THE IMMEDIATE PRODUCTS OF POST-MORTEM GLYCOGENOLYSIS IN MAMMALIAN MUSCLE AND LIVER.

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In the present investigation evidence is furnished to show that carbohydrate substances which are intermediary between glycogen and lactic acid are formed during post-mortem glycogenolysis in the muscles. It is also shown that this process yields different end products in the muscles as compared with the liver.

That intermediary substances are formed in muscle has already been shown by several investigators, among whom may be mentioned Laqueur(1) and Lohmann(2). Laqueur found that the total yield of lactic acid could exceed that possible from pre-existing glycogen and lactacidogen, and Lohmann has succeeded in identifying as a triose the dialysable, yeast-unfermentable reducing substance which is formed by the action of muscle extract on glycogen. This triose is not, however, further split into lactic acid.

Because of the possibility that formation of such intermediary substances might, in part at least, account for the disappearance of sugar from the organism during the action of insulin, warm blooded animals have been used in the investigation in preference to cold blooded, on which insulin acts very slowly. The first step consisted in finding the conditions under which minimal values for lactic acid could be obtained in mammalian muscle. In the earlier experiments rabbits were used, the animals being killed by stunning. The legs were immediately skinned and pieces of muscle removed as quickly as possible and frozen in liquid air. The time from stunning of the animal to complete freezing of the muscle was less than two minutes. After being ground to a fine powder in an ice-cold iron mortar, samples of the frozen muscle powder were weighed out in previously cooled and tared tubes. An equal volume of 0·9 p.c. sodium chloride solution was then added and the tubes let stand in a water bath at 25° C. Sample tubes were taken every few minutes and the contents precipitated with hydrochloric acid-mercuric chloride

solution. Lactic acid was then determined by the von Fürth-Charnass method, after precipitation with $CuSO_4$ and milk of lime as recommended by Clausen, and in another portion of the solution phosphorus was measured by the Doisy-Bell method. Free sugar was determined in a second portion of the muscle powder by macerating it in 70 p.c. alcohol at -2 to $-3°$ C., and centrifuging, the residue being similarly treated several times until no more reducing substance could be detected in the extract. The supernatant fluids were combined, evaporated to dryness at low temperature, the residue dissolved in water and the solution mixed with a solution of lead acetate and alumina cream until no further precipitation occurred. Excess of lead was then removed by adding a few crystals of potassium oxalate and, after centrifuging, the reducing power of an aliquot portion of the clear supernatant fluid was determined by the Shaffer Hartman method. The control samples were mixed with the reagents immediately after weighing, while the muscle was still in a frozen state.

The controls of the first two rabbits showed "initial" muscle lactic acid contents of 0·388 and 0·353 p.c. respectively, indicating, in view of the low resting lactic acid level of amphibian muscle observed by Fletcher and Hopkins, that considerable post-mortem change had already occurred. In order to reduce this change to a minimum and to find the normal resting lactic acid level for mammalian muscle, experiments were performed in which great pains were taken to excise the muscle as quickly and with as little cutting as possible, and as our technique became better and better, lower and lower lactic acid values were obtained, viz. — 0·239, 0·198, 0·173 p.c.

Evidently the process by which the acid is produced is one that occurs with lightning rapidity immediately after death, and as we had reached the shortest time possible for removal of the muscle it became necessary to look for a method in which the muscle could be removed without previous disturbance of its blood supply. Knowing that the percentage of glycogen remains constant in the muscles of decapitated cats after they have been dissected free from their neighbours without disturbance of their blood supply, it was decided to use such preparations. The gracilis and semitendinosus muscles were chosen, their removal after dissection being very quickly effected by snipping across the insertions. They were then plunged in liquid air, ground to a powder and analysed as described above. By this method we have been able in one observation to obtain resting values for lactic acid as low as 0·024 p.c., so that it seems probable that the normal resting lactic acid in mam-

malian, as in frog muscle, is only a bare trace. In most of our observations the initial lactic acid content varied between 0·050 and 0·090 p.c. Glycogen under these conditions varied between 0·6 and 0·8 p.c., so that even in our best rabbit experiments, in which the muscle was removed and frozen in liquid air within a minute, the process of destruction must already have progressed to a considerable degree. Similar low lactic acid and high glycogen values for resting mammalian muscle have been published by Beatty and Milroy(3). These authors also emphasise the importance of rapidity in technique in order to obtain true results.

