THE RÔLE OF THE PHOSPHATES IN CARBOHYDRATE METABOLISM IN SKELETAL MUSCLE. Part II. A comparison between the muscles of normal, fatigued and depancreatised animals.

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In our earlier paper[1] dealing with this subject the muscles of normal animals were compared with those from animals subjected to the action of adrenaline, insulin, and adrenaline plus insulin, attention being directed specially to the synthesis of the hexosediphosphate. The amount of the pre-existing and of the added precursors of the ester (glycogen and phosphoric acid) which can be synthetised in the presence of the fluoride anion by minced normal muscle may be taken as a standard of efficiency, so far as one link in the chain of the carbohydrate transformations in the muscle is concerned. Under normal conditions within the muscle the bound phosphate remains at a fairly constant level, although the ester may be readily broken down by fatigue (Embden and Jost[2], Andrews[3]), or in the condition of rigor mortis (Deuticke[4]). This constancy is undoubtedly due to the fact that the compound appears as an intermediary in the transformation of glycogen to lactic acid, and as this process is a reversible one, its concentration is determined by the balance struck between the hydrolytic and the synthetic processes. While the glycogen content of skeletal muscle may show wide variations even in well-fed cats, say from 0·5 to over 1 p.c., the amounts of the bound phosphate in the normal muscle, as a rule only vary within quite a narrow range, say from 0·18 up to 0·22 p.c. Under the action of insulin there may be, and indeed there usually is, an increase in the amount of bound phosphate, but even were such an increase not detectable on analysis, one cannot conclude that the process of esterification has not been aided by this hormone as the transformation rate through the later stages leading to the partial combustion of the glucose may also be quickened. In order to detect whether the esterification process itself has been altered during some disorder of carbohydrate metabolism, the

method of checking both hydrolysis of the ester and also oxidation by the use of the fluoride anion has been made use of. Embden and his school have drawn attention to the marked synthetic effect of this anion, giving rise to a very great decrease in the concentration of the free inorganic phosphates. Not only is such the case but the normal muscle of the cat can synthetise in addition large quantities of added phosphate, if a sufficiency of glycogen be supplied. One may therefore determine the optimal capacity for esterification of the muscles of the normal animal, and if this be found to be fairly constant under certain fixed conditions, it may be used as a standard with which the muscles of animals suffering from various disturbances in carbohydrate metabolism may be compared. If the hexosediphosphate be an essential link in the chain of carbohydrate transformations in the active muscle, it is necessary to gain as much knowledge as possible of the conditions under which the combination between glycogen (the precursor of the hexose) and phosphate is most readily brought about, and also of the conditions which interfere with the esterification. The fact that the synthetic process under fluoride takes place with a concomitant disappearance of glycogen and a check in lactic acid formation shows that one stage, and one alone, in the cycle is being dealt with. This synthetic process is seriously interfered with if there be traces of metallic impurity (copper) in the distilled water, and in our opinion it is advisable to use tap-water to make up all the solutions with which the minced muscle is brought into contact.

Throughout the course of the investigation the normal cat's muscle was analysed frequently and the effects of variations in the nutritive condition, as well as the effects of slight and prolonged stimulation were also studied in order that a knowledge of the specific effects of depancreatisation might be compared with those resulting from a simple lowering in the muscle glycogen store. The differences between the muscles of the normal and depancreatised animals were studied in the following ways:

I. The amounts of phosphoric acid, glycogen, and lactic acid were determined in the muscle immediately after removal, the so-called "existing condition."

II. The "breakdown process," when the minced muscle was kept in 2 p.c. bicarbonate solution at 45° C. for two hours, was investigated.

III. The "synthetic process," as it occurs when the fluoride acts upon the intrinsic constituents, without further addition of the components of the ester; and

IV. The synthetic action on the pre-existing plus added precursors of the ester (glycogen plus phosphate) were also studied.

In addition to analyses of the free phosphoric acid, glycogen and lactic acid, under these various conditions, the blood sugar and the liver glycogen were also determined whenever necessary. The methods employed for the analyses were those described in the earlier paper. The pancreas was removed in its entirety by one of us (T. H. M.) in more than twenty animals in the following way. The glandular substance was cauterised lightly in the first place and, after tying the ducts, the gland substance was broken down carefully with small portions of sterile gauze and removed, leaving the duodenal circulation as far as possible intact. After removal, any small particles of gland substance on the duodenal wall and along the course of the vessels were carefully cauterised, and finally, when all bleeding had ceased, the duodenum was carefully wrapped round with the omentum which was held in position by a few catgut stitches. The animals recovered perfectly and immediately after the operation moved about freely. They were kept warm and given hot milk and water at the outset, and whole milk during the rest of the period. There were no signs of sickness. The muscles were examined after varying periods, from one to ten days after the operation.

