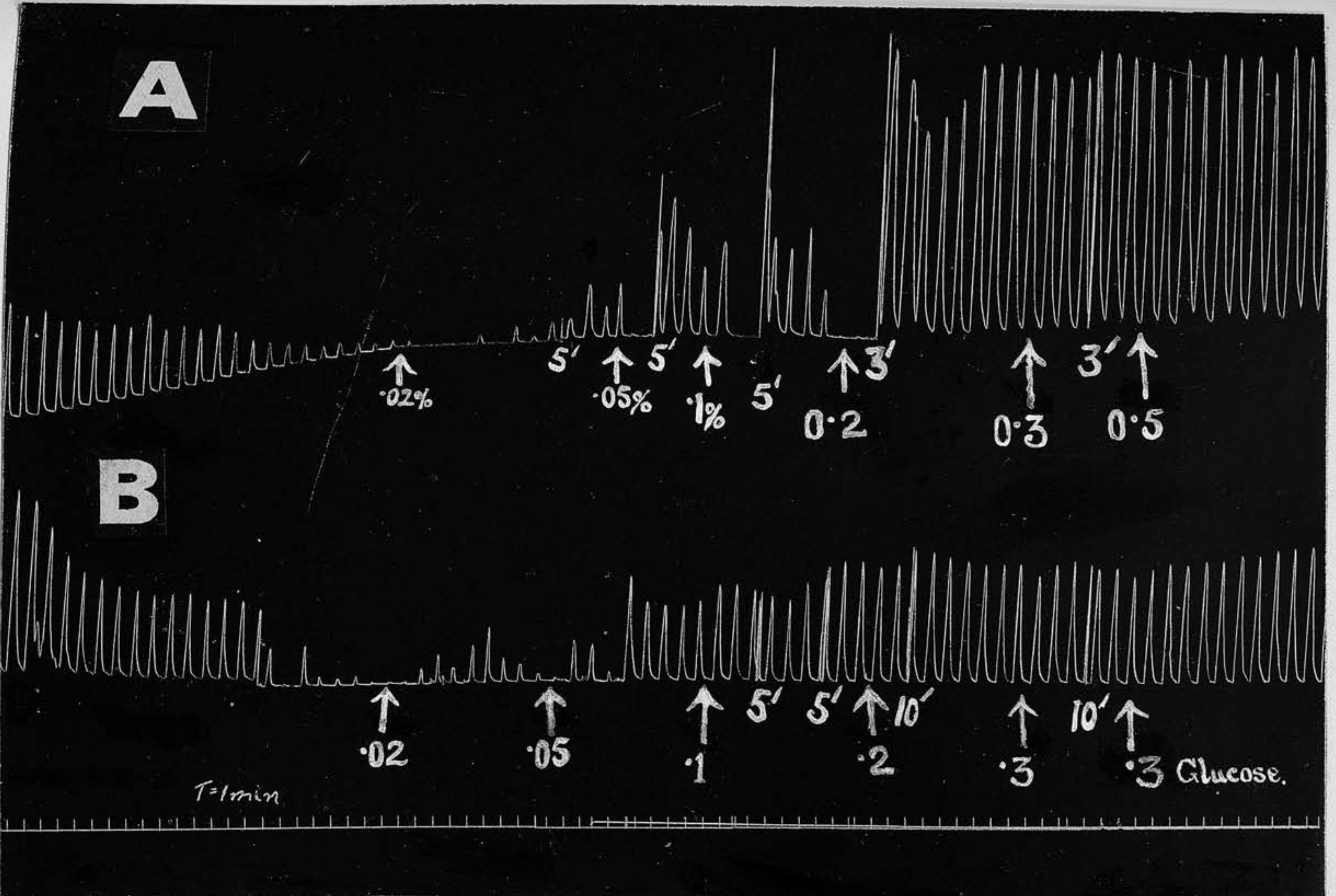


Fig. 18. Effects of asphyxia on stimulated fresh colon of cat. (Time = 1 min.)



<u>Fig. 20</u>. Beneficial effects of glucose and mannose on asphyxiated and stimulated cat's colon. Concentrations attained in the perfusing fluid are indicated at arrows in per cent. A = Glucose; B = Mannose.

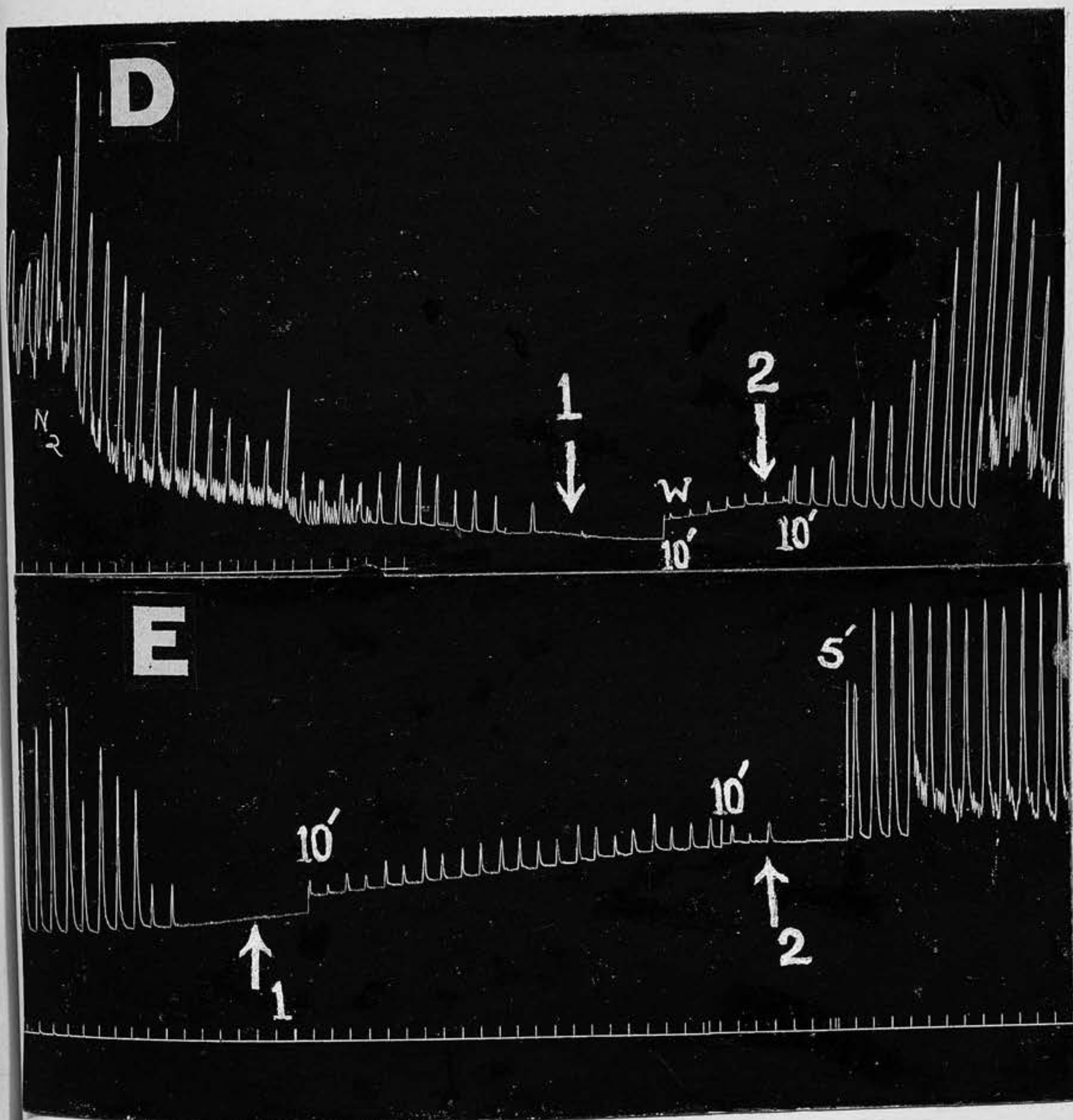


Fig. 21. Effect of galactose and fructose on asphyxiated and stimulated cat's colon. Different substances were added at arrow marks to obtain 0.1 per cent. final concentration. D. - 1 = galactose, 2 = glucose. E - 1 = fructose, 2 = glucose. Time marked in the figures indicates stoppage of drum. (Time = 1 min.)

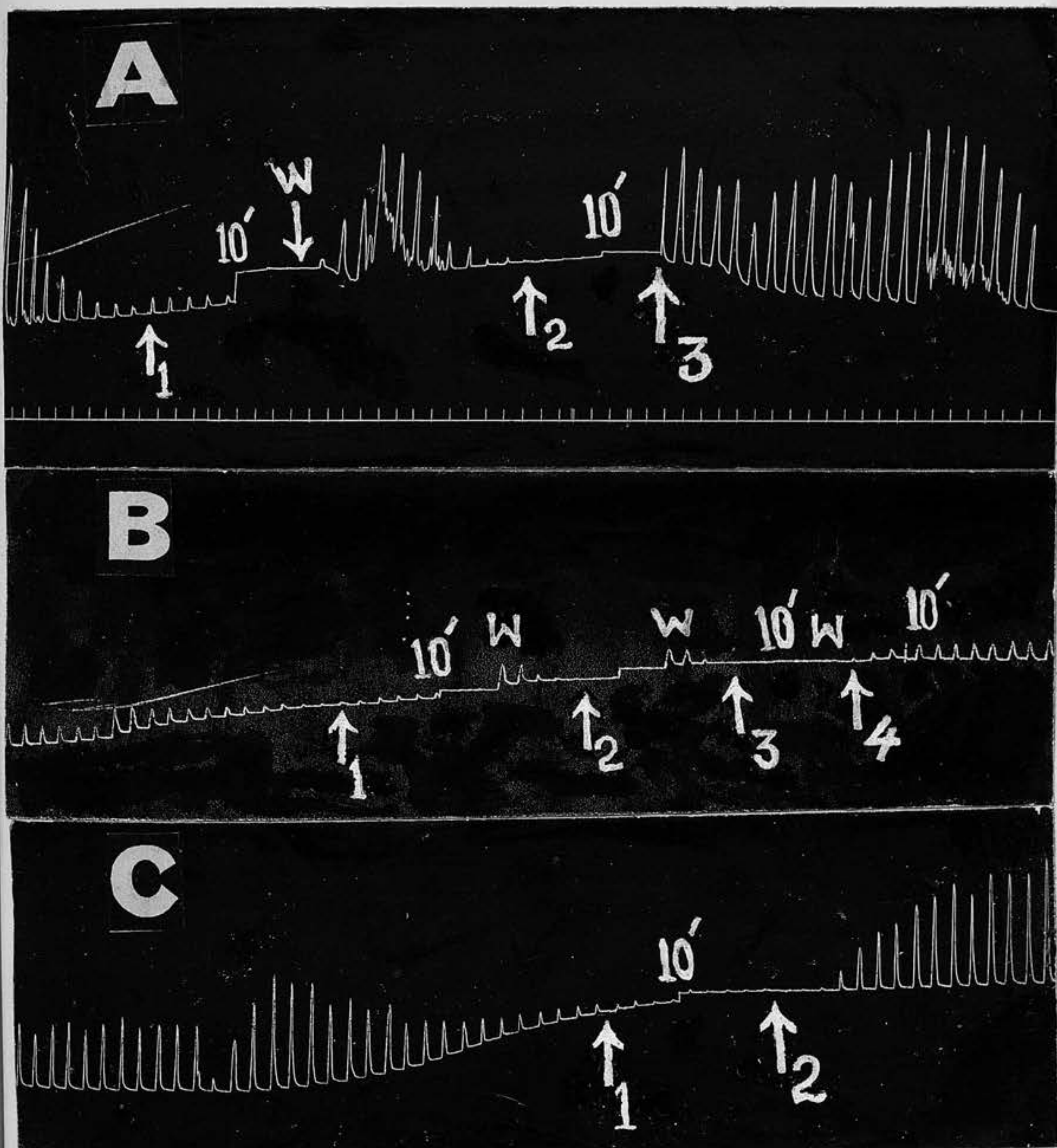


Fig. 22. Effect of carbohydrate etc. on asphyxiated and stimulated cat's colon. Different substances were added at arrow marks to obtain 0.1 per cent. final concentration.

