CLIV. THE GLYCOGEN OF MAMMALIAN MUSCLE AND ITS BEHAVIOUR AFTER DEATH.

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(*Received August 12th, 1930.*)

EVIDENCE has been accumulating which indicates that intermediate substances other than hexosediphosphate may play a part in the breakdown of glycogen to lactic acid in mammalian muscle. Simpson and Macleod [1927] showed that when muscle is removed from decapitated cats with precautions to prevent twitching, frozen in liquid air, pulverised, and then allowed to thaw, glycogen disappears much more rapidly than lactic acid accumulates. At the same time the inorganic phosphorus shows a slight increase instead of the decrease which would be expected if the glycogen which disappeared had been broken down to hexosediphosphate. In more recent experiments Thomas and Macleod [see Macleod, 1929] found that if the pulverised muscle were allowed to stand for a longer period the amount of lactic acid which finally accumulated sometimes exceeded the initial amount of glycogen. These results seem to show that the glycogen breaks down rapidly into some intermediate substance from which lactic acid is derived more slowly. The initial presence of this substance in muscle would explain the excess of lactic acid produced over the glycogen originally present. The presence of a trisaccharide during the breakdown of glycogen by muscle enzyme has recently been demonstrated by Barbour [1930].

In intact muscle after death glycogenolysis sets in very slowly. Mackay [1928] compared the percentages of glycogen in certain muscles removed under ether anaesthesia from one hind-limb in rabbits with the corresponding muscles of the opposite limb removed at varying intervals after death and found, in most cases, that up to 1 hour after death there was no appreciable difference. Sometimes there was an apparent increase in the muscles which had stood after death. This result was confirmed by Macleod and Fraser [see Macleod, 1929] who eliminated the possibility that the difference might be due to there being more blood in the living muscle by removing two control muscles immediately after death and the two corresponding muscles of the opposite side 1 hour after death in rabbits killed by an overdose of chloroform. In a series of 22 such experiments it was found that the glycogen of the muscle removed 1 hour after death exceeded that of the corresponding muscle of the opposite

side removed immediately in 12 cases, and was less in 10 cases; in every case there was a distinct but relatively small increase in lactic acid in the muscle which had stood for 1 hour after death.

These results suggest the presence in mammalian muscle of an intermediate substance from which lactic acid and sometimes glycogen can be formed after death. In drawing such a conclusion however it has to be assumed that the corresponding muscles of the two sides contain approximately the same percentages of glycogen at the time of death, and the first part of the present paper shows to what extent this assumption is justified. For other reasons also it is most important to have accurate information regarding the quantitative distribution of glycogen in the musculature of mammals [see Macleod, 1929].

Part I.

A comparison of the glycogen content of corresponding muscles of opposite sides of the body.

Pflüger [1903] found close agreement in the glycogen content of the musculature of opposite limbs in different animals, and Elias and Schubert [1918] found, in animals under urethane anaesthesia, that the mean difference in glycogen content between corresponding muscles of opposite sides was only 2·3 %; in one experiment "without anaesthesia" however a difference of as much as 16 % is recorded. Long [1928] obtained similar results in cats under amytal anaesthesia, the average difference in the two gastrocnemii being 3·2 %. Best, Hoet and Marks [1926] found in the decapitated, eviscerated cat preparation differences between individual corresponding muscles of the opposite sides of as much as 20 %, but, when the average values for five muscles on each side were compared, the differences were small (4 % and 7 % in the two experiments recorded). This suggests that the differences between corresponding muscles may be partly due to unavoidable variations in the technique of removal.

Rabbits fed on the usual stock ration were used in the first experiments. They were anaesthetised with chloroform, administered slowly to reduce struggling to a minimum, and then killed by giving an overdose. Immediately after death three corresponding muscles were dissected out on each side with as much care as possible to avoid mechanical irritation. The dissected muscles were then dropped into hot 60 % potassium hydroxide and the glycogen content determined by Pflüger's method, the reducing substance being estimated by the modified Shaffer-Hartmann method[1]. The results of four such experiments are given in Table I.