The first table shows the relationship between the rates of breakdown of glycogen and the appearance of lactic acid, free sugar and phosphoric acid in excised *rabbit* muscle kept after thawing at a temperature of 25° C.

TABLE I. Post-mortem changes in rabbit muscle.

	Time	Glycogen p.c.	Lactic acid p.c.	Free sugar p.c.	Phosphorus p.c.
I.	Immediate	0·240	0·388	0·134	0·107
	10 min.	—	0·669	0·146	0·114
	15 „	0·024	—	—	—
	20 „	—	0·671	0·145	0·121
	40 „	0·006	0·665	0·150	0·127
	60 „	0·003	0·710	0·163	0·128
II.	Immediate	0·308	0·353	—	0·110
	5 „	0·091	0·488	—	0·104
	10 „	0·053	—	—	—
	20 „	0·012	0·700	—	0·128
	30 „	zero	0·710	—	0·133
	60 „	zero	0·718	—	0·139

The high initial lactic acid, as well as the low initial glycogen, as compared with the corresponding values found when decapitate preparations were used, shows that the breakdown process had already proceeded well on its course by the time the tissue was fixed in the chemical reagents. The subsequent speed of glycogen disappearance was also very great, as is shown by a drop of 200 mgm. in five minutes (Rabbit no. 2).

The change in the percentage of free sugar was very slight and came about slowly and gradually, evidently bearing no relationship to the disappearing glycogen. The increase in free phosphorus was also a slow and steady one and might be considered as due to break-down of hexose phosphate, although in view of the work of Eggleton and Eggleton and of Fiske and Subbarow, this part of the work requires repetition.

In both experiments, as in several others of a similar nature, more lactic acid accumulated than could be accounted for by the glycogen which disappeared, thus confirming Laqueur. This excess of lactic

acid comes in all probability from intermediary substances arising as a first stage in the breakdown of glycogen, a process which must have been proceeding extensively before the "immediate" samples were taken, as evidenced by the low initial values for glycogen and the high ones for lactic acid.

Observations on cat muscle. In these the immediate values for glycogen and lactic acid show, for reasons already given, that post-mortem changes had not proceeded to any great extent by the time the muscle was frozen and ground up. In most of the observations the ground muscle, after mixing with Locke's solution, was kept *in vacuo* at 25° C. for 20 min. In others, the muscle was exposed to air and typical results of both groups of observations are shown in Table II.

TABLE II. Post-mortem changes in cat muscle.

		Initial p.c.	Final (20 mins.) p.c.	Phosphorus increase p.c.	Lactic acid increase p.c.	Glycogen decrease p.c.	Balance mgms.
I. *In vacuo.*							
1.	Lactic acid	0·055	0·420	—	0·365	—	—
	Glycogen	0·533	0·000	—	—	0·533	168
	Phosphorus	0·117	0·132	+0·015	—	—	—
2.	Lactic acid	0·070	0·436	—	0·366	—	—
	Glycogen	0·503	0·048	—	—	0·455	89
	Phosphorus	0·110	0·135	+0·025	—	—	—
3.	Lactic acid	0·065	0·402	—	0·337	—	—
	Glycogen	0·507	0·056	—	—	0·451	117
	Phosphorus	0·111	0·119	+0·008	—	—	—
4.	Lactic acid	0·059	0·378	—	0·319	—	—
	Glycogen	0·520	0·158	—	—	0·362	43
	Phosphorus	0·103	0·114	+0·011	—	—	—
II. In air.							
5.	Lactic acid	0·030	0·160	—	0·130	—	297
	Glycogen	0·804	0·377	—	—	0·427	—
	Phosphorus	0·113	0·104	−0·009	—	—	—
6.	Lactic acid	0·054	0·243	—	0·189	—	190
	Glycogen	0·633	0·254	—	—	0·379	—
	Phosphorus	0·116	0·146	+0·030	—	—	—
7.	Lactic acid	0·054	0·118	—	0·064	—	—
	Glycogen	0·793	0·328	—	—	0·465	461
	Phosphorus	0·115	0·115	0·000	—	—	—
8.	Lactic acid	0·040	0·220	—	0·180	—	350
	Glycogen	0·795	0·265	—	—	0·530	—
	Phosphorus	0·103	0·115	+0·012	—	—	—