(A) 1 = alanine, 2 = glycine, 3 = glucose.
(B) 1 = sodium lactate, 2 = sodium pyruvate, 3 = sodium oleate, 4 = glucose.
(C) 1 = maltose, 2 = glucose.

Time marked in figures indicates stoppage of drum. (Time = 1 min.)

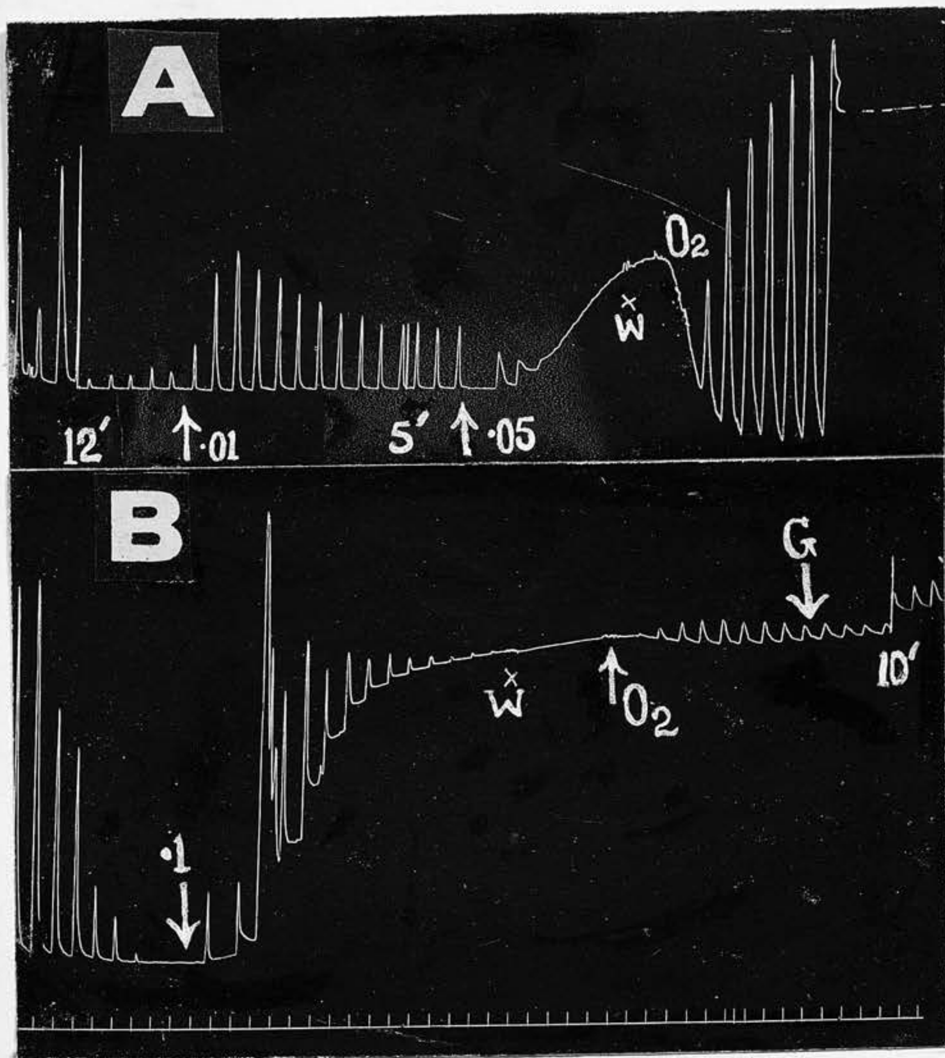


Fig. 23. Effect of methylglyoxal on stimulated cat's gut muscle.
A = asphyxiated muscle shows beneficial effect in low concentration of 0.01 p.c. and rigor effect in 0.05 p.c.
B = muscle passes into rigor in high concentration 0.1 p.c. and is not reversible.
Time marked in figures indicates stoppage of drum. (Time = 1 min.)

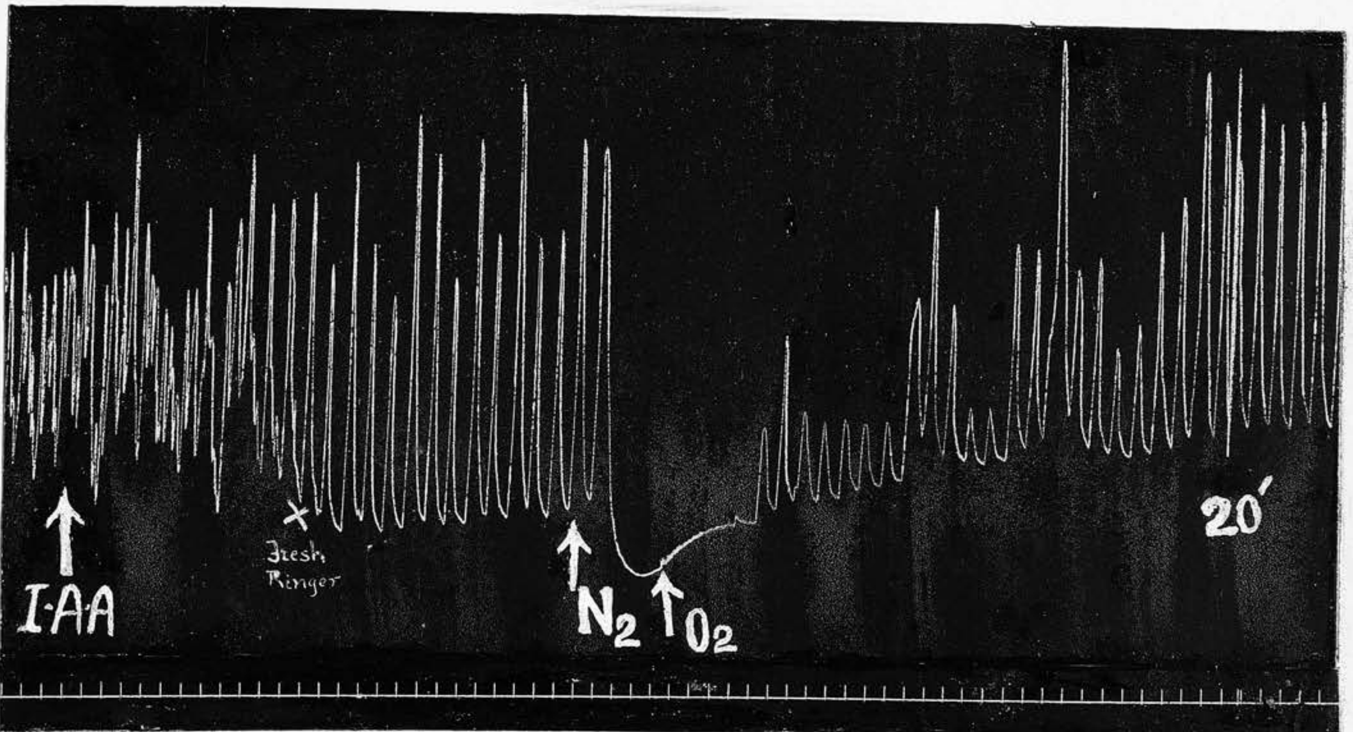


Fig. 24. Effect of I.A.A. (1:10,000) on stimulated cat's colon. Arrested movements are revived if oxygenation is resumed soon. (Time = 1 min.)

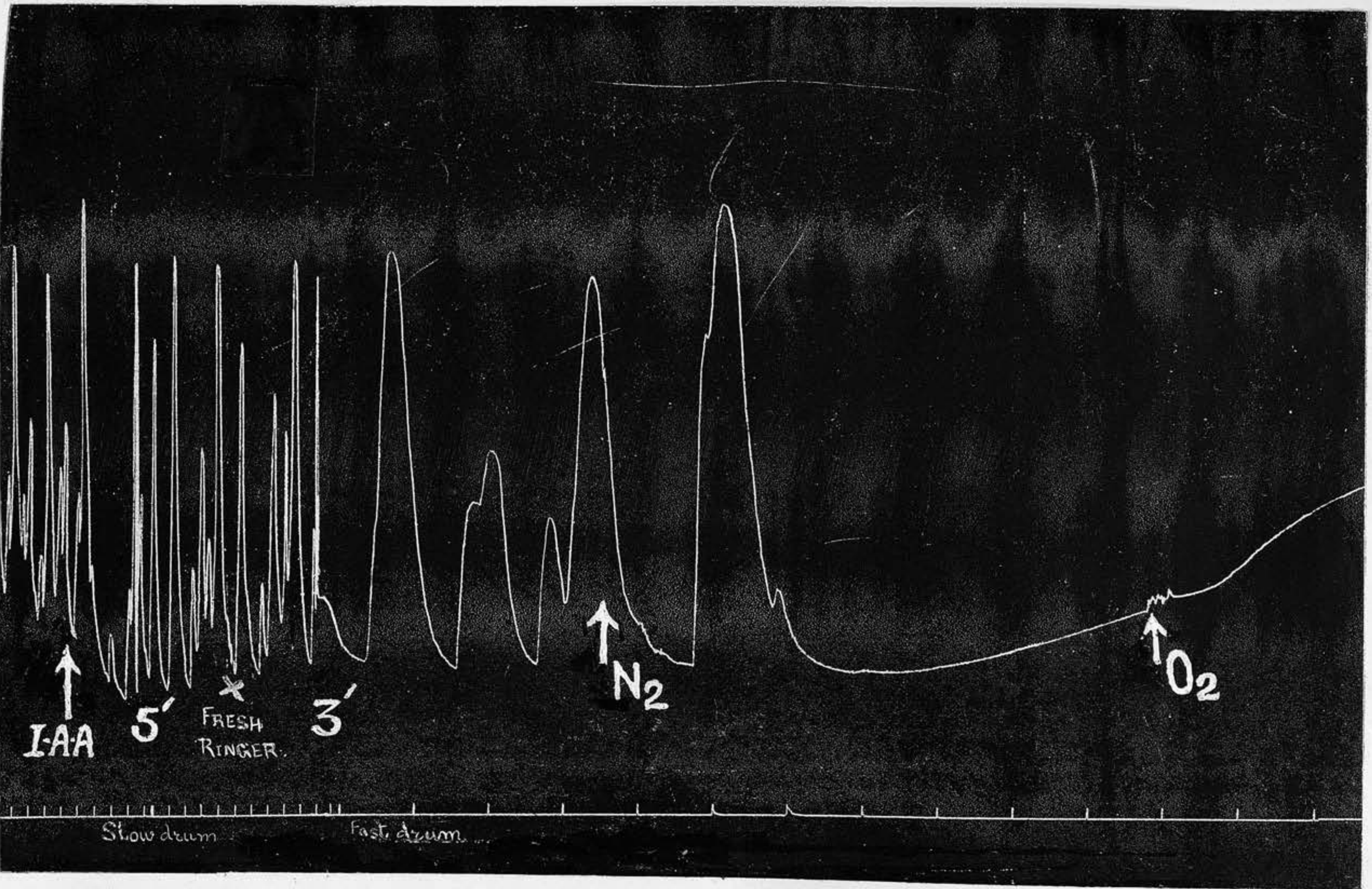
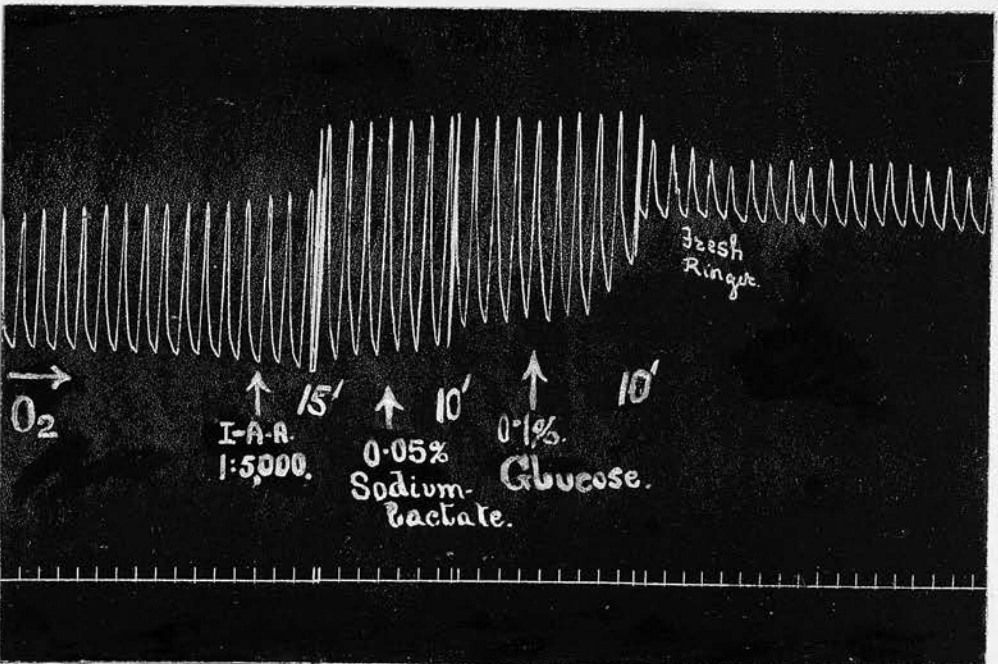


Fig. 26. Effect of I.A.A. (1:10,000) on stimulated cat's colon. Time of asphyxial arrest about 3 min. The drum was accelerated to note the exact time of cessation in oxygen lack. (Time = 1 min.)