The degree of disturbance produced in the muscle did not appear to bear a quantitative relationship to the interval allowed to elapse between the operation and the removal of the muscle, nor to the degree of hyperglycæmia produced. In almost every case, even after a short period, the liver glycogen had fallen to a low level (0·1 p.c. and under) while the muscle glycogen was much more resistant, rarely falling below 0·2 p.c., the average value in normal animals being about 0·6 to 0·7 p.c.

To avoid repetition, the results obtained for the normal well-fed animal's muscles will in each case be compared with those found after starvation, stimulation and pancreatectomy. The average results obtained for the normal well-fed animal differ in certain respects from those given in the earlier paper, and since these were derived from a much larger series of experiments which were carried out during the course of the work embodied in this paper, they are more suitable for comparison.

I. *The existing condition in the muscle (adductors).*

1. Normal well-fed animal. The liver glycogen varied usually between 3–6 p.c., the blood sugar (under light ether anæsthesia) averaged 0·15 p.c.

Analyses of muscle constituents.

(*a*) The glycogen percentage ranged from 0·5 to 1 (average 0·64 p.c.), the store rarely falling to the lower level, even when the liver glycogen in the less well-fed animals had fallen to 1 p.c. or under.

(*b*) The lactic acid varied from 0·075 p.c. to 0·25 p.c., the average being 0·117 p.c. The higher values were obtained with very excitable muscle which also showed more active synthesis under fluoride.

(*c*) The free phosphate concentration is the most constant and appears to be independent of the nutritive condition of the animal. Per gramme muscle the average content was 2·94 mg. H_3PO_4, with a range from 2·59 to 3·38.

The value of the bound phosphate will be given under the breakdown process.

The averages referred to above were obtained from a series of twenty normal animals.

Two types of normal animals may be compared, both well-fed but differing greatly in their liver glycogen.

TABLE I.

	I	II
	p.c.	p.c.
Liver glycogen	5·240	1·00
Muscle glycogen	0·530	0·48
Free phosphate (H_3PO_4)	0·260	0·31
Lactic acid	0·085	0·24

A high muscle glycogen, low free phosphate, and low lactic acid often are indications of less excitable muscle. The bound phosphate in I was also much higher (0·222) than in II (0·187). Evidently the former offered greater resistance to the natural breakdown process which accompanies the mincing of the cooled muscle.

2. The starved animal.

(*a*) The muscle glycogen percentage after 3–4 days' starvation never fell below 0·2 and after a shorter starvation period (2 days) it remained about 0·5.

(*b*) The lactic acid average percentage was slightly over that found in the well-fed animal, namely 0·15.

(*c*) The free phosphate average value was the same in both.

3. The effects of stimulation of the muscle on the existing condition will be dealt with more fully in another communication, but an example of the effect of prolonged stimulation may be given. Table II gives the values obtained for the gastrocnemius muscle where the sciatic nerve on

one side was stimulated for two hours by slow interrupted faradic stimulation.

TABLE II.

	Stimulated p.c.	Unstimulated p.c.
Glycogen	0·024	0·541
Free phosphate (H_3PO_4)	0·357	0·282
Lactic acid	0·221	0·057

The rise in the free phosphate was naturally due to the breakdown of hexosediphosphate. When the stimulation was for a much shorter period, 10–15 minutes, the glycogen always fell slightly with an accompanying slight lactic acid rise, while the free phosphate remained practically unaltered.

4. The depancreatised animal.

(a) Glycogen. The liver glycogen even after two days was usually brought down to a very low level (0·1 p.c. or under), while the muscle glycogen, although it might fall from 0·6 to 0·12 p.c. within two days, was usually maintained at a much higher percentage, for example 0·3, even after six days had elapsed since the removal of the pancreas and with a blood sugar of 0·42 p.c.

(b) Lactic acid. As in normal muscle there is a wide range of variation, but in the depancreatised state the original value is often much lower than is ever found in the normal muscle, 0·05 p.c. and under. The exceptionally low values are found in cases where the bound phosphate is diminished, while the higher values (0·1 p.c. or slightly over) are met with in cases where, if any phosphate diminution has occurred, it has done so at the cost of the free form.

(c) Free phosphate. The average percentage is slightly higher than in normal muscle, namely 0·308 p.c. (range from 0·259 to 0·377).