There is, as a rule, marked inequality of glycogen content in corresponding muscles of opposite sides, the variations being as great as the *post mortem* changes found by Macleod and Fraser. When the average of the three muscles on each side is taken, correspondence on the two sides is reasonably close.

[1] The experimental error of the entire method does not exceed ± 0·02 %.

Table I. *Glycogen (as glucose) % in corresponding muscles of legs of chloroformed rabbits.*

No.	Muscle	Glycogen-glucose (%) Right	Glycogen-glucose (%) Left	Difference Left compared with right	Remarks
I.	Gastrocnemius	0·41	0·41	—	—
	Gracilis	0·43	0·31	−0·12	—
	Tib. anticus	0·52	0·49	−0·03	No contractions
	Average	0·45	0·40		
II.	Gastrocnemius	0·48	0·20	−0·28	—
	Gracilis	0·43	0·58	+0·15	—
	Tib. anticus	0·27	0·26	−0·01	Contractions
	Average	0·39	0·35		
III.	Gastrocnemius	0·62	0·47	−0·15	$CHCl_3$ given very gradually
	Gracilis	0·63	0·48	−0·15	—
	Tib. anticus	0·48	0·79	+0·31	No contractions
	Average	0·58	0·58		
IV.	Gastrocnemius	0·41	0·63	+0·22	$CHCl_3$ given rapidly
	Gracilis	0·59	0·47	−0·12	—
	Tib. anticus	0·57	0·34	−0·23	No contractions
	Average	0·52	0·48		

In the next experiments the decapitated cat preparation was used. The animals were anaesthetised with chloroform, decapitated and, as a rule, eviscerated, after which they were allowed to rest for an hour on a warmed table; artificial respiration was maintained throughout and it was assumed that at the end of the hour the anaesthetic would have been removed from the tissues. Three corresponding muscles on each side were dissected out and removed immediately before or after death by bleeding. (To study the *post mortem* changes in glycogen, three other muscles on the right side were removed at the same time, and the corresponding muscles on the left side removed 1 hour after death. The results of these observations on *post mortem* behaviour of glycogen are given separately in Part II of this paper, Table V.)

The results of six experiments are given in Table II.

In two cases only is there agreement within the experimental error between individual corresponding muscles of opposite sides and the differences expressed as a percentage of the larger amount not infrequently exceed the maximum (20 %) mentioned by Best, Hoet and Marks. If, as these authors assume, the differences are largely due to difference in handling of the muscles, they cannot be satisfactorily allowed for by averaging the results of three muscles. In view of the foregoing results it was decided to study the glycogen of the entire musculature of opposite legs. For this purpose rats, previously fed on a uniform (McCollum) diet, were chosen.

In the first observations the animals were killed by stunning, the abdomen was opened, and the hind-quarters were separated by cutting through the vertebral column; after skinning, the sacrum was bisected longitudinally with

Table II. *Glycogen (as glucose) °/₀ in simultaneously removed corresponding muscles of legs of decapitated cats.*

No.	Muscle	Glycogen-glucose (%) Right	Glycogen-glucose (%) Left	Difference Left compared with right	Remarks
I.	Gracilis	0·51	0·54	+0·03	—
	Tib. anticus	0·37	0·26	−0·11	One hour after decapitation
	Flexor carpi ulnaris	0·33	0·38	+0·05	and evisceration
	Average	0·40	0·39		
II.	Gracilis	0·55	0·49	−0·06	—
	Tib. anticus	0·41	0·36	−0·05	One hour after decapitation
	Flexor carpi ulnaris	0·45	0·49	+0·04	
	Average	0·47	0·45		
III.	Gracilis	0·41	0·38	−0·03	One hour after decapitation
	Tib. anticus	0·41	0·45	+0·04	and evisceration
	Flexor carpi ulnaris	0·27	0·39	+0·12	—
	Average	0·36	0·41		
IV.	Gracilis	0·59	0·61	+0·02	One hour after decapitation
	Tib. anticus	0·49	0·62	+0·13	and evisceration
	Flexor carpi ulnaris	0·53	0·58	+0·05	—
	Average	0·54	0·60		
V.	Gracilis	0·14	0·18	+0·04	One hour after decapitation
	Tib. anticus	0·21	0·28	+0·07	and evisceration
	Flexor carpi ulnaris	0·38	0·42	+0·04	Asphyxiated
	Average	0·24	0·29		
VI.	Gracilis	0·50	0·50	—	One hour after decapitation
	Tib. anticus	0·59	0·50	−0·09	—
	Flexor carpi ulnaris	0·32	0·33	+0·01	Asphyxiated
	Average	0·47	0·44		