<u>Fig. 27</u>. Effects of sodium lactate and glucose on I.A.A. poisoned cat's gut stimulated in oxygenated Ringer's fluid; shows no beneficial effect. (Time = 1 min.)

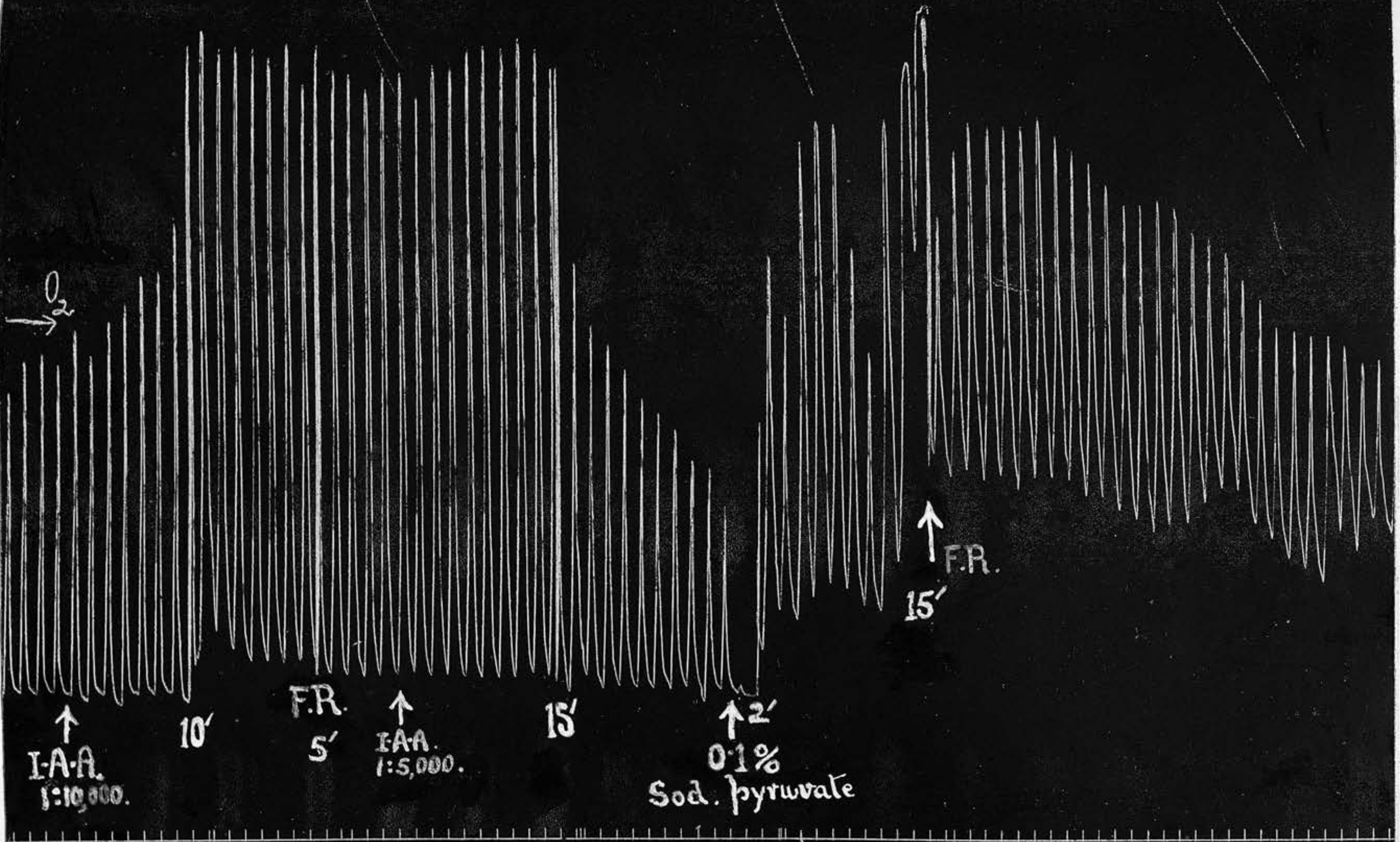


Fig. 28. Effects of sodium pyruvate on I.A.A. poisoned cat's gut stimulated in oxygenated Ringer's fluid; shows beneficial effect. (Time = 1 min.)

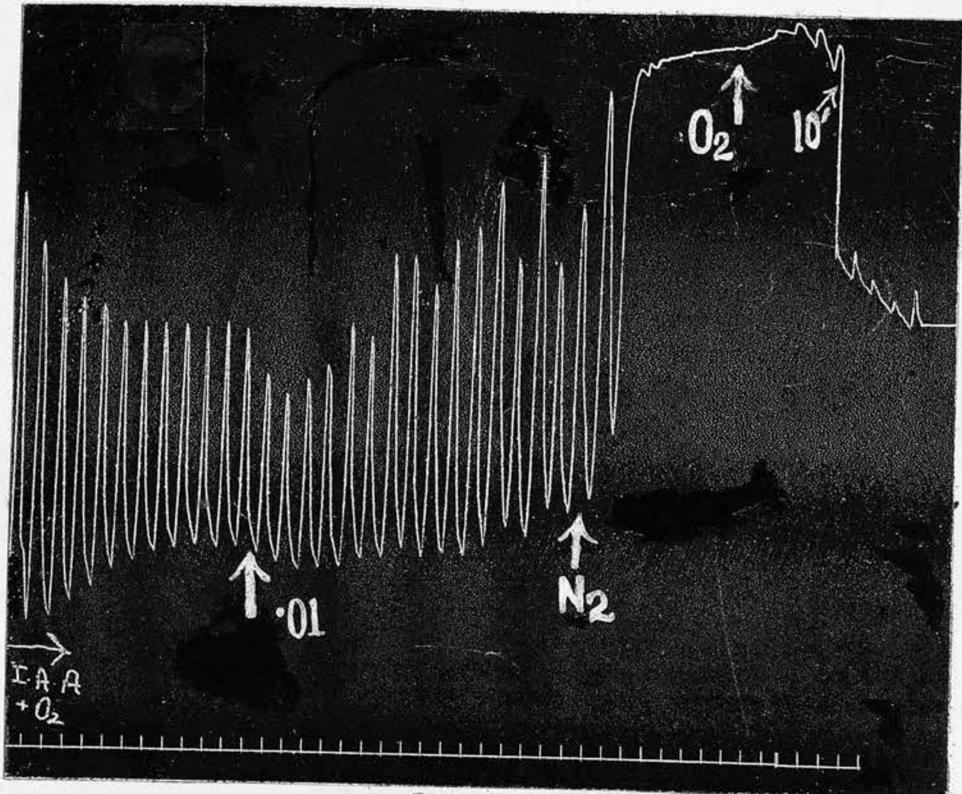


Fig. 29. Effect of methylglyoxal on I.A.A.(1:10,000) poisoned cat's gut stimulated in oxygenated Ringer's fluid; muscle shows some beneficial effect but introduction of anaerobiosis sends it into sudden rigor. (Time = 1 min.)

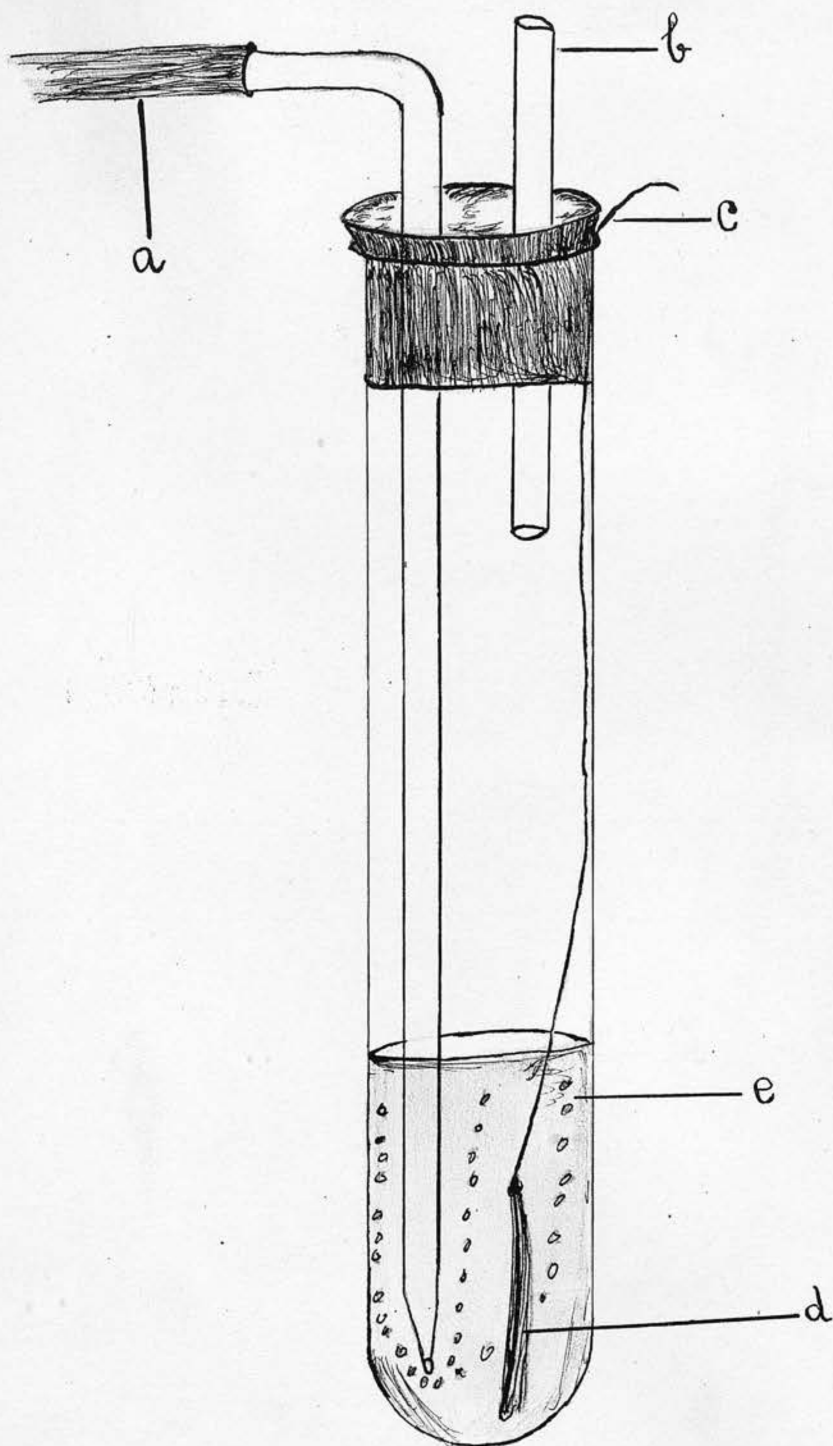


Fig. 30. Aeration or nitrogenation of suspended smooth muscle.
a = Inlet for oxygen or nitrogen.
b = Free outlet.
c = Thread.
d = Strip of smooth muscle.
e = Perfusion fluid.

X. APPENDIX.

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612.734.11

THE CARBOHYDRATE METABOLISM OF GUT MUSCLE.

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(*Received July* 5, 1935.)

INTRODUCTION.