II. *The breakdown process.*

1. In the normal well-fed animal's muscles.

(a) Glycogen practically disappeared entirely.

(b) Lactic acid reached on an average 0·5 to 0·6 p.c. although in some cases with an original high glycogen value, a greater production was met with. A lower maximal acid production (0·4) was met with in certain cases where the original lactic acid value was low, and the muscle fairly well stored with glycogen.

(c) Free phosphate. The increase in free phosphate produced by hydrolysis was derived from the originally bound form, and amounted

on an average to 0·188 p.c. Of the total phosphate present in the normal muscle 36–40 p.c. was in the bound form.

2. In the starved animal.

(*a*) Glycogen disappeared, as always occurs during hydrolysis.

(*b*) Lactic acid production reached as an average maximal value 0·5 to 0·6 p.c., which was in part derived from the original small glycogen store, and in part from the lactacidogen.

(*c*) The increase in the free phosphate was the same as in the well-fed animal, the hexosediphosphate thus being present in the same amount in both. An example of the breakdown process in the starved animal's muscle will show the nature of the changes produced.

TABLE III.

	H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.
Original condition	0·313	0·139	0·234
After hydrolysis	0·540	0·612	—

During the hydrolysis, per gramme muscle, over 4 mg. lactic acid have appeared, 2 mg. of which could have been supplied by the glycogen and 2 mg. from the hexosediphosphate.

3. The stimulated muscle. After prolonged stimulation (2 hours), hydrolysis produced very little further change as the glycogen and the hexose diphosphate had both reached a low value. Thus in the case of the muscle referred to in Table II the lactic acid showed no further increase while the free phosphoric acid value rose from 0·357 to 0·422 p.c., corresponding to a lactacidogen phosphoric acid value of 0·065 p.c. When the stimulation was of very short duration (10–15 mins.) the existing condition of the muscle was found to be the same on the stimulated as on the unstimulated side, and therefore hydrolysis brought about the same changes in both sets of muscles. When stimulation was rather more prolonged the glycogen and the hexosediphosphate percentage in the muscle gradually fell and the lactic acid rose, so that on subsequent hydrolysis the lactic acid and free phosphoric rise showed a smaller increase than in the case of the normal rested muscle.

4. After pancreatectomy. The breakdown process showed certain variations which indicate that there are at least two different types of muscle met with after pancreatectomy. In these types both the breakdown and synthetic processes differ. The first type is met with in animals in very good condition, apart from the high blood sugar and low liver glycogen. There is no appearance, so far as the general behaviour and

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muscular movements are concerned, of any departure from the normal. Three examples of the breakdown process in this type may be given. In (a) the muscles were examined 24 hours after removal of the pancreas, in (b) after 48 hours and in (c) after 75 hours.

TABLE IV.

	H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.	Blood sugar p.c.
(a) Before hydrolysis	0·314	0·03	0·27	
After hydrolysis	0·487	0·54	0·03	0·32
(b) Before hydrolysis	0·308	0·03	0·44	
After hydrolysis	0·490	0·66	0·04	0·47
(c) Before hydrolysis	0·318	0·18	0·37	
After hydrolysis	0·493	0·51	—	0·36

In all the specimens the hexosediphosphate, as determined from the increase in free phosphate on hydrolysis, and the glycogen are below the normal. The increment in lactic acid is the same as would occur on hydrolysis of normal muscle.

The other type is mainly characterised by a small lactic acid increase during the breakdown process. The animals were more lethargic than those of the other type, one (C_1) dying suddenly under ether just as the blood was being withdrawn.

Five examples of this type will be given.

TABLE V.

	H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.	Blood sugar p.c.	Time depan-creatised
(a) Before hydrolysis	0·297	0·09	0·565		
After hydrolysis	0·459	0·32	—	0·27	48 hours
(b) Before hydrolysis	0·377	0·09	0·379		
After hydrolysis	0·401	0·27	—	0·29	4 days
(c) Before hydrolysis	0·287	0·05	0·488		
After hydrolysis	0·455	0·17	—	0·35	$2\frac{1}{2}$,,
(d) Before hydrolysis	0·313	0·14	0·472		
After hydrolysis	0·477	0·37	0·204	0·25	4 ,,
(e) Before hydrolysis	0·335	0·15	0·482		
After hydrolysis	0·471	0·35	0·208	0·38	2 ,,

These five cases, and there were many more of the same type, show the following peculiarities in the breakdown process. There is a distinct diminution in the amount of phosphate set free by hydrolysis and hence a lessened lactacidogen content. The increase in lactic acid is extremely small and can nearly, or in one case altogether, be accounted for by the hexosediphosphate breaking down with only a small portion derivable from the glycogen.

