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THE CARBOHYDRATE METABOLISM OF GUT MUSCLE.

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Introduction.

The experiments described below were undertaken for the purpose of analysing the sources of energy utilized in the contraction process of smooth muscle. The methods used were similar to those which have been found of value in analysing the metabolism of skeletal and cardiac muscle. The effect of anaerobiosis on the metabolic changes was determined firstly on normal muscle and then on muscle poisoned with iodoacetic acid. The plain muscle studied was that of the rabbit's ileum and the cat's colon. The biochemical analyses described in this paper were undertaken as a preliminary to the study of the effect of asphyxia under various conditions on the mechanical response of smooth muscle. These results will be described in a later paper.

Literature.

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 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. Their results also 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.

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.

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

In most of the experiments the gut was allowed to contract spontaneously, but in a few experiments (Table IV) it was stimulated electrically. The stimulus was an alternating current at 16 volts which was applied through the length of the muscle for 5 sec. every minute. The results in Table IV indicate that the metabolism of the stimulated gut muscle 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 weight was determined on a torsion balance. The tissue was then put into ice-cold tungstic acid and ground with sand in a cooled mortar. The method of estimation used was the modification of Friedemann, Cotonio and Shaffer's method [1927] used and described by Kerley [1931]. In this method manganese sulphate is used to catalyse the reaction and the aldehyde formed is distilled over a current of steam instead of air. The distilled aldehyde is absorbed in bisulphate and the bound sulphate is titrated with iodine.

The estimation of total carbohydrate.

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

The carbohydrate and lactic acid content of fresh gut muscle.

The average results obtained by the author with fresh muscle are shown in Table I. These results agree fairly well with the results obtained by previous workers. The figures for lactic acid content 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. 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		
	(a)	(b)	(c)	(a)	(b)	(c)
Tissue	No. of observations	Average value	Range of results	No. of observations	Average value	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 g. per 100 g. muscle			Lactic acid g. per 100 g. muscle					
Duration in hours	No. of exps.	Control	Exp.	Carbohydrate loss	No. of exps.	Control	Experimental: Muscle	Experimental: Fluid	Total	Lactic acid production
					A. Cat's colon.					
					(a) Oxygenated.					
1	4	0·760	0·542	0·218	6	0·123	0·097	0·078	0·175	0·052
2	5	0·607	0·465	0·142	4	0·110	0·130	0·156	0·286	0·176
1		(Stimulated muscle)			3	0·089	0·063	0·083	0·146	0·057
					(b) Anaerobic.					
1	4	0·760	0·542	0·218	3	0·158	0·092	0·204	0·296	0·138
					(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	0·186
					B. Rabbit's ileum.					
					(a) Oxygenated.					
1	9	0·592	0·437	0·155	7	0·213	0·123	0·105	0·228	0·015
					(b) Anaerobic.					
1	9	0·592	0·348	0·244	7	0·213	0·183	0·165	0·348	0·135
					(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	0·133

into lactic acid. The results are summarized in Table II. The result of outstanding importance is that although the total carbohydrate content of the gut amounted in some cases to as much as 0·760 p.c., yet under no conditions was it possible to cause a loss of more than 0·26 p.c. The amount of carbohydrate in the gut available for conversion into lactic acid is therefore about 0·25 p.c. The gut suspended for 1 hour in oxygenated Ringer's fluid utilized, however, from 0·16 to 0·22 p.c. 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 following average values were obtained in three experiments. The total carbohydrate content (mg. per 100 g. muscle) was: after 1 hour 0·437; after 3 hours, 0·426. The lactic acid in the fluid (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 formation of lactic acid. These results indicate that most of the carbohydrate breakdown and lactic acid excretion occurs during the first hour of the isolation.

The lactic acid found in the control strips of the cat's colon (about 0·12 p.c.) was three times that found by Evans [1925] in the dog's intestine. It is probable therefore that between 0·05 and 0·10 p.c. of lactic 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. of labile carbohydrate, that about 0·10 p.c. of this is changed to lactic acid during manipulation, and that from 0·15 to 0·25 p.c. is broken down during the first hour of isolation irrespective of whether the conditions are aerobic or anaerobic. Table II shows that there was a considerable variation in the amount of lactic acid formed under aerobic conditions. This suggests that a variable portion of the tissue was receiving an inadequate oxygen supply even in oxygenated fluid. The oxygen consumption of the tissue may be assumed to be about 0·5 c.c. per g. per hour [Rosenthal and Lasnitzki, 1928] which equals 0·008 c.c. per g. per min. The thickness of typical pieces of colon as estimated from their weight and area was 0·14 cm.

Warburg [1923] calculated that the thickness of tissue (d) which would receive an adequate oxygen supply when suspended in fluid saturated with oxygen, and using a quantity (A) of oxygen per g. per min., 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]

found that the lactic acid production per hour of the rabbit's colon muscle in presence of glucose was 0·03 p.c. in aerobiosis and 0·49 p.c. in anaerobiosis. My figures (0·255 p.c. in oxygen and 0·616 p.c. 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 of the tissue.

The essential fact shown by my figures is that large quantities of lactic acid are produced by the gut muscle in the 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.

A few experiments were made to see if the gut metabolized lactates when supplied with oxygen. Colon strips were suspended for 3 hours in glucose-free Ringer's fluid containing 0·004 p.c. sodium lactate and oxygen was passed. 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 p.c. 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, 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.

The action of sodium iodoacetate (S.I.A.).

Experiments in which the mechanical response of gut muscle was measured showed that when this was poisoned with S.I.A. (1 in 10,000) it continued to contract in an 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 to study the lactic acid production of the S.I.A. poisoned muscle during asphyxia, and this was therefore studied in muscles suspended in oxygenated Ringer's fluid containing glucose, a condition under which normal gut muscle produces a considerable amount of lactic acid. S.I.A. (1 in 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 IV).

It was thought possible that the failure of 0·01 p.c. S.I.A. to abolish completely 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

30 min. 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.

Discussion.

The results of my analyses suggest that most of the lactic acid found in the gut muscle prepared in the manner described in this paper 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 the cat's colon the true resting value for reducing substances is probably about 0·9 p.c.; from 0·10 to 0·15 p.c. undergoes glycolysis during isolation and a further 0·25 p.c. is readily glycolysed, but the remaining 0·5 p.c. is not glycolysed even after prolonged exposure to anaerobic conditions. The gut muscle when isolated contains therefore only about 0·25 p.c. 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 min.

If glucose is added to Ringer's fluid the gut can convert considerable quantities to lactic acid. Glycolysis occurs in presence of glucose even when oxygen is perfused through the fluid, and this suggests that the muscle does not obtain an adequate supply of oxygen throughout its thickness. The application of Warburg's formula confirms this conclusion.

The results shown in Tables IV and V suggest that S.I.A. acts immediately on the surface of the muscle and inhibits glycolysis of sugar present in the fluid but that 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 the first hour of isolation, and it is difficult to determine 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 available for glycolysis.

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

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