HE experiments described below were undertaken for the purpose of alysing the sources of energy utilized in the contraction process of nooth muscle. The methods used were similar to those which have been und of value in analysing the metabolism of skeletal and cardiac muscle. he effect of anaerobiosis on the metabolic changes was determined stly on normal muscle and then on muscle poisoned with iodoacetic id. The plain muscle studied was that of the rabbit's ileum and the t's colon. The biochemical analyses described in this paper were underken as a preliminary to the study of the effect of asphyxia under rious conditions on the mechanical response of smooth muscle. These sults will be described in a later paper.

LITERATURE.

Rosenthal and Lasnitzki [1928] found that the oxygen consumpn of the smooth muscle of the rabbit's colon was 2·64 c.c. per g. dry ight per hour (=0·53 c.c. per g. wet weight per hour), while the ygen consumption of the colon mucosa was about four times as great. is result shows that in metabolic studies it is essential to use smooth uscle free from mucosa, since when both are present the results will press chiefly the changes occurring in the mucosa. Their results also ow the following values for lactic acid production (g. per 100 g. wet ight per hour): rabbit's stomach muscle, anaerobic, (*a*) in glucose free nger's fluid 0·04, (*b*) in Ringer's fluid with 0·2 p.c. glucose 0·6; rabbit's lon muscle in glucose Ringer's fluid, (*a*) aerobic 0·031, (*b*) anaerobic 49.

The energy set free by the use of 0·53 c.c. of oxygen in oxidizing carbohydrate is about 2·5 cal., whilst in the case of skeletal muscle the energy set free by the conversion of 5 mg. glycogen to lactic acid and by the neutralization of the acid is 1·5 cal. Hence the energy release in the anaerobic metabolism of plain muscle in presence of glucose Ringer's fluid is probably of the same order as that in the aerobic metabolism. On the other hand, the energy release under anaerobic conditions in absence of glucose must be less than one-tenth of these amounts, and hence it appears that the gut muscle has relatively little power to convert its own carbohydrate to lactic acid. Their figures also show that even under aerobic conditions the isolated gut muscle produces a certain amount of lactic acid.

Haarmann [1932] who used dog's gut and human uterine muscle, found that under anaerobic conditions there was little formation of lactic acid in absence of glucose, but that when glucose was added there was a large formation. Saiki [1908] found that there was very little glycogen in the frog's stomach and bladder and that the lactic acid content in fresh specimens was about 0·06 p.c. Evans [1925] found in the retractor penis of dogs 0·06 p.c. soluble carbohydrate and 0·2 p.c. lactic acid, and the latter figure rose to 0·3 p.c. in rigor. In the fresh dog's intestine he found 0·005 p.c. glycogen and 0·05 p.c. lactic acid, and the latter figure rose to 0·08 p.c. in rigor. In a later communication Evans [1926] stated that his figure for glycogen in the intestine was too low. Horne and Magee [1933] found 0·008–0·025 p.c. glycogen in the gut (muscle plus mucosa) of the rabbit.

Methods.

Cat's colon and rabbit's ileum were used in the present investigation. Rabbits were killed by breaking their necks and cats were first stunned with an electro-lethaler and were then killed by cutting the carotids. In some cases the colon was removed under ether anæsthesia. The gut was removed and placed in Ringer's fluid at 0° C., and muscle was separated from the mucosa at this temperature.

Ringer's fluid of the following percentage composition was used: NaCl 0·9, KCl 0·042, CaCl 0·024 and $NaCHO_3$ 0·05. Glucose free Ringer's solution was used except when otherwise stated.

In all cases sufficient muscle was isolated to serve for the experiment and for the control. Care was taken to obtain both sets of muscle from adjacent portions of the gut. The gut strips were suspended in Ringer's fluid in a bath at 37° C. The temperature was controlled within 0·1° C.

y an electrical thermostat. Aerobic and anaerobic conditions were oduced by passing oxygen or nitrogen respectively.

In most of the experiments the gut was allowed to contract sponneously, but in a few experiments (Table IV) it was stimulated elecically. The stimulus was an alternating current at 16 volts which was plied through the length of the muscle for 5 sec. every minute. The sults in Table IV indicate that the metabolism of the stimulated gut uscle is about 12 p.c. greater than that of the unstimulated gut muscle.

The estimation of lactic acid.

The tissue was dried gently between moist filter paper and the moist eight was determined on a torsion balance. The tissue was then put into e-cold tungstic acid and ground with sand in a cooled mortar. The method estimation used was the modification of Friedemann, Cotonio and haffer's method [1927] used and described by Kerley [1931]. In is method manganese sulphate is used to catalyse the reaction and the dehyde formed is distilled over a current of steam instead of air. The stilled aldehyde is absorbed in bisulphate and the bound sulphate is trated with iodine.

The estimation of total carbohydrate.

The method for determining the total carbohydrate is the same as nployed by Clark *et al.* [1931] in this laboratory for the frog's heart. he method was described by Ochoa [1930]. All the carbohydrate is nverted to reducing dextrose by treating the tissue with 6 p.c. sulphuric id for 3 hours in a boiling water bath. The dextrose is estimated by the ethod of Hagedorn and Jensen [1923*a*, 1923*b*]. The total reducing bstance present is calculated as dextrose. It is described as "total rbohydrate", although it would be more accurate to speak of "total ducing substance" because it probably includes reducing substances her than carbohydrates.

The carbohydrate and lactic acid content of fresh gut muscle.

The average results obtained by the author with fresh muscle are own in Table I. These results agree fairly well with the results obtained previous workers. The figures for lactic acid content are higher than ose obtained by other workers, but this is probably due to the inevite injury produced by separating the muscle from the mucous memane. This was carried out in iced Ringer's fluid, but as the purpose of

TABLE I. Carbohydrate and lactic acid content of freshly isolated gut muscle.

	Total carbohydrate g. per 100 g. muscle			Lactic acid g. per 100 g. muscle		
Tissue	(*a*) No. of obser-vations	(*b*) Average value	(*c*) Range of results	(*a*) No. of obser-vations	(*b*) Average value	(*c*) Range of results
Colon of cat	16	0·684	0·490–0·947	19	0·117	0·074–0·175
Ileum of rabbit	10	0·595	0·418–0·943	11	0·173	0·073–0·300

these estimates was to serve as controls for experiments made with surviving strips, it was not possible to use more drastic methods (such as freezing with carbon dioxide snow) to arrest lactic acid production during isolation of the muscle. The variation in the values obtained is considerable, but fortunately it was possible always to make control estimations in all the experiments described below and thus to eliminate the effect of individual variation.

THE UTILIZATION OF TISSUE CARBOHYDRATE.

A series of experiments was made with gut muscle suspended in glucose-free Ringer's fluid. The general object of these experiments was to determine how much of its own carbohydrate the muscle could convert

TABLE II. Utilization of carbohydrate by gut muscle in Ringer's fluid without glucose.

	Total carbohydrate				Lactic acid				
		g. per 100 g. muscle				g. per 100 g. muscle			
							Experimental		
Duration in hours	No. of exps.	Control	Exp.	Carbohydrate loss	No. of exps.	Control	Muscle	Fluid	Total
				A. Cat's colon.					
				(*a*) Oxygenated.					
1	4	0·760	0·542	0·218	6	0·123	0·097	0·078	0·175
2	5	0·607	0·465	0·142	4	0·110	0·130	0·156	0·286
1		(Stimulated muscle)			3	0·089	0·063	0·083	0·146
				(*b*) Anaerobic.					
1	4	0·760	0·542	0·218	3	0·158	0·092	0·204	0·296
				(*c*) In Ringer's fluid at room temperature.					
24	4	0·760	0·503	0·257	3	0·158	0·100	0·244	0·344
				B. Rabbit's ileum.					
				(*a*) Oxygenated.					
1	9	0·592	0·437	0·155	7	0·213	0·123	0·105	0·228
				(*b*) Anaerobic.					
1	9	0·592	0·348	0·244	7	0·213	0·183	0·165	0·348
				(*c*) In Ringer's fluid at room temperature.					
24	4	0·573	0·316	0·257	3	0·233	0·113	0·253	0·366

to lactic acid. The results are summarized in Table II. The result of tstanding importance is that although the total carbohydrate content the gut amounted in some cases to as much as 0·760 p.c., yet under no nditions was it possible to cause a loss of more than 0·26 p.c. The nount of carbohydrate in the gut available for conversion into lactic id is therefore about 0·25 p.c. The gut suspended for 1 hour in oxygened Ringer's fluid utilized, however, from 0·16 to 0·22 p.c. carbohydrate. here appears therefore to be a small quantity of labile carbohydrate in e gut, and this is fairly rapidly exhausted even under aerobic conditions.

This conclusion was confirmed by the following experiment. Two eces of cat's colon were suspended in oxygenated Ringer's fluid and re removed after 1 hour and after 3 hours respectively. The following erage values were obtained in three experiments. The total carbodrate content (mg. per 100 g. muscle) was: after 1 hour 0·437; after hours, 0·426. The lactic acid in the fluid (g. per 100 g. muscle) was: ring the first hour 0·100 and during the next 2 hours 0·039. Since the id was changed at the end of the first hour the cessation of carbodrate breakdown was not due to the formation of lactic acid. These ults indicate that most of the carbohydrate breakdown and lactic id excretion occurs during the first hour of the isolation.

The lactic acid found in the control strips of the cat's colon (about 12 p.c.) was three times that found by Evans [1925] in the dog's estine. It is probable therefore that between 0·05 and 0·10 p.c. of tic acid was formed during the manipulation of the muscle.

The figures suggest that in the muscle *in situ* there is about 0·35 p.c. labile carbohydrate, that about 0·10 p.c. of this is changed to lactic d during manipulation, and that from 0·15 to 0·25 p.c. is broken down ring the first hour of isolation irrespective of whether the conditions e aerobic or anaerobic. Table II shows that there was a considerable riation in the amount of lactic acid formed under aerobic conditions. is suggests that a variable portion of the tissue was receiving an dequate oxygen supply even in oxygenated fluid. The oxygen conmption of the tissue may be assumed to be about 0·5 c.c. per g. per hour osenthal and Lasnitzki, 1928] which equals 0·008 c.c. per g. per n. The thickness of typical pieces of colon as estimated from their ight and area was 0·14 cm.

Warburg [1923] calculated that the thickness of tissue (d) which uld receive an adequate oxygen supply when suspended in fluid urated with oxygen, and using a quantity (A) of oxygen per g. per n., was given by the following formula: $d=\sqrt{8D/A}$; D being Krogh's

constant for oxygen diffusion, which at 37° C. is about $1 \cdot 7 \times 10^{-5}$. In this case $d = \sqrt{\frac{8 \times 1 \cdot 7 \times 10^{-5}}{0 \cdot 008}} = 0 \cdot 13$ cm. Since the average thickness of the tissue was about 0·14 cm. it seems probable that when oxygen was perfused some pieces got an oxygen supply adequate to prevent lactic acid formation, whilst others slightly thicker got insufficient oxygen.

It has been pointed out that under anaerobic conditions the gut can convert only about 0·15 p.c. or 1·5 mg. per g. of its carbohydrate to lactic acid. The production of 1·5 mg. lactic acid from glycogen provides energy equivalent to about 0·4 cal. The gut under aerobic conditions uses about 0·5 c.c. oxygen per g. per hour, and this would suffice to oxidize about 0·7 mg. carbohydrate. The oxidation of this amount is equivalent to an energy release of about 3 cal. Hence the anaerobic glycolysis of the available carbohydrate of the gut is only adequate to supply an amount of energy equal to that released under aerobic conditions in $\frac{60 \times 0 \cdot 4}{3} = 8$ min. This is a remarkable contrast to the skeletal muscle and cardiac muscle of the frog, where the tissue carbohydrate available for utilization is only exhausted after some hours of anaerobic activity. The figures also show that under aerobic conditions nearly the whole of the carbohydrate utilization occurs during the first hour, and since an isolated gut can continue to function in glucose-free Ringer's fluid for several hours it is evident that it must be able to oxidize other material in addition to the carbohydrates.

Utilization of added glucose.

A series of experiments was made in which cat's colon muscle was suspended in Ringer's fluid containing 0·1 p.c. glucose. Table III shows the amounts of lactic acid produced under aerobic and anaerobic conditions.

Table III. Lactic acid production of cat's colon suspended for 3 hours in Ringer's fluid containing 0·1 p.c. glucose (control value lactic acid 0·135 p.c.).