a sharp scalpel, the feet were cut off, and the remainder of each hind-leg was dropped into hot 60 % potassium hydroxide in tared flasks. After the usual heating followed by precipitation of the glycogen, the bones were collected on a filter paper along with the precipitate, and after the glycogen had been dissolved with several small quantities of hot water the bones were weighed moist and their weight was subtracted from that of the limb to give the weight of muscle.

The results obtained on 12 rats are given in Table III.

It is seen that there is in many cases marked inequality between the two legs. Spasmodic muscular contractions always occurred at death and this rendered the experiment inconclusive, since these contractions, occurring in the absence of an adequate blood flow, would cause a rapid disappearance of glycogen.

A method of killing was however found which usually eliminated any muscular contractions at death. The rats were placed on a block and, when quiet, were cut in two by a smart blow with a heavy butcher's cleaver. Provided transection was performed well behind the fore-limbs, there was usually

Table III. *Glycogen (as glucose) °/₀ in muscles of hind-legs of stunned rats.*

No.	Glycogen-glucose (%) Right	Left	Difference Left compared with right
1	0·31	0·30	−0·01
2	0·14	0·18	+0·04
3	0·18	0·18	—
4	0·28	0·32	+0·04
5	0·22	0·26	+0·04
6	0·34	0·25	−0·09
7	0·29	0·33	+0·04
8	0·17	0·22	+0·05
9	0·25	0·24	−0·01
10	0·22	0·23	+0·01
11	0·27	0·24	−0·03
12	0·21	0·25	+0·04

no movement of the hind-limbs after death. The hind quarters were then rapidly skinned, the sacrum was bisected, and the legs were treated as described above.

Table IV. *Glycogen (as glucose) °/₀ in leg muscles of rats killed by body transection.*

	No.	Glycogen-glucose (%) Right	Left	Difference Left compared with right
Part 1. Without contractions				
	1	0·207	0·183	−0·024
	2	0·217	0·218	+0·001
	3	0·121	0·127	+0·006
	4	0·270	0·275	+0·005
	5	0·233	0·245	+0·012
	6	0·264	0·249	−0·015
	7	0·246	0·253	+0·007
	8	0·211	0·210	−0·001
	Average	0·220	0·221	
Part 2. With contractions				
	1	0·145	0·211	+0·066
	2	0·205	0·137	−0·068
	3	0·178	0·141	−0·037
	4	0·143	0·177	+0·034
	5	0·228	0·305	+0·077
	6	0·103	0·081	−0·022
	7	0·195	0·224	+0·029
	Average	0·171	0·182	

Table IV, Part 1, shows the results obtained in a series of eight rats in which no movements of the hind-limbs were visible after transection; it will be noticed that the differences between the two legs are very small, not exceeding 0·02 % except in one case when it was 0·024 %. In Table IV, Part 2, obtained in a series of seven rats in which spasmodic movements of the hind-limbs occurred, the differences are much greater, the average being 0·049 %. The general conclusion may be drawn that the glycogen content of the entire musculature of the hind-limbs is equal on the two sides, provided that there have been no spasmodic muscular movements of the limbs at the time of

death. It will also be noted that with one exception the muscle glycogen in different rats is fairly constant in the absence of contractions but varies greatly when these occur. The average for the former group is 0·22 %, or excluding No. 3 0·235 %, which is somewhat below that given by Barbour, Chaikoff, Macleod and Orr [1926], viz. 0·25 %. The difference is probably due to the fact that fasting in the present observations occurred at room temperature, whereas in those of Barbour, etc. it occurred at 29°.

Part II.

The behaviour of glycogen in intact mammalian muscle after death.

The *post mortem* behaviour of glycogen was next studied. Two preparations were used.