The original glycogen content of the muscle, even four days after removal of the pancreas, has remained at a fairly high level. As will be seen later, the synthetic processes in these muscles have been very seriously interfered with in most cases. The glycogen after hydrolysis was only determined in the last two, because unfortunately it was taken for granted in the others that it would have disappeared entirely, but in the two mentioned there was still a fair amount of glycogen present so that the diminution in lactic acid production can be partly explained in this way.

III. *The synthetic process under fluoride as it affects the intrinsic precursors of the ester.*

1. In the normal animal in good condition. Three examples will be given, chosen because of the differences in the glycogen store in the muscle. The amounts of the free phosphate, lactic acid and glycogen before (A) and after (C) the action of the fluoride will be given.

TABLE VI.

		H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.
(1)	A	0·309	0·242	0·489
	C	0·085	0·129	0·084
(2)	A	0·296	0·252	0·711
	C	0·051	0·191	0·356
(3)	A	0·281	0·145	1·300
	C	0·040	—	1·000

Per gramme muscle in (1) 2·24 mg. have been synthetised out of 3·09 with a consumption of 4·05 mg. glycogen along with a decrease of 1·13 mg. lactic acid, in (2) 2·45 mg. out of 2·96 with a consumption of 3·55 mg. glycogen and a decrease of 0·61 mg. lactic acid, in (3) 2·41 mg. have been synthetised out of 2·81 and 3 mg. glycogen have disappeared. The lactic acid in this muscle was not determined after synthesis. Of the intrinsic free phosphate, between 72·5 and 85·7 p.c. has been synthetised and during this synthesis lactic acid formation is not only checked but there is also a disappearance of the acid while an amount of glycogen has been consumed which is more than sufficient to supply the carbohydrate component of the ester formed.

The best synthesis has occurred when the glycogen consumption has closely followed in amount the removal of the free phosphate.

The effect of impoverishment of the muscle glycogen store on the synthetic process will now be referred to.

2. After starvation for four days (two examples).

TABLE VII.

		H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.
(1)	A	0·293	0·209	0·486
	C	0·058	0·190	0·367
(2)	A	0·313	Lost	0·234
	C	0·081	0·139	0·130

In (1) the muscle glycogen store was practically the same as in one of the well-fed animals.

In (1) 2·35 mg. out of 2·93 and in (2) 2·32 mg. out of 3·13 were synthetised by 1 gm. muscle with a consumption only of 1·19 mg. glycogen in (1) and of 1·04 mg. in (2).

Less glycogen has been consumed than would be required for synthesis of the hexosediphosphate. From some experiments dealing with the time factor in this synthetic process there was evidence of a synthesis of glycogen under the action of fluoride. It is probable therefore that, for the synthesis of the hexosediphosphate, glycogen formation during the esterification process is required. As has been shown by Embden, the sugars, as such, cannot replace glycogen in facilitating synthesis.

3. The effect of stimulation of the muscle on the synthetic process. If the stimulation is brief and does not result in a distinct decrease in the muscle glycogen store, the synthesis of the intrinsic precursors is as good as in the unstimulated muscle. Three examples of the effect of stimulation will be given.

TABLE VIII.

		H_3PO_4 p.c.	Lactic acid p.c.	Glycogen p.c.
(a)	A	0·278	0·159	1·220
	C	0·023	—	0·980
25 minutes' direct stimulation of exposed muscle				
(b)	A	0·279	0·330	0·152
	C	0·093	—	0·033
35 minutes' stronger direct stimulation of exposed muscle				
(c)	A	0·357	0·220	0·024
	C	0·314	0·210	0·054
2 hours' stimulation of sciatic nerve until gastronemius showed marked fatigue				

With progressive diminution in the glycogen store the synthetic process deteriorates, but even when it is lowered to 0·152 p.c. one gm. muscle can still synthetise 1·86 mg. out of 2·79 mg. H_3PO_4. In order that this quantity of phosphate should be changed to the hexose-diphosphate form, at least 1·52 mg. glycogen would be required, and slightly less than this, 1·19 mg. have been used up. In the first example

the glycogen store in the muscle at the close of stimulation was very high and the synthesis was extremely good, 2·55 mg. out of 2·78 with a consumption of 2·4 mg. glycogen, while 2·09 mg. would have, theoretically, been sufficient.