Gas perfused	No. of exps.	Lactic acid in g. per 100 g. muscle			
		In muscle	In fluid	Total	Increase
Oxygen	4	0·09	0·30	0·390	0·255
Air	4	0·111	0·354	0·465	0·330
Nitrogen	4	0·147	0·604	0·751	0·616

The substitution of air for nitrogen reduced the lactic acid production to one-half, but even when oxygen was perfused there was still a considerable lactic acid production. Rosenthal and Lasnitzki [1928]

und that the lactic acid production per hour of the rabbit's colon muscle presence of glucose was 0·03 p.c. in aerobiosis and 0·49 p.c. in anaero-osis. My figures (0·255 p.c. in oxygen and 0·616 p.c. in nitrogen) show much smaller difference, and the probable reason for this is that the robic lactic acid production is unduly high owing to the thickness of e tissue.

The essential fact shown by my figures is that large quantities of tic acid are produced by the gut muscle in the presence of glucose, and nce the failure of the gut to produce similar quantities in absence of ucose is due to exhaustion of the available carbohydrate and not to the cumulation of lactic acid preventing further production.

A few experiments were made to see if the gut metabolized lactates hen supplied with oxygen. Colon strips were suspended for 3 hours in ucose-free Ringer's fluid containing 0·004 p.c. sodium lactate and ygen was passed. Three experiments were made and the amount of tic acid recovered from the muscle and fluid in excess of the lactate iginally present was 0·20 p.c. of the muscle weight. Control experi-ents showed that oxygenation for 3 hours of lactate solutions of the same rength did not cause any loss of lactate. This result indicates that ere is no extensive oxidation of lactates by the gut, but the experiment es not prove that no oxidation occurs, because it has already been own that even when oxygen is perfused the gut may produce a certain nount of lactic acid.

THE ACTION OF SODIUM IODOACETATE (S.I.A.).

Experiments in which the mechanical response of gut muscle was asured showed that when this was poisoned with S.I.A. (1 in 10,000) it ntinued to contract in an apparently normal manner as long as oxygen s supplied, but that asphyxia caused arrest in a few minutes. The pidity of arrest made it impracticable to study the lactic acid produc-n of the S.I.A. poisoned muscle during asphyxia, and this was therefore idied in muscles suspended in oxygenated Ringer's fluid containing icose, a condition under which normal gut muscle produces a consider-le amount of lactic acid. S.I.A. (1 in 10,000) reduced the lactic acid oduction to less than one-quarter of the value obtained with the rmal muscle both when the muscle contracted spontaneously and en it was stimulated electrically (Table IV).

It was thought possible that the failure of 0·01 p.c. S.I.A. to abolish mpletely the lactic acid production might be due to a large initial

TABLE IV. Cat's colon strip poisoned with sodium iodoacetate (S.I.A.) and suspended for 3 hours in oxygenated Ringer's fluid containing 0·1 p.c. glucose.

Conc. of S.I.A. p.c.	Duration of exp. in hours	No. of exps.	Lactic acid content (g. per 100 g. muscle) (a) Control	(b) Exp. muscle and fluid	(c) Increase
A. Spontaneous contractions.					
0	3	5	0·153	0·463	+0·310
0·01	3	3	0·152	0·229	+0·077
0·03	3	3	0·226	0·283	+0·055
B. Electrical stimulation.					
0	2	3	0·100	0·566	+0·463
0·01	2	3	0·100	0·150	+0·050

TABLE V. Cat's colon strips poisoned with S.I.A. (N/2080) and suspended in oxygenated Ringer's fluid without glucose.

Nature of exps.	No. of exps	Control	Normal muscle: Oxygenation for 10 min.	Normal muscle: Oxygenation for 2 hours	S.I.A. poisoned muscle: Oxygenation for 10 min.	S.I.A. poisoned muscle: Oxygenation for 2 hours
Total carbohydrate content in g. per 100 g. muscle	5	0·607	0·556	0·465	0·503	0·424
Total carbohydrate breakdown =			0·051	0·142	0·104	0·183
Lactic acid content in g. per 100 g. muscle	4	0·110	0·131	0·286	0·130	0·234
Lactic acid production =			0·021	0·176	0·020	0·124

lactic acid production during the period of poisoning. The effect of 1 in 10,000 (N/2090) sodium iodoacetate on carbohydrate breakdown and lactic acid production was studied in glucose-free Ringer's fluid. The results (Table V) show that S.I.A. does produce a slight increase in the carbohydrate breakdown in the first 10 min. of its action, but that it produces no corresponding increase in lactic acid production. The lactic acid production during the first 2 hours is, however, only 30 p.c. less in the S.I.A. poisoned muscle than in the normal muscle. The results in Tables IV and V show therefore that S.I.A. has a powerful action in reducing the glycolysis of sugar in the fluid surrounding the muscle, but has a less marked action on the glycolysis of the carbohydrate contained in the muscle.

The most probable reason for this result is that S.I.A. penetrates muscles slowly. Gaffar [1935] has shown that at 40° C. N/1000 S.I.A. takes about 20 min. to reduce the lactic acid formation of frog's skeletal muscles to one-half normal. His results are in accordance with those of Meyerhof and Boyland [1931] who found that I.A.A. took about

min. to penetrate fully the sartorius of *Rana temporaria*, whilst ohmann [1931] found that I.A.A. took nearly an hour to inhibit mpletely the lactic acid formation of the muscle pulp.

DISCUSSION.

The results of my analyses suggest that most of the lactic acid found the gut muscle prepared in the manner described in this paper is rmed during manipulation after isolation. Hence the true value of the ducing substances in the fresh gut muscle is probably the sum of the nounts found of reducing substances and lactic acid. In the case of the t's colon the true resting value for reducing substances is probably bout 0·9 p.c.; from 0·10 to 0·15 p.c. undergoes glycolysis during isolation d a further 0·25 p.c. is readily glycolysed, but the remaining 0·5 p.c. is t glycolysed even after prolonged exposure to anaerobic conditions. he gut muscle when isolated contains therefore only about 0·25 p.c. of rbohydrate that is available for the supply of energy, and under aerobic conditions this supply is only adequate to support the normal tivity of the gut for from 5 to 15 min.

If glucose is added to Ringer's fluid the gut can convert considerable antities to lactic acid. Glycolysis occurs in presence of glucose even hen oxygen is perfused through the fluid, and this suggests that the uscle does not obtain an adequate supply of oxygen throughout its ickness. The application of Warburg's formula confirms this con-usion.

The results shown in Tables IV and V suggest that S.I.A. acts im-ediately on the surface of the muscle and inhibits glycolysis of sugar esent in the fluid but that it takes the greater part of an hour to abolish l glycolysis in the interior of the muscle. This hypothesis is difficult to ove, because in the unpoisoned muscle very little glycolysis of muscle rbohydrate occurs after the first hour of isolation, and it is difficult to termine whether this small glycolysis is further reduced by S.I.A.

SUMMARY.

1. Isolated gut muscle contains only about 0·25 p.c. of carbohydrate ailable for glycolysis.

2. Isolated gut muscle in presence of oxygen oxidizes about 1 mg. rbohydrate per g. per hour.

3. Isolated gut muscle in presence of glucose produces considerable antities of lactic acid both under aerobic and anaerobic conditions.

The deeper portions of the muscle probably do not obtain an adequate oxygen supply even in oxygenated fluid. Under anaerobic conditions about 2 mg. glucose per g. per hour is glycolysed.

4. Sodium iodoacetate (1:10,000) inhibits glycolysis of glucose in the Ringer's fluid in contact with the gut muscle.

5. Periodic electrical stimulation increases the glycolysis by about 12 p.c.

The author wishes to acknowledge his great indebtedness to Prof. A. J. Clark for his advice throughout the course of this investigation, and to thank the Moray Research Fund of Edinburgh University for a grant in aid of the expenses.

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THE MECHANICAL ACTIVITY OF GUT MUSCLE UNDER ANAEROBIC CONDITIONS.

By B. N. PRASAD.

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Appendix B.

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THE MECHANICAL ACTIVITY OF GUT MUSCLE UNDER ANAEROBIC CONDITIONS.

BY B. N. PRASAD.

(*From the Department of Pharmacology, University of Edinburgh.*)

(*Received July* 5, 1935.)

ANY writers have observed that lack of oxygen causes a fall in tonus of ut muscle and after a certain interval causes cessation of movements. ross and Clark [1923], Hoskins and Hunter [1924] and Garry 928] observed these phenomena in isolated gut. Mikulicz-Radecki d Lueg [1924] found temporary increases in tonus and rhythmicity llowed by diminution in both with the uteri of rabbits and cats *in vivo*. risler, van Liere and Booher [1932] found with dog's stomach *vivo* decreased movement with oxygen lack. Gross and Clark [1923] so found that asphyxia abolished the response of the gut to adrenaline d pilocarpine, and Schmitt and Nicoll [1933] have shown that anide, hydrogen sulphide and carbon monoxide inhibit the response isolated intestine to a variety of drugs that normally cause tonic ntraction.

A number of other observers have shown that a moderate change in H towards acidity causes loss of tonus and diminution of mechanical tivity in plain muscle [Hatai and Hammett, 1920; Hammett, 22; Evans and Underhill, 1923; and Gruber, 1927]. Gaskell 880] and Bayliss [1901] found vaso-dilatation in the frog with weak tic acid. Young [1915] and Bottazzi [1916–17] confirmed these sults on mammalian intestine. Wild and Platt [1902] found that idity caused vaso-constriction in the frog, but that very weak acid metimes caused a preliminary dilatation. Fardon [1908] obtained wered tonus and diminished spontaneous contraction of the mammalian erus with acid. Contraction of the smooth muscle with acid has been served by Fleisch [1918, 1921], Hooker [1912], Ishikawa [1914] d Fraenkel and Morita [1925]. McSwiney and Newton [1927, 28] have investigated elaborately the effect of great and moderate idity and found that the latter produced relaxation of tonus and

diminution in the rate of contraction of the smooth muscle. Ets and Hemwall [1933] found that 1 in 100,000 NaCN abolished the rhythm of the rabbit's ileum, but that this effect was decreased by addition of dextrose.

METHODS.

Rabbit's ileum (complete) and cat's colon (free from mucous coat) were used for this investigation; in the former spontaneous mechanical activity and in the latter electrically stimulated activity were studied.

(*a*) *Spontaneous activity*. A modified Trendelenburg's technique was employed in this case. The arrangement of bath (25 or 10 c.c.) and thermostat used is a modification of that described by Burn and Dale [1922]. The Ringer's fluid was the same as described previously [Prasad, 1935].

(*b*) *Electrical stimulation* of smooth muscle has been used by various workers. Evans [1925] stimulated the stomach of the tortoise and frog and the bladder of the cat in a moist chamber with a galvanic current of sufficient strength to cause maximum contraction. Winton [1926] stimulated the retractor penis of the dog in a special narrow chamber in which the muscle was suspended in Burn and Dale's solution. Sometimes faradic but usually direct currents were used. Winton found that relatively strong currents were required and that the tissue could easily be injured by an unduly intense stimulus. M. G. Eggleton [1934] measured the effect of stimulation on the content of phosphagen in the retractor muscle of the foot of *Mytilus edulis*. She used alternating current (50 cycles) at 16 volts, and the amount of current passing between the electrodes, through the sea water in which the tissue was suspended, was of the order of 60 milliamperes. Only a small fraction of this passed through the muscle itself.

The author first tried stimulation of strips of cat's colon with a direct current. A stimulus of 70 volts for 6 sec. every minute was found adequate for the purposes of the investigation. The high intensity of current was found, however, to produce injury to the muscle, which was shown by abnormal behaviour and by irregular response to drugs. Polarization of the electrodes also was found to be a serious source of error with direct current at this high voltage. Alternating current (50 cycles) at a low voltage (16–18) was therefore tried. This gave an adequate stimulus and did not injure the tissue, nor did serious polarization occur. The electrodes showed blackening after a day's use but could be cleaned quite easily. Observations on the muscle stimulated in this way could be carried on for 6 hours, and at the end of such a period the tissue still showed no sign

f injury. In order to facilitate electrical stimulation narrow tubes of 0 c.c. capacity were used for the bath. Smaller tubes were found difficult o use because the passage of gas bubbled tended to blow the fluid out of he tube when this latter was reduced to too small a diameter. Elecrodes of coiled silver wire were used. They did not touch the muscle, ut the current passed through the whole length of the fluid in which he muscle was suspended. The muscle was stimulated for 6 sec. every inute with an automatic make and break arrangement fixed on a evolving plate. It was found that muscle when stimulated would lift a onsiderable weight, and a weight of 5 g. was hung on the recording lever t a distance from the pivot equal to the distance between the pivot and he attachment of the muscle. This arrangement had the advantage of bolishing effects due to slight changes in tonus. The current passing etween the electrodes was measured and found to be of the order of -10 milliamperes of which only a small fraction passed through the uscle.