(1) *The decapitated cat.* Three muscles of the right side were removed at

Table V. *Glycogen (as glucose) % in corresponding muscles of the two sides removed either immediately or 1 hour after death (in decapitated cats).*

No.	Muscle	Glycogen-glucose %: Immediately after death (Right)	Glycogen-glucose %: 1 hour later (Left)	Difference Left compared with right	Lactic acid in blood (mg. per 100 cc.)	Remarks
I	Rectus femoris	0·20	0·29	+0·09	—	Muscular contractions during bleeding
	Gastrocnemius	0·30	0·47	+0·17	—	
	Biceps	0·30	0·46	+0·16	25·26	
	Average	0·27	0·41			
II	Rectus femoris	0·69	0·53	−0·16	—	During dissection muscles of fore-limbs showed twitching
	Gastrocnemius	0·36	0·40	+0·04	—	
	Biceps	0·62	0·47	−0·15	7·12	
	Average	0·56	0·47			
III	Rectus femoris	0·34	0·35	+0·01	—	No muscular contractions
	Gastrocnemius	0·42	0·48	+0·06	—	
	Biceps	0·33	0·25	−0·08	15·03	
	Average	0·36	0·36			
IV	Rectus femoris	0·65	0·54	−0·11	—	Feeble contractions during bleeding
	Gastrocnemius	0·47	0·47	—	—	
	Biceps	0·45	0·42	−0·03	17·81	
	Average	0·52	0·48			
V	Rectus femoris	0·25	0·29	+0·04	—	Preparation asphyxiated prior to bleeding
	Gastrocnemius	0·32	0·30	−0·02	—	
	Biceps	0·27	0·32	+0·05	20·38	
	Average	0·28	0·30			
VI	Rectus femoris	0·41	0·36	−0·05	—	Preparation asphyxiated prior to bleeding
	Gastrocnemius	0·57	0·49	−0·08	—	
	Biceps	0·38	0·30	−0·08	25·12	
	Average	0·45	0·38			

death (see p. 1411) and three on the left side were left *in situ* at room temperature for 1 hour, being wrapped in gauze damped with physiological saline so as to avoid loss of water.

Table V, which should be examined along with Table II, shows the same lack of agreement between corresponding muscles of opposite sides removed an hour apart as was observed in muscles removed simultaneously. On the other hand, the averages for the muscles removed 1 hour after death when compared with those removed at death, show close agreement of glycogen on the two sides in three cases (3, 4 and 5); a considerable decrease after death in two cases (2 and 6), and an increase after death in one case (1). No evidence of *post mortem* synthesis of glycogen in intact mammalian muscle is therefore furnished by these experiments.

(2) *The rat killed by spinal transection.* Eight rats were killed by spinal transection and, provided no contractions occurred, the right leg of each was immediately thrown into hot 60 % potassium hydroxide, while the left leg was kept at room temperature covered with damp gauze for 1 hour after death; the foot was then cut off and the remainder of the leg thrown into hot potassium hydroxide.

Table VI. *Comparison of glycogen (as glucose) % in leg muscles of rats immediately after and 1 hour after death by body transection.*

	Glycogen-glucose %		
No.	Immediately after death (Right)	1 hour later (Left)	Difference Left compared with right
1	0·299	0·308	+0·009
2	0·368	0·363	−0·005
3	0·293	0·281	−0·012
4	0·184	0·175	−0·009
5	0·222	0·264	+0·042
6	0·301	0·299	−0·002
7	0·295	0·307	+0·012
8	0·301	0·239	−0·062
Average	0·279	0·283	

Table VI gives the results and it can be seen by comparison with Table IV, Part 1, that with two exceptions, Nos. 5 and 8, the differences between the two legs are insignificant, as also is that of the averages of the two groups. In one of the exceptional results the glycogen percentage was much greater in the leg which had stood for 1 hour (5) but in the other exception it was much less (8). The conclusion is drawn that no change occurs in the amount of glycogen in intact mammalian muscle as a result of standing at room temperature for 1 hour after death.

Part III.

The behaviour of glycogen, lactic acid and free sugar in intact mammalian muscle after death.