Referring first to the observations *in vacuo* it can be seen that, with one exception, the glycogen had very largely disappeared in the 20 min. during which the muscle stood after thawing, but that lactic acid had not increased to a corresponding degree, the extent of the difference

being shown by the figures of the last column. This confirms the results obtained on rabbit muscle in showing that some intermediary substance must be formed. The small, though somewhat irregular increase in phosphorus does not support the view that this intermediate substance may be of the nature of lactacidogen, for if such were the case the free phosphorus would presumably be less, instead of greater, at the end of the observation. Of course it is possible that all available free phosphorus was already used up at the time the muscle was frozen and that, as the hexose phosphate formed from it was subsequently broken down, the liberated inorganic phosphorus was immediately used to form new hexose phosphate. In such a case the balance of free phosphorus at the time the post-mortem process was terminated might be practically the same as in the beginning.

In the observations in air the initial glycogen figures were decidedly higher than those *in vacuo*, but this we believe is merely a coincidence. The amount of glycogen which disappeared in 20 min. was practically the same in air as *in vacuo*, the average of the four experiments of both groups being 0·450 p.c. The increase in lactic acid, on the other hand, was decidedly less marked in the "air" experiments (average 0·347 p.c. "*in vacuo*," as compared with 0·141 p.c. "in air"). When this difference was first noted we thought that it might be of significance as indicating that a certain amount of lactic acid was removed by some oxidative process, but in the light of more recent experiments we are very doubtful of this explanation. In the experiments referred to we have compared the amounts of lactic acid formed in two samples of the same ground muscle placed in tubes so arranged that either air or nitrogen could be bubbled through their contents during the period of incubation. As the following figures will show, Table III, equal quantities of lactic acid were

TABLE III. Post-mortem formation of lactic acid in muscle kept either in oxygen or nitrogen.

	Initial lactic acid p.c.	Final lactic acid: 20 mins. oxygen p.c.	Final lactic acid: 20 mins. nitrogen p.c.	Lactic acid increase: In oxygen p.c.	Lactic acid increase: In nitrogen p.c.
1.	0·080*	0·500	0·475	0·420	0·405
	0·073	0·495	0·490		
2.	0·086	0·315	0·300	0·234	0·214
		0·325			
3.	0·058	0·400	0·395	0·324	0·327
	0·050	0·380	0·380		

* The duplicate analyses are given here to indicate the extent of the experimental error of the methods used.

formed in both tubes. We are at present at a loss to explain the greater formation of lactic acid in the vacuum tubes. During evacuation of these tubes apparently large bubbles of gas were evolved in the mixture. These were collected, but were not found to contain more than traces of CO_2. It is possible that the nitrogen used in the experiments of Table III may not have been strictly free of oxygen, since only the usual chemical procedure was employed to purify it.

The evidence which we have so far given for the presence of an intermediate substance between glycogen and lactic acid has been obtained, in most of the experiments, by comparisons made after glycogenolysis had proceeded almost to completion, and it is open to the criticism that the apparently greater disappearance of glycogen as compared with the appearance of lactic acid was really due to inaccuracies in the measurement of small traces of glycogen and of lactic acid. In the method used for the determination of lactic acid (Charnass-von Fürth) there is known to be an error which may amount to 10 p.c., so that if, in experiment 4 of Table II for example, in which the glycogen disappearance comes closest to the lactic acid formation, we add 10 p.c. to each of the lactic acid figures, the balance (last column) comes out at only 0·011 instead of 0·043. We have not applied this correction throughout, because we cannot be certain that it is constant and moreover, even if we did apply it, the majority of the balances would obviously still show a marked deficit in lactic acid formation as compared with glycogen disappearance.

Since the intermediate substance accumulates much more rapidly than lactic acid is formed from it, it must be present in relatively larger amounts at an early stage of the breakdown process than at later stages, such as those chosen (20 min.) in the above experiments. To demonstrate that this is the case we did several experiments in which the muscle was allowed to stand after thawing for only 5 min., by which time only