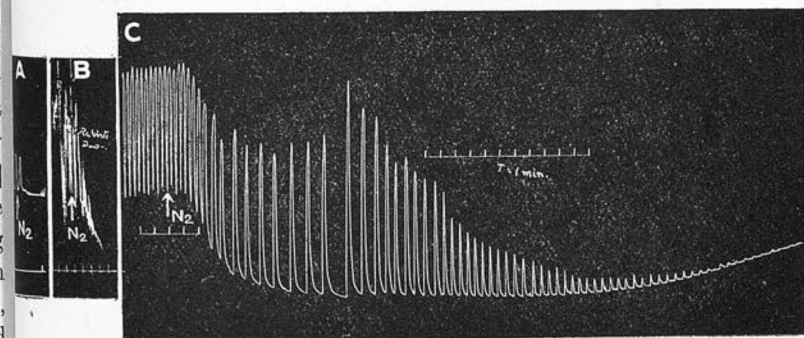


Fig. 1. Effect of asphyxia on spontaneous activity of A = rabbit's stomach, B = rabbit's duodenum, C = cat's colon. (Time = 1 min.)

THE EFFECT OF ASPHYXIA IN GLUCOSE-FREE RINGER'S FLUID.

Fig. 1 shows the effect of asphyxia on the spontaneous activity of gut. he rabbit's stomach (Fig. 1 A) is paralysed more rapidly than is the bbit's duodenum (Fig. 1 B) or the rabbit's ileum (Fig. 5), and the cat's olon is paralysed much more slowly (Fig. 1 C). The essential effects are n immediate fall in tonus, followed by a continuous decrease in the mplitude of the contractions, which terminates in arrest. Fig. 2 shows hat asphyxia produces the same sequence of effects in the colon when his is stimulated electrically.

The introduction of oxygen causes rapid recovery both of tonus and contractions, but subsequent asphyxia produces arrest much more

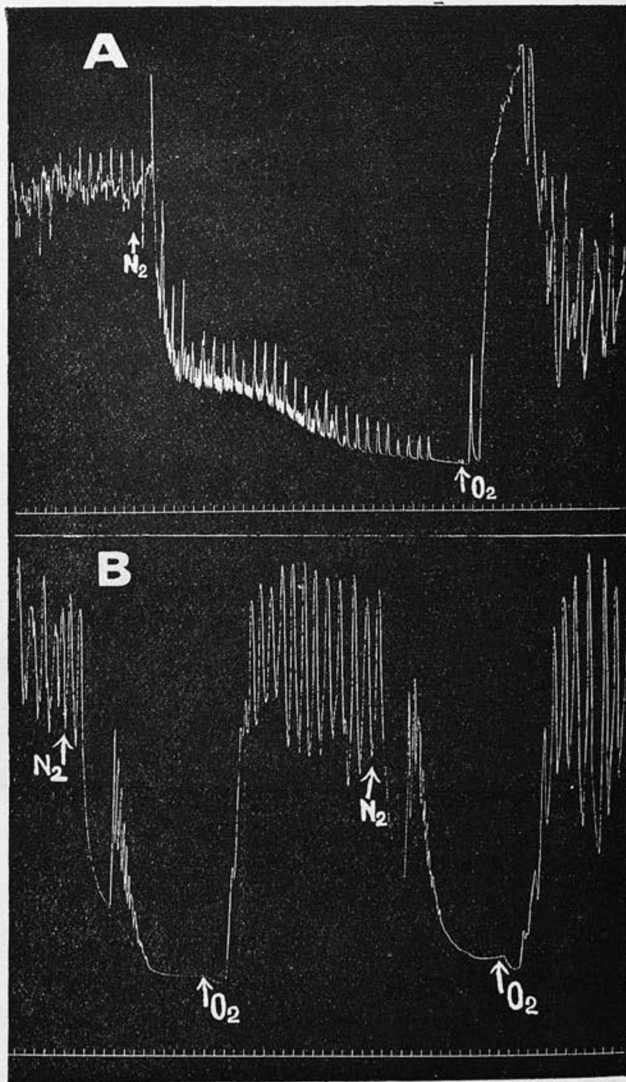


Fig. 2. Effect of asphyxia on stimulated colon of cat. A = for the first time after removal from the animal, B = for the third and fourth time, same strip. (Time = 1 min.)

rapidly than does the first asphyxia. This decrease in the power to maintain anaerobic activity confirms the conclusion drawn from chemical

nalysis in the preceding paper, that the gut has only a small store of arbohydrate which is available as a supply of anaerobic energy, and hat this is depleted by asphyxia after a few minutes.

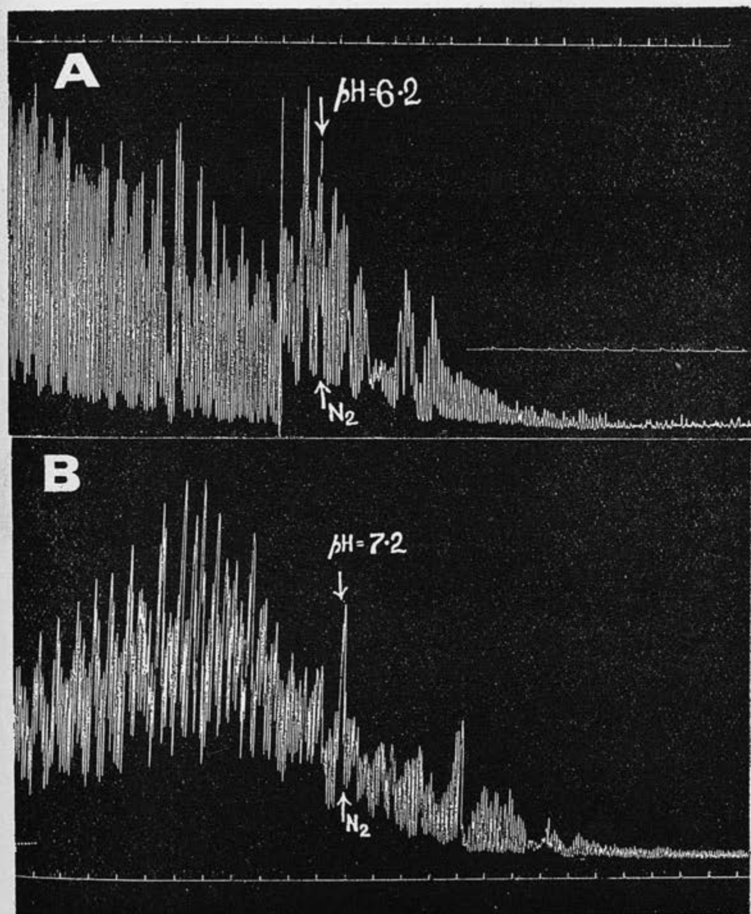


Fig. 3. Effect of asphyxia on spontaneous activity of rabbit's ileum in A, acid fluid (pH 6·2); B, neutral fluid (pH 7·2). (Time = 1 min.)

When ordinary Ringer's fluid was used (cf. Fig. 5) the reaction at the ime of asphyxial arrest was never found to be below pH 7·8. Fig. 3 shows he effect of asphyxial arrest in neutral (pH 7·2) and acid (pH 6·2) fluid. This shows that the rate at which asphyxial arrest occurs is not markedly ffected by changes in the pH of the perfusion fluid. This result is in

sharp contrast to the behaviour of the frog's heart in which asphyxia produces rapid arrest in neutral Ringer's fluid but in alkaline Ringer's fluid only produces arrest after some hours [Clark, Eggleton and Eggleton, 1932].

The difference in the behaviour of these tissues is explained by the fact that the gut muscle has a much smaller carbohydrate reserve than has the frog's heart, and hence the gut cannot maintain anaerobic activity for long even when conditions are favourable for the glycolysis of its own carbohydrate.

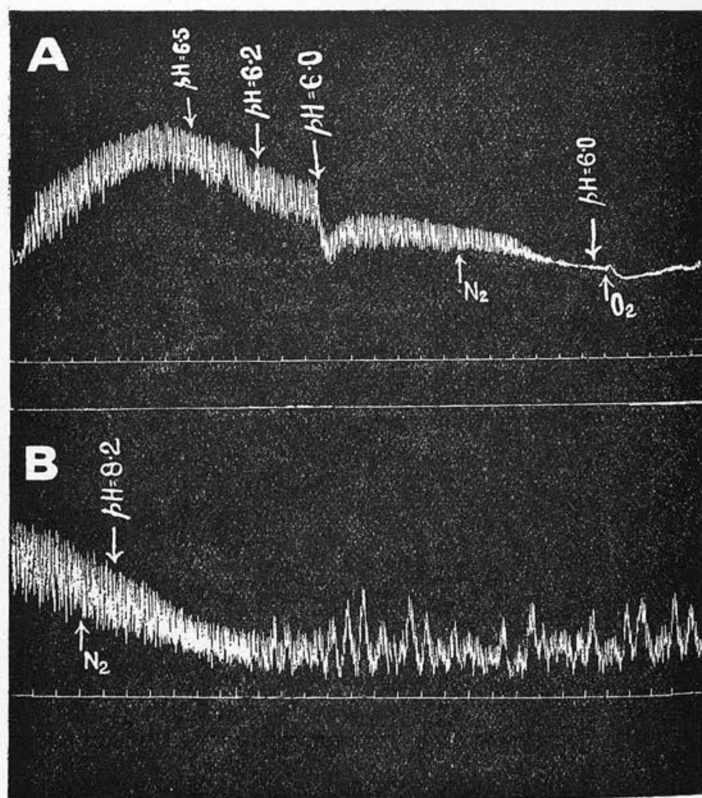


Fig. 4. Effect of asphyxia on spontaneous activity of rabbit's ileum in 0·1 p.c. glucose Ringer's fluid: A, acid fluid (*p*H 6·0); B, alkaline fluid (*p*H 8·2). (Time = 1 min.)

THE EFFECT OF GLUCOSE IN RESPONSE TO ASPHYXIA.

The addition of glucose prevents asphyxial arrest as long as the fluid surrounding the gut remains alkaline (Fig. 4B), but in acid Ringer (Fig. 4A)

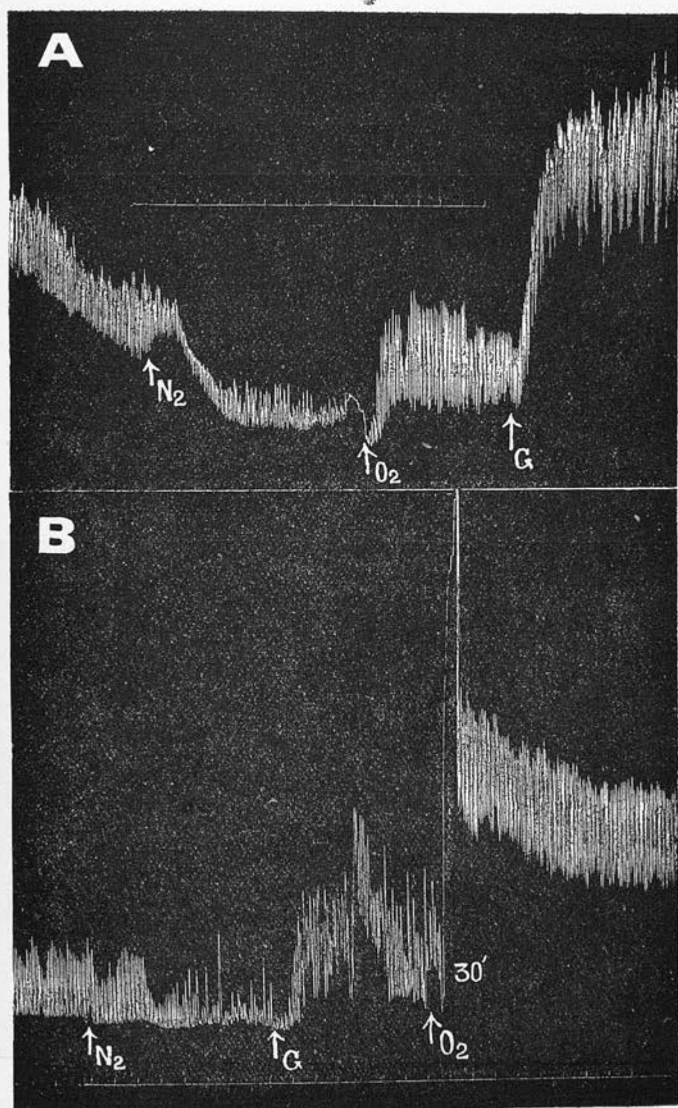


Fig. 5. Effect of oxygen and glucose (0·1 p.c.) on revival of arrested spontaneous movements and tonus of rabbit's ileum. (Time = 1 min.)

glucose has little beneficial action. Similarly the addition of glucose to a gut arrested in glucose-free Ringer causes partial restoration of tonus and of amplitude. Fig. 5 shows that both oxygen and glucose are needed

for the full restoration of activity after asphyxia, but that either causes a partial restoration.