An attempt was next made to see whether any change occurs in the amounts of lactic acid and free sugar in intact mammalian muscle on standing for 1 hour after death. If either of these should accumulate, their source must be some substance other than glycogen, since we know that this remains unchanged in amount during this time. No attention has been paid to the behaviour of inorganic phosphorus, since this has previously been investigated by Simpson and Macleod [1927] and by Irving [1928], who were unable to detect any significant changes under the conditions of these experiments.

In order to rule out any unusual behaviour of glycogen it was deemed necessary to determine this substance as well as lactic acid and free sugar, but it has been found impossible to determine the three substances simultaneously, on account of the tendency of glycogen to change rapidly in amount in the presence of the extraction agents used, ice-cold alcohol or trichloroacetic acid.

A word is necessary regarding the method used for determining free sugar. Previous workers who have estimated free sugar in tissue have done so usually by making an alcoholic extract of the tissue, evaporating to dryness, dissolving the residue in water, and, after precipitation of proteins and other interfering substances, determining the amount of reducing substance in the solution. It is certain however that this reducing substance is not all free sugar, as is shown by using the technique described by Holmes and Gerard [1929], which was employed in the present experiments.

In the first experiment rats were killed by spinal transection, and, after skinning, the legs were separated by longitudinal section of the sacrum and rapidly weighed. The right leg was immediately thrown into ice-cold 95 % alcohol, while the left leg was kept for 1 hour after death at room temperature and covered with damp gauze before being treated in a similar fashion. The muscles were cut off the bones under the alcohol and finely minced with sharp scissors, and the bones were removed and weighed. The finely divided muscle was extracted for 24 hours in three changes of alcohol, after which the glycogen in the muscle residue was determined. The alcoholic extracts were combined and evaporated at a low temperature to a syrup, which was then dissolved in water and made up to a definite volume. An aliquot portion was treated with "colloidal iron", and the protein-free filtrate, after being made up to known volume, was treated with copper sulphate and calcium hydroxide. The mixture, after standing on ice for 30 minutes, was centrifuged; lactic acid was determined in the filtrate [Friedmann, Cotonio and Schaffer, 1927], while the copper-lime precipitate was utilised for the determination of free sugar.

The results are given in Table VII, Part 1. The glycogen content is unusually high and is much more variable on the two sides than in the observations

Table VII. *Part 1. Glycogen, free sugar and lactic acid (mg. per 100 g.) in intact leg muscles of rats immediately (right) and 1 hour after death (left). Extraction with chilled alcohol.*

No.	Limb	Glycogen	Difference Left compared with right	Free sugar	Difference Left compared with right	Lactic acid	Difference Left compared with right
1	Right	486		14		316	
	Left*	507	+21	26	+12	502	+186
2	Right	444		21		221	
	Left*	415	-29	39	+18	234	+ 13
3	Right	273		8		188	
	Left*	209	-64	11	+ 3	257	+ 69
4	Right	240		16		434	
	Left*	298	+58	21	+ 5	508	+ 74
5	Right	369		25		297	
	Left*	352	-17	24	- 1	442	+145

* 1 hour after death.

Part 2. Glycogen, free sugar and lactic acid (mg. per 100 g.) in leg muscles of rats removed immediately (right) and after maceration and standing for 1 hour after death (left). Extraction with chilled alcohol.

No.	Limb	Glycogen	Difference Left compared with right	Free sugar	Difference Left compared with right	Lactic acid	Difference Left compared with right
1	Right	286		—		328	
	Left*	94	-192	—	—	381	+53
2	Right	276		—		220	
	Left*	107	-169	—	—	226	+ 6
3	Right	321		—		225	
	Left*	94	-227	—	—	249	+24
4	Right	277		16		222	
	Left*	114	-163	23	+7	247	+25
5	Right	342		11		167	
	Left*	175	-167	14	+3	218	+51

* Minced, bruised and left for 1 hour after death.