In the last example where fatigue had set in after the prolonged stimulation, the muscle glycogen store was practically exhausted and after the synthesis the amount had apparently risen slightly, although 0·43 mg. H_3PO_4 had been synthesised out of 3·57. The glycogen values are however too low for accurate estimation. In all three cases the amount of glycogen which disappeared during the synthesis of the ester was small, and in the last two examples carbohydrate other than the original glycogen must have been used for the synthesis. It certainly appears as if the synthetic process is governed not only by the amount of the components of the ester available but also, in accordance with the view of Embden, by the effect produced on the enzymatic process by changes in the colloidal intrafibrillar mechanism.

4. The effect of pancreatectomy on the synthesis of the intrinsic precursors of the ester. The effect was, in general, a diminution in the synthetic power. This diminution was not due to the exhaustion of the carbohydrate stores, as these were usually no more depleted than in the muscles of starved animals. The deterioration was most marked in the cases where the original lactic acid value was low and where, on hydrolysis, the total lactic acid production and the free phosphate increase were smaller than in the muscles of normal animals. Thus a muscle in which the breakdown process was interfered with showed a disturbance also in the synthetic process while in cases where the former differed but slightly from that of the normal animal the latter also approached the normal type. Two types of muscle in the depancreatised animal then are met with both as regards hydrolytic and synthetic changes. The synthetic changes in the intrinsic components will be given for the two types.

The best type which approaches closely the normal (the breakdown process in which is given in Table IV) is shown in the following examples.

TABLE IX.

		H_3PO_4 p.c.	Glycogen p.c.
(a)	A	0·314	0·270
	C	0·071	0·180
(b)	A	0·308	0·441
	C	0·056	0·098
(c)	A	0·318	0·378
	C	0·072	0·17

The synthesis is as good as in normal animals so far as capacity to synthetise the intrinsic free phosphate is concerned. In (b) and (c), but not in (a), the glycogen consumption is sufficient for esterification.

Examples of the second type in which esterification as well as the breakdown process has been interfered with will now be given (the breakdown process is given in Table V).

TABLE X.

		H_3PO_4 p.c.	Glycogen p.c.
(a)	A	0·297	0·565
	C	0·139	0·109
(b)	A	0·377	0·379
	C	0·170	—
(c)	A	0·287	0·488
	C	0·284	0·342
(d)	A	0·313	0·472
	C	0·096	0·250
(e)	A	0·335	0·482
	C	0·147	0·300

The lactic acid production under the fluoride was checked in all the specimens. The phosphate synthesis was much worse than in the normal muscle. In (c) there was practically no synthesis, in (a), (b) and (e) between 52 and 56 p.c., and in (d) 67 p.c. of the intrinsic phosphate was synthetised. The glycogen consumption was sufficient to cover synthetic requirements. In (c) where synthesis was practically absent there must have been a serious disturbance in the muscle colloids associated with the enzymatic process, as there was a fairly high glycogen store in the muscle, a small amount being used up under the action of the fluoride without beneficial effect.

IV. *The synthetic process after the addition of glycogen and phosphate.*

From the earlier work it had been found that the normal muscle was capable of synthetising a larger quantity of phosphate than was originally present and that this synthesis was greatly improved by adding glycogen as well as phosphate.

In all cases examined the amount of the intrinsic plus added phosphate was approximately 13 mg. (stated as H_3PO_4) per gm. muscle, sufficient glycogen being added to synthetise completely this quantity. As in all cases, the lactic acid production, even with the excess of glycogen and phosphate, was completely checked, it is not necessary to give the amounts of this acid before and after synthesis.

The glycogen (intrinsic and added) was almost completely removed

during the esterification. The amount of phosphate synthetised was always of the same order, the disappearance of the added free phosphate being most complete in those cases where the intrinsic phosphate and glycogen had been most efficiently esterified. The results may be given in condensed form.

1. One gm. minced muscle taken from an animal in good condition will synthetise on an average 11·5 mg. out of 13 mg. H_3PO_4, the phosphate being present in the form of Na_2HPO_4. The lowest values are slightly over 10 mg. and the highest slightly over 12 mg.

2. The average synthesis in the case of muscle from a starved animal is approximately 10 out of 13 mg. In both cases a sufficiency of glycogen disappears for the formation of the hexosediphosphate.

3. After stimulation of the muscle for a short time (10–15 mins.) the synthesis is quite as good as with the unstimulated muscle, in certain cases rather better, but, when the stimulation is prolonged and the intrinsic glycogen store brought down to a low level, the simple addition of glycogen along with the phosphate does not lead to the usual improved synthesis. In one case of extreme fatigue, only 1·72 mg. were synthetised out of 13 mg., although the added glycogen had almost entirely disappeared under the action of the fluoride. Syntheses of 6–7 mg. out of 13 mg. were obtained after direct stimulation of the muscle for slightly over half an hour.

4. After pancreatectomy. In two cases only did the synthesis of the intrinsic plus added phosphate approach that obtained in the case of the muscles of the normal animal. In each of these the synthesis was 10 out of 13 mg. In one of these, examined three days after the removal of the pancreas, the muscle glycogen was 0·378 p.c., the blood sugar 0·36 p.c., and the liver glycogen had almost entirely disappeared.

In the other, examined four days after removal of the pancreas, the muscle glycogen was much higher (0·693 p.c.), the blood sugar low (0·23 p.c.) and the liver glycogen 0·14 p.c.

The results obtained in thirteen depancreatised animals are given in the following table, the muscle and liver glycogen and the blood sugar as well as the phosphate synthetised being included.

The animals which were in the best condition after removal of the pancreas synthetised from 6–8 mg. out of 13 mg. while those which were in poor condition and lethargic (therefore killed earlier) synthetised 3–5 mg. out of 13. Usually the best syntheses occurred in cases where the muscle glycogen was about 0·4 p.c., although the percentage might be above that figure and esterification bad or below it and yet fairly good.

TABLE XI.

	Muscle glycogen p.c.	Liver glycogen p.c.	Blood sugar p.c.	mg. H_3PO_4 synthetised out of 13 mg. per gm. muscle	Duration of depancreatisation
(1)	0·28	0·08	0·20	3·94	28 hours
(2)	0·12	0·12	0·20	4·80	48 ,,
(3)	0·48	0·12	0·35	3·27	54 ,,
(4)	0·46	0·06	0·27	5·70	48 ,,
(5)	0·28	—	0·21	5·06	48 ,,
(6)	0·29	0·03	0·30	6·08	48 ,,
(7)	0·48	0·05	0·38	7·00	48 ,,
(8)	0·44	0·06	0·47	8·20	48 ,,
(9)	0·38	1·07	0·30	5·10	4 days
(10)	0·50	0·02	0·25	7·06	4 ,,
(11)	0·27	—	0·32	7·38	4 ,,
(12)	0·40	0·04	0·34	8·10	4 ,,
(13)	0·32	0·15	0·40	4·17	6 ,,

In none was the synthesis so good as with the muscles of the normal animal.

In conclusion a brief reference may be made to a short series of experiments which was carried out to determine the changes which the free phosphate, glycogen and lactic acid undergo during the course of the fluoride action on the intrinsic, and the intrinsic plus added glycogen and phosphate. These experiments, dealing both with normal and diabetic animals, were carried out in the following way.

The fluoride action on the minced muscle was checked in the usual way after ½ hour, 1 hour, 1½ hours and 3 hours, and after each of these intervals the free phosphate, lactic acid and glycogen were determined so that with the original (A) values the course of the reaction changes might be investigated.

The results obtained in the case of normal cat's muscle are shown in Fig. 1 (action on intrinsic components) and Fig. 2 (action on intrinsic plus added components, 13 mg. H_3PO_4 and 13 mg. glycogen per gm. muscle).

The comparable results in the depancreatised animal are shown in Figs. 3 and 4.

Fig. 1 shows the following:

1. A rapid glycogen consumption during the first half hour, followed by a synthesis of glycogen and finally its disappearance.

2. The synthesis of the phosphate has been completed within the first hour. The primary glycogen disappearance has occurred before the phosphate synthesis has been completed.

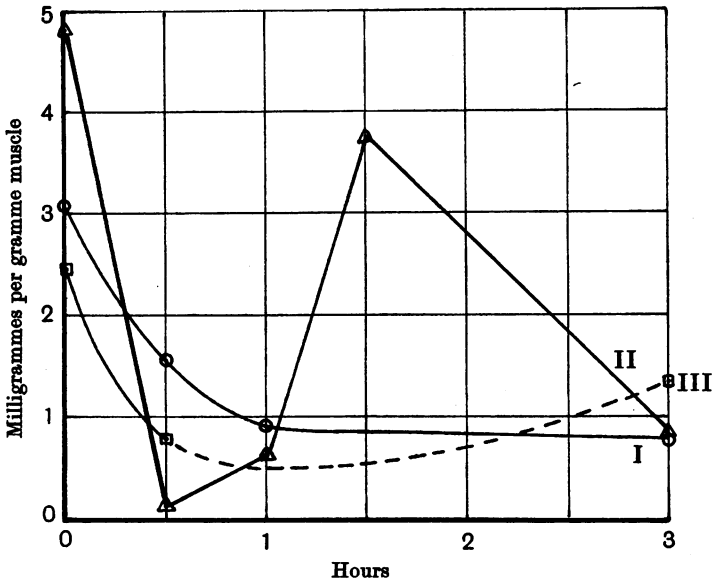3. A disappearance of lactic acid during the early part of the synthetic process.

Fig. 1. Normal cat's muscle. Synthesis under fluoride.

I = Phosphate (intrinsic), as H_3PO_4. II = Glycogen (intrinsic).
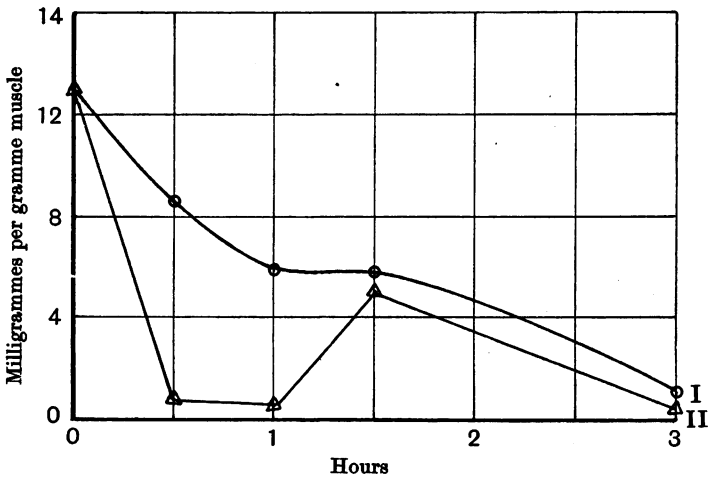III = Lactic acid.

Fig. 2. Normal cat's muscle. Synthesis under fluoride.

I = Phosphate (intrinsic + added), as H_3PO_4.
II = Glycogen　　　　,,　　　　,,

Comparing this with the behaviour of muscle from the depancreatised animal, the following differences are to be seen (Fig. 3):
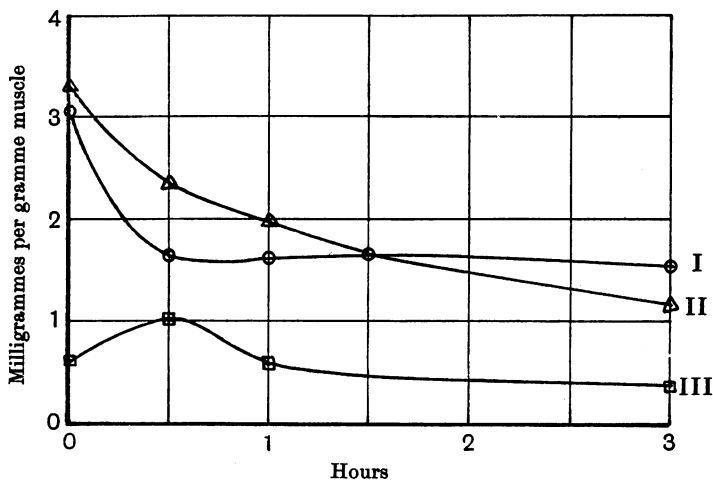

Fig. 3. Depancreatised cat's muscle. Synthesis under fluoride.
I = Phosphate (intrinsic), as H_3PO_4. II = Glycogen (intrinsic).
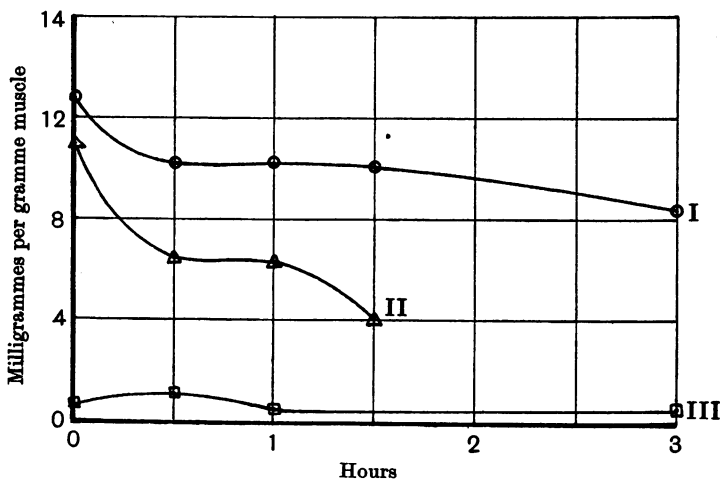III = Lactic acid.

Fig. 4. Depancreatised cat's muscle. Synthesis under fluoride.
I = Phosphate (intrinsic + added), as H_3PO_4. II = Glycogen (intrinsic + added).
III = Lactic acid.

1. The glycogen consumption is more gradual and less complete. There is no evidence of a glycogen synthesis.

2. The maximal phosphate synthesis has occurred early but to a lesser degree than in normal muscle. The lactic acid production is checked.

The syntheses of the intrinsic plus added components in the normal and depancreatised animals are shown in Fig. 2 and Fig. 4.

In the normal muscle the same rapid consumption of glycogen followed by a synthesis is to be seen, and the phosphate synthesis is not completed at the end of the 1½ hours period.

In the depancreatised animal the same type of reaction changes is seen with the added as with the intrinsic components alone, namely, a holding up of the glycogen, the consumption of which corresponds more closely to the amount required for the production of the hexosediphosphate than in the case of the normal muscle. There is also a great decrease in the amount of free phosphate synthetised.

DISCUSSION.

From a study of the existing condition in muscle the carbohydrate mechanism appears to be a very stable one. The resistance offered to depletion of the glycogen store and the maintenance of the phosphate, organic and inorganic, at such a constant level indicate that reversible processes play important parts in the transformations.

The changes produced in the existing condition of the muscle by starvation and depancreatisation are, in certain respects, very similar, but when the diabetic condition is accompanied by great weakness and lethargy the changes produced in the muscle by the hydrolytic action of weak bicarbonate solutions (the breakdown process) are very different. In the case of the starved animal the breakdown process is the normal one, while, in the diabetic, there is a much smaller production of lactic acid, the value after hydrolysis being as low as from 0·17 to 0·3 p.c. The difference in behaviour of these muscles is of interest, since, at the outset, both have a similar glycogen store. In some cases the amount of lactic acid produced in the depancreatised animal's muscle could be accounted for by the breakdown of hexosephosphate alone so that the glycogen must have been changed into some form other than lactic acid.

As regards the synthetic process, the muscles in which the hydrolytic change was abnormal showed a reduced power to synthetise both the intrinsic and the added precursors of the ester. That the failure to synthetise was not due to lack of the components is evident. The possible cause might be one or other of the following:

(*a*) An interference with the action of the esterifying enzyme or an alteration in the associated muscle colloids.

(*b*) A change in the form of combination of the glycogen in the muscle.

Undoubtedly the most marked interference with the synthetic mechanism is found in muscles which have been exhausted by prolonged stimulation or as a result of severe pancreatic diabetes especially in animals of poor condition, or in rigor mortis. In all of these conditions alterations in the muscle proteins are to be expected, so that Embden's hypothesis may furnish the correct explanation of these disturbances in synthesis, especially in the case of overstimulated muscle and rigor. The disturbances after pancreatectomy may however be due to a change in the form in which the glycogen is bound in muscle or an interference with the preliminary storage of the glycogen.

The evidence of synthesis of glycogen by normal muscle during the course of the fluoride action and the absence of such evidence in the case of diabetic muscle are of importance, but further experiments are certainly necessary in order to elucidate the nature of the changes which occur during the progress of the fluoride action.

In conclusion, the muscle of the cat may be regarded as normal if it shows the following properties:

(*a*) In the existing condition.
    (1) A lactacidogen value not less than 0·18 p.c. (H_3PO_4).
    (2) A lactic acid content of 0·08–0·2 p.c.
    (3) Glycogen 0·5 p.c. and over.

(*b*) After hydrolysis.
    (1) Lactic acid 0·5–0·8 p.c. (or over).
    (2) Total phosphate 0·48 p.c.

(*c*) After synthesis of the intrinsic components a lactacidogen value of not less than 75 p.c. of the total phosphate.

(*d*) In the synthesis of the intrinsic and added free phosphate and glycogen.
    (1) 1 gm. muscle transforming 11–12 mg. H_3PO_4 (as phosphate) out of 13 mg. from the free to the bound form with
    (2) Practically complete consumption of the glycogen.

The factors which indicate disturbances in the metabolism such as are met with in severe pancreatic diabetes are:

(1) A low original lactic acid value (·03–·06 p.c.).
(2) Lactacidogen H_3PO_4 below 0·17 p.c. in the original muscle.
(3) A lactic acid maximum after hydrolysis of less than 0·4 p.c.

(4) Lessened synthesis of both the intrinsic and the added phosphate with the carbohydrate component along with

(5) A tendency to hold up the glycogen during the synthetic processes.

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