The effects of sodium cyanide are similar to the effect of asphyxia; it causes paralysis which is abolished by glucose. Ets and Hemwall

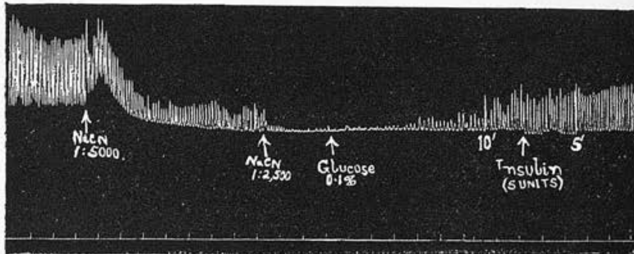


Fig. 6. Effect of sodium cyanide and insulin on spontaneous movements of rabbit's ileum. (Time = 1 min.)

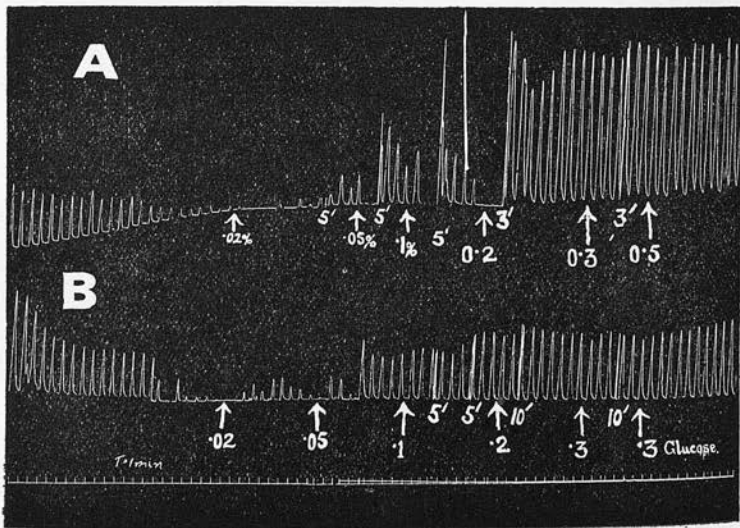


Fig. 7. Effects of glucose and mannose on asphyxiated and stimulated cat's colon. Concentrations attained in the perfusing fluid are indicated at arrows in p.c. A = glucose, B = mannose. (Time = 1 min.)

[1933] found a beneficial effect of glucose and insulin with gut muscle poisoned by NaCN. I was unable to find any indication that the utilization of glucose by plain muscle was improved by addition of insulin. These results are illustrated in Fig. 6.

THE EFFECT OF VARIOUS CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES, ETC., ON THE ASPHYXIATED GUT.

The stimulant action of glucose on the asphyxiated gut is rapid and finite, and hence this preparation is a favourable one on which to test power of the gut to obtain anaerobic energy from carbohydrates, etc. gs. 7 and 8 show the type of results obtained, and these are summarized

TABLE I. Beneficial action of carbohydrates, etc., on (*a*) the asphyxiated gut and (*b*) the asphyxiated and exhausted frog's heart.

Substance	(*a*) Asphyxiated cat's colon	(*b*) Asphyxiated and exhausted frog's heart [Gaddie and Stewart, 1934]
Polysaccharides:		
Starch	–	–
Disaccharides:		
Maltose	–	–
Lactose	–	–
Sucrose	–	–
Monosaccharides:		
Glucose	+ + +	+ + +
Mannose	+ + +	+ + +
Fructose	+	–
Galactose	–	–
Pentoses:		
Arabinose	–	–
Xylose	–	–
Possible breakdown products of carbohydrates:		
Dihydroxy acetone	–	–
Glyceric aldehyde	+ +*	+ +*
Methyl glyoxal	+ +*	+ +
Pyruvate	–	–
Various substances:		
Amino acids, glycine and alanine	–	–
Lipoids, sodium oleate	–	–

+ + + = complete revival.
+ + = partial revival or temporary revival.
+ = feeble and short revival.
– = no revival.
* = poisoning in higher concentration.

Table I. The results given in Table I show that glucose and mannose utilized equally rapidly by the gut muscle, that fructose is utilized a small extent, but that the gut muscle cannot utilize any of the other rbohydrates tested. The results with possible breakdown products of

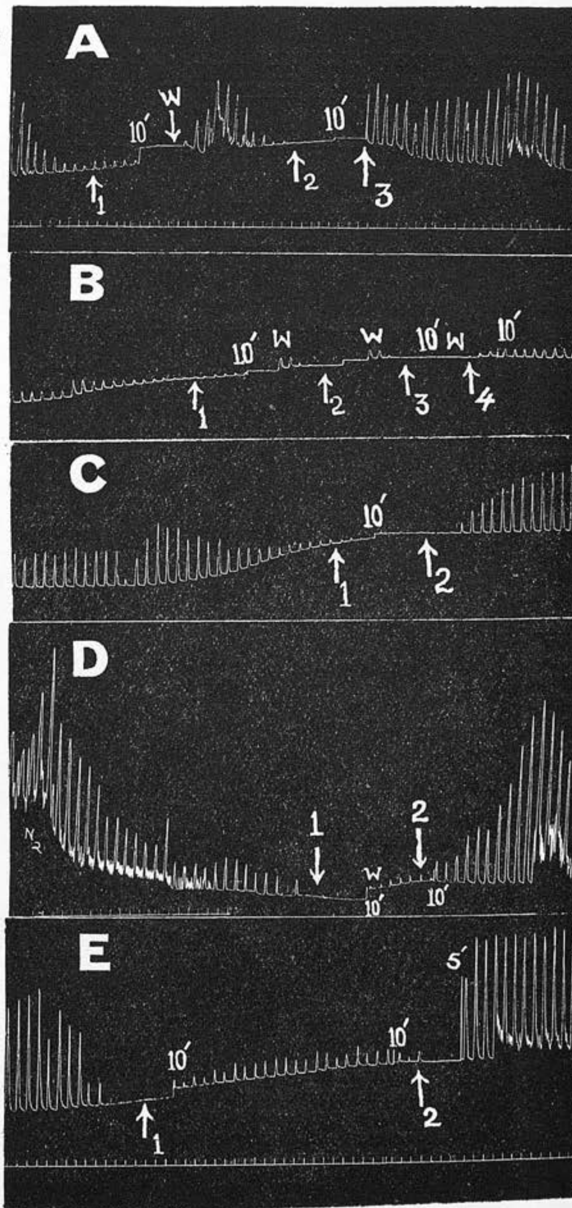
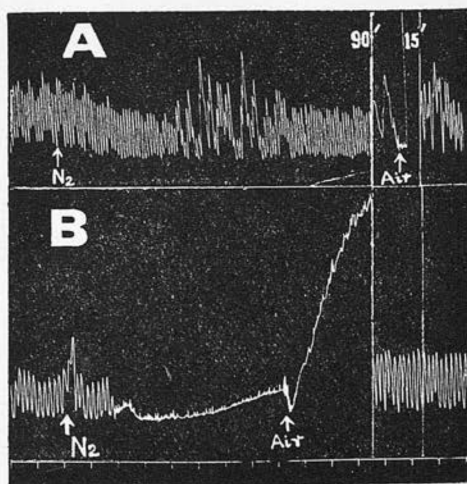


Fig. 8. Effect of carbohydrates, etc., on asphyxiated and stimulated cat's colon. Different substances were added at arrow marks to obtain 0·1 p.c. final concentration. (A) 1 = alanine, 2 = glycine, 3 = glucose. (B) 1 = sodium lactate, 2 = sodium pyruvate, 3 = sodium oleate, 4 = glucose. (C) 1 = maltose, 2 = glucose. (D) 1 = galactose, 2 = glucose. (E) 1 = fructose, 2 = glucose. (Time marked in figures indicates stoppage of drum.) (Time = 1 min.)

arbohydrate show that gut can utilize glyceric aldehyde and methyl-
lyoxal in very low concentrations, but in higher concentrations these
ubstances produce spasmodic contracture and injure the muscle.

THE ACTION OF IODOACETIC ACID.

Iodoacetic acid produces no certain effect on gut as long as this is upplied with oxygen, but asphyxia causes rapid arrest, and the presence f glucose does not delay the asphyxial arrest. Fig. 9 shows the effect of sphyxia in presence of glucose in the normal and in the I.A.A. poisoned ut. In the absence of glucose the contrast is less marked because pshyxia arrests the normal gut fairly rapidly.



ig. 9. Effects of asphyxia on spontaneous movements of rabbit's ileum in 0·1 p.c. glucose Ringer's fluid. A = without I.A.A., B = with I.A.A. (1:10,000). (Time = 1 min.)

The I.A.A. poisoned colon is arrested by asphyxia in about 3 min. Fig. 10 A). The tonus first falls and then rigor occurs. Introduction of ir before the rigor develops restores the activity (Fig. 10 B), but once igor has occurred the gut cannot be revived (Fig. 10 C). The rapid sphyxial arrest of the I.A.A. poisoned muscle shows that this contains nly a small store of non-carbohydrate substance that can be utilized s the source of energy under anaerobic conditions.

The action of various substances was tested on the I.A.A. poisoned ut both in presence of oxygen and during asphyxia; the results obtained

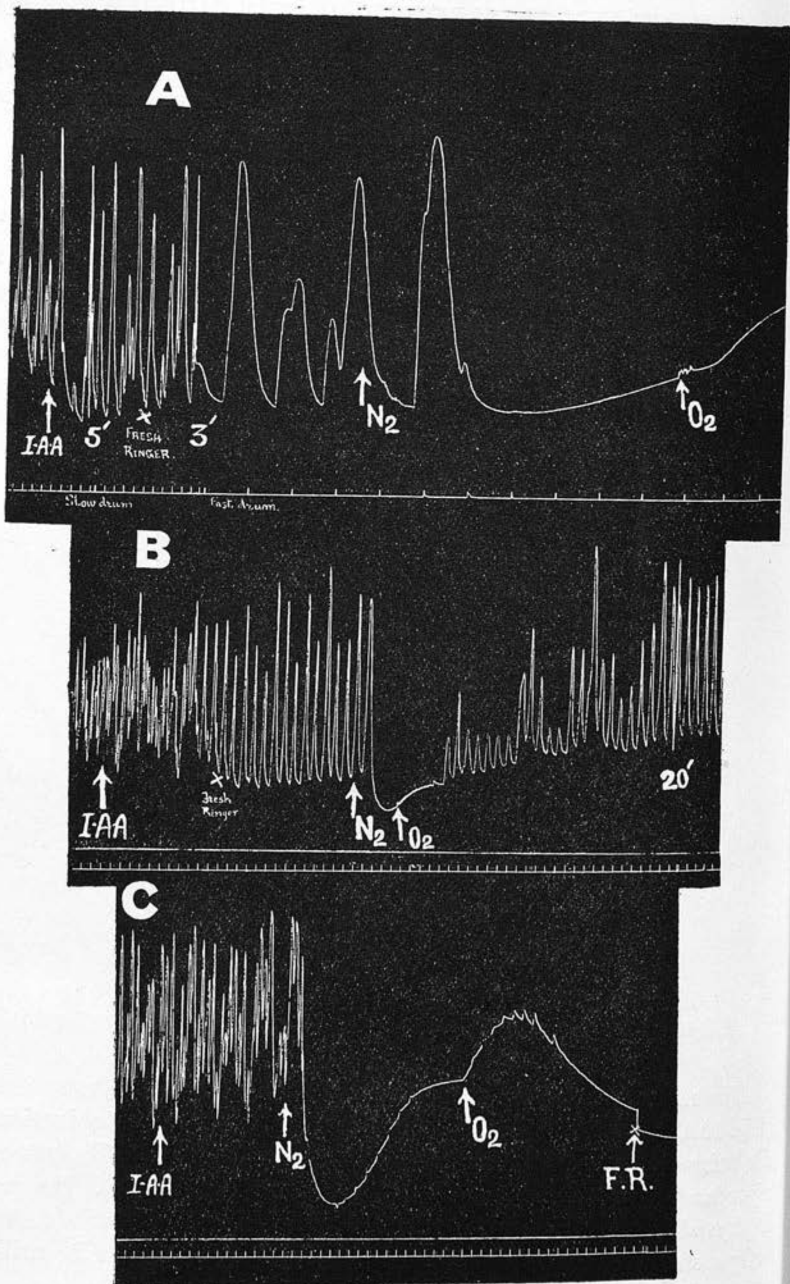


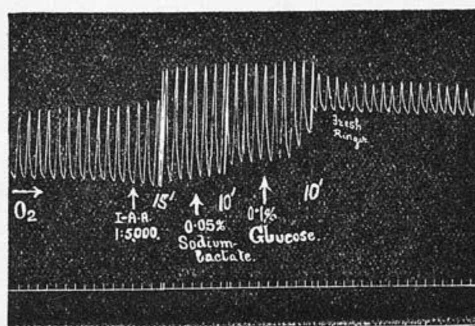
Fig. 10. Effect of I.A.A. (1:10,000) on stimulated cat's colon. A = time of asphyxial arrest about 3 min. B = arrested movements are revived if oxygenation is resumed soon. C = when muscle is passing into rigor, oxygen does not revive its movements. (Time = 1 min.)

re summarized in Table II. In presence of oxygen glucose did not roduce any beneficial action nor did sodium lactate (Fig. 11). In resence of oxygen sodium pyruvate (Fig. 12) and methylglyoxal

TABLE II. Action of different substances on I.A.A. poisoned and normal cat's gut with and without oxygen.

Substances tested and conc. in p.c.	Normal gut		I.A.A. poisoned gut	
	With oxygen	Without oxygen	With oxygen	Without oxygen
Glucose (0·1 p.c.)	+ + +	+ + +	–	–
Methylglyoxal (0·01 p.c.)*	+	+	+	– (†)
Glyceric aldehyde (0·01 p.c.)*	+	+	+	– (†
Sodium pyruvate (0·1 p.c.)	+ +	–	+	–
Sodium lactate (0·1 p.c.)	–	–	–	–

+ + + = good beneficial effect.
+ + = moderate beneficial effect.
+ = slight beneficial effect for short time.
– = no effect.
* = poisonous in higher concentration.
† = sudden contracture.



ig. 11. Effects of sodium lactate and glucose on I.A.A. poisoned cat's gut stimulated in oxygenated Ringer's fluid; shows no beneficial effect. (Time = 1 min.)

Fig. 13 C) produced a slight increase in the amplitude of contractions, ut in the absence of oxygen these substances produced no beneficial ction.

The action of methylglyoxal on the I.A.A. poisoned gut is of interest, ecause Barrenscheen *et al.* [1931] showed that methylglyoxal accumulated in skeletal muscle poisoned with I.A.A. and Ledebur [1933] ointed out that in skeletal muscle methylglyoxal produced a rigor imilar to that produced by I.A.A. In the case of normal asphyxiated gut

methylglyoxal in low concentration (0·01 p.c.) produced a beneficial action, and in larger concentrations (0·05 p c.) it produced a tonic contraction which might or might not be reversible (Fig. 13 A and B). In the I.A.A. poisoned gut it produced increased contractions and rise of tonus when oxygen was present, but when the gut was asphyxiated it caused immediate rigor (Fig. 13 C). These results support the possibility that the rigor which occurs in asphyxiated I.A.A. poisoned gut muscle may be associated with the accumulation of methylglyoxal. Goldenberg *et al.* [1935] have, however, been unable to demonstrate any such accumulation in animals *in vivo*.

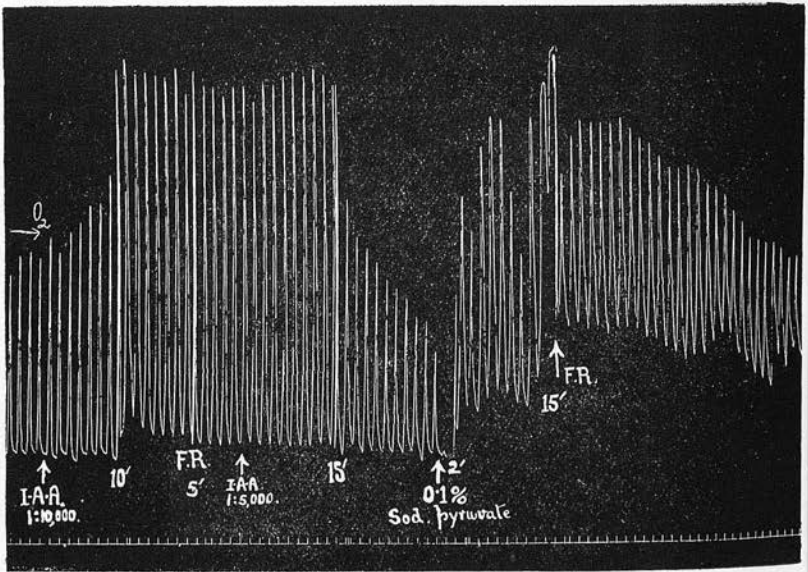
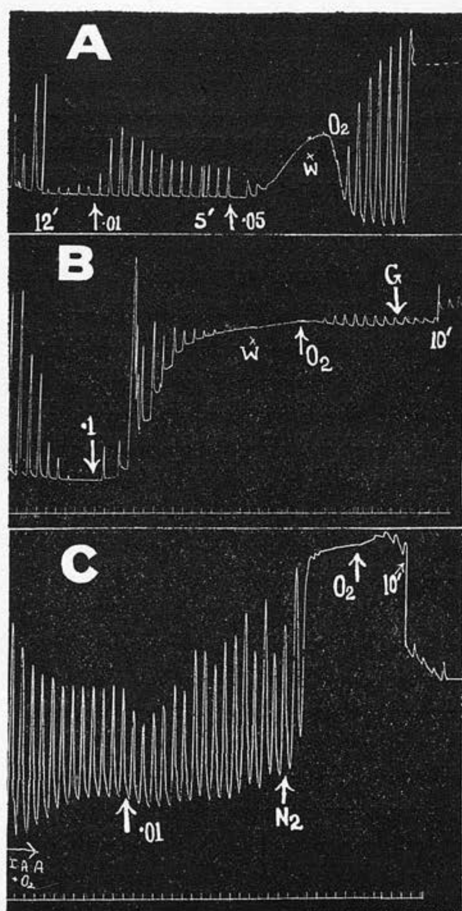


Fig. 12. Effects of sodium pyruvate on I.A.A. poisoned cat's gut stimulated in oxygenated Ringer's fluid; shows beneficial effect. (Time = 1 min.)

Gaddie and Stewart [1934] showed that the sodium iodoacetate poisoned frog's ventircle when arrested in nitrogen could be revived with glutathione, but the recovery was never complete. They suggested that I.A.A. had other effects than that of inactivating co-glyoxalase, and that these other effects were not reversible by any means yet discovered. In the case of gut muscle the author was unable to detect any beneficial action produced by addition of glutathione to I.A.A. poisoned and asphyxiated muscle.



ig. 13. Effect of methylglyoxal on stimulated cat's gut muscle. A, asphyxiated muscle shows beneficial effect in low concentration of 0·01 p.c. and rigor effect in 0·05 p.c. B, muscle passes into rigor in high concentration 0·1 p.c. and is not reversible; C, in aerobiosis and poisoned with I.A.A. (1:10,000) muscle shows some beneficial effect but introduction of anaerobiosis sends it into sudden rigor. (Time = 1 min.)

DISCUSSION.

Experiments on the response of the gut muscle to asphyxia at first ggested that its behaviour was quite unlike that of the frog's heart. he biochemical analyses, described in my previous paper, explained hese differences, since they showed that unlike the heart, the gut muscle ossessed no large store of carbohydrate available for the production of

anaerobic energy by glycolysis. Another difference is that under aerobic conditions the frog's heart does not oxidize or glycolyse glucose present in the perfusion fluid, whereas the gut muscle usually glycolyses a considerable amount of glucose even under aerobic conditions. This difference is explained by the fact that the gut muscle is too thick to obtain an adequate oxygen supply throughout its substance even when oxygen is supplied freely.

Apart from these two important differences the metabolism of the mammalian gut muscle shows a very close resemblance to that of the frog's heart. The frog's heart under aerobic conditions utilizes a mixed diet of carbohydrate and non-carbohydrate material. This must also be true of the mammalian gut muscle for the following reasons:

(*a*) The gut muscle under aerobic conditions exhausts nearly the whole of its available carbohydrate in an hour [Prasad, 1935], but it can continue regular activity for many hours without the addition of glucose.

(*b*) I.A.A. abolishes glycolysis and yet does not markedly depress the activity of gut muscle provided that this is supplied with oxygen (Fig. 10B).

The activity of the gut muscle is, however, maintained best when it is supplied with both glucose and oxygen (Fig. 6 A and B). Therefore the optimum condition for the activity of the gut muscle appears to be when it is utilizing both carbohydrate and non-carbohydrate sources of energy.

The gut muscle when poisoned with I.A.A. and deprived of oxygen is arrested in about 3 min. The sources of available anaerobic energy other than carbohydrate are, therefore, very small. This agrees with the finding of Eggleton and Eggleton [1929] that the phosphagen content of mammalian plain muscle is only 2·3–5·1 mg. of phosphagen per 100 g., whilst Eggleton [1934] has shown that the arginine phosphate of *Mytilus* muscle is reduced in asphyxia. It is reasonable, therefore, to assume that the short anaerobic activity in the I.A.A. poisoned gut muscle measures the time required to exhaust its small store of phosphagen. The effect of I.A.A. on gut muscle is in all respects similar to its action upon the frog's heart.

The power of mammalian gut muscle to utilize various carbohydrates and breakdown products of carbohydrate appears to be identical with the powers of the frog's heart (cf. Table I).

It is of interest to note that the effect of acidity on the gut muscle is similar to its effect on the heart. An acidity of pH 6·0 does not markedly affect the activity of the gut as long as this is supplied with oxygen, but

hen the oxygen supply is cut off the gut is rapidly arrested, presumably ecause the acidity inhibits glycolysis.

The power of methylglyoxal and glyceric aldehyde to restore partial ctivity of gut muscle in nitrogen suggests the probability of these being ntermediate products in the path of glycolysis.

Sodium pyruvate produces a definite beneficial effect on I.A.A. poioned gut muscle (Fig. 13). This suggests that I.A.A. stops the process of lycolysis before this stage. Haarmann [1932] found that lactic acid ould be produced by muscle poisoned with bromacetic acid on addition f pyruvate.

SUMMARY.

1. Asphyxial arrest of the mechanical movements of gut muscle is ot due to accumulation of acid but to exhaustion of its labile carboydrate store.

2. The mechanical experiments confirm the biochemical findings that he gut muscle has only a small reserve of available carbohydrate.

3. Gut muscle probably utilizes a mixed diet of carbohydrate and on-carbohydrate material in aerobiosis.

4. The activity of the gut muscle is maintained best when it is pplied with both glucose and oxygen.

5. I.A.A. poisoned gut muscle has a very limited activity under naerobiosis; this suggests a small phosphagen content.

The author wishes to acknowledge his great indebtedness to Prof. A. J. Clark for his dvice throughout the course of this investigation, and to thank the Moray Research Fund Edinburgh University for a grant in aid of the expenses.

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