recorded in Table VI. This irregularity is no doubt due to the twitching which occurred when the muscles were placed in ice-cold alcohol, and the decidedly higher values found in the muscles of the left side in two of the animals (1 and 4) are to be accounted for by these having decreased in irritability on standing. The lactic acid values are also very high, even immediately after death, the twitching combined with the unavoidable *post mortem* handling being no doubt responsible for this. The glucose value of combined glycogen and lactic acid in four of the animals is over 650 mg. which is so high as to suggest some unknown error in the determinations. In all cases there is an increase in the lactic acid on standing for 1 hour after death, and usually (1, 4 and 5) the increase is considerably greater than that which could have been derived from the glycogen which disappeared. The free sugar, determined by the Shaffer-Hartmann method, immediately after death varied from 8 to

25 mg. per 100 g., and it only increased significantly in two out of the five cases (30–52 mg.). It is decidedly higher than that found by Simpson and Macleod [1927] who used lead acetate and alumina cream for removal of the interfering substances.

The experiment was repeated with the difference that the muscles of the left leg were minced and bruised before being allowed to stand for 1 hour after death. The results are shown in Table VII, Part 2. The initial glycogen is of the usual order of magnitude but, in contrast with its behaviour in intact muscle, a marked decrease is shown on standing. The lactic acid, also high to start with, increases as before, but in every case the increase only accounts for a small part of the glycogen which has disappeared. This result is of a similar nature to that found by Simpson and Macleod in pulverised frozen muscle allowed to stand after thawing; it points to the existence of an intermediary substance between glycogen and lactic acid, which is obviously not free sugar (*cf.* 4 and 5).

The lactic acid values immediately after death are far above several of those mentioned in the literature in cases where the muscle has been frozen on removal from the animal. Cori [1925], using carbon dioxide snow as a freezing agent, obtained values ranging from 83 to 120 mg. per 100 g. in rats, while Fletcher [1913] obtained values of about 80 mg. by dropping rabbit muscle into liquid air immediately on removal, and Simpson and Macleod [1927], using a similar method with cat muscle, obtained values averaging about 70 mg. in muscles removed from decapitated cats.

In all these cases a certain amount of the lactic acid was probably formed at death or on removal of the muscles from the animal. Davenport and Davenport [1928] describe a technique by which they obtained very low resting lactic acid values, averaging about 15 mg. per 100 g., by freezing the gastrocnemii *in situ* in rats and guinea-pigs previously anaesthetised with "amytal." By adopting this technique, it was hoped to obtain a more accurate picture of the glycogen, lactic acid and free sugar estimated simultaneously. Rats were anaesthetised by intraperitoneal injection of "luminal," and the skin was removed from the right hind-leg, which was then thoroughly frozen by packing it round with carbon dioxide snow. Freezing usually took about 3 minutes, and after this the frozen limb was amputated with a bone-forceps and kept packed in carbon dioxide snow, while the animal was killed by spinal transection. The left hind-leg was then removed by cutting through the vertebral column and sacrum, and allowed to stand for 1 hour under damp gauze and at room temperature before being frozen. A portion of frozen muscle was removed from each leg with a bone-forceps, weighed, and ground up in a mortar surrounded by ice with 20 cc. of 10 % trichloroacetic acid[1]. The contents of the mortar were transferred to a graduated cylinder made up to a volume of 50 cc. with the acid, and left to stand overnight. After filtering,

[1] This strength was chosen because we found that lactic acid formation went steadily on for several hours when cut up muscle was allowed to stand in 5 % trichloroacetic acid.

an aliquot portion of the filtrate was brought to p_H 5 by cautious addition of 60 % potassium hydroxide, the volume was readjusted, and copper sulphate and calcium hydroxide were added. After centrifuging, free sugar was determined in the precipitate and lactic acid in the filtrate. Glycogen was determined separately by the usual procedure. The results of an experiment on six rats are given in Table VIII.

Table VIII. *Glycogen, free sugar and lactic acid (mg. per 100 g.) in leg muscles of anaesthetised rats frozen* in situ *during life (right) and 1 hour after death (left). Extraction with 10 % trichloroacetic acid.*

No.	Limb	Glycogen	Difference Left compared with right	Free sugar	Difference Left compared with right	Lactic acid	Difference Left compared with right
1	Right	198		92		49	
	Left*	277	+79	104	+12	112	+ 63
2	Right	117		110		134	
	Left*	33	−84	106	− 4	260	+126
3	Right	126		136		175	
	Left*	84	−42	138	+ 2	227	+ 52
4	Right	236		138		86	
	Left*	204	−32	148	+10	197	+111
5	Right	208		150		144	
	Left*	195	−13	162	+12	176	+ 32
6	Right	147		190		216	
	Left*	118	−29	226	+36	235	+ 19

* 1 hour after death.

They show much higher lactic acid values than those recorded by Davenport and Davenport, and in every case there is an increase on standing after death. The free sugar values are much higher than those found when muscle is extracted with alcohol, and this is probably due to a certain amount of hydrolysis of glycogen by the trichloroacetic acid[1]. The glycogen values, in striking contrast to those in Table VI, besides being very low are very divergent on the two sides, and in five cases out of the six show a marked *post mortem* decrease, although not sufficient in Nos. 2, 3, 4 and 5 to account for the increase in lactic acid. Long [1928] has also found that amytal causes a decrease in glycogen in resting muscle and inhibits restoration of glycogen in muscle after stimulation. In view of these results we conclude that investigations on the behaviour of muscle glycogen cannot be of value when conducted on animals narcotised by barbituric acid compounds.

The lactic acid values previously obtained by freezing the muscles immediately after death were much lower than those obtained by chopping the muscle in ice-cold alcohol, and an endeavour was next made to estimate glycogen and lactic acid simultaneously by freezing the muscle in carbon dioxide snow immediately after death. The rats were killed by spinal tran-

[1] In another series of observations in which hot alcohol was used as the extractive and the Hagedorn-Jensen method for estimating the reducing power, values ranging from 17 to 25 mg. per 100 g. were found.

section and the hind-quarters were rapidly skinned and placed in carbon dioxide snow. When completely frozen, the hind-legs were cut off with a bone-forceps and treated as described previously. The results are given in Table IX.

Table IX. *Glycogen, free sugar and lactic acid (mg. per 100 g.) in leg muscles of rats frozen simultaneously immediately after death.*

Rat No.	Limb	Glycogen	Difference Left compared with right	Free sugar	Difference Left compared with right	Lactic acid	Difference Left compared with right
1	Right	130		47		121	
	Left	169	+39	41	−6	123	+ 2
2	Right	219		39		74	
	Left	183	−36	46	+7	81	+ 7
3	Right	201		36		122	
	Left	203	+ 2	32	−4	132	+10

It is seen that while the lactic acid and free sugar values agree within reasonable limits on the two sides, the glycogen values, except in the last case, show wide divergence. The muscles in every case twitched during the freezing and this is evidently sufficient to destroy the equality of glycogen in the two sides.

None of the three methods described can therefore be counted upon to give an accurate picture of the behaviour of both glycogen and lactic acid in muscle after death. Until the simultaneous estimation of glycogen and lactic acid can be carried out in muscle by a method which kills the muscle without stimulating the highly labile reaction which leads to the disappearance of glycogen, the conclusion that muscle glycogen does not disappear from intact mammalian muscle within 1 hour after death while lactic acid accumulates must depend on results obtained from separate experiments.

Summary.

1. In rabbits killed by chloroform or in decapitated cats corresponding muscles of opposite sides do not necessarily contain equal percentages of glycogen, although the averages of three corresponding muscles on each side usually agree.

2. In rats killed by spinal transection with a cleaver the two hind-limbs contain equal percentages of glycogen.

3. The glycogen of intact mammalian muscle does not appreciably diminish when the muscle is kept for 1 hour at room temperature after death.

4. Lactic acid accumulates under the foregoing conditions and this is interpreted as evidence that an intermediate substance exists in mammalian muscle, from which lactic acid can be derived.

5. A suitable method for the simultaneous determination of glycogen and lactic acid in muscle has been sought in order that their behaviour after death might be studied in the same muscle. No method was found in which

the muscle could be killed without causing stimulation of the reaction leading to the disappearance of glycogen. A *post mortem* increase of lactic acid, usually out of proportion to any glycogen decrease, was always found under these conditions.

We are indebted to the Medical Research Council for a grant made to one of us (J. J. R. M.) in support of this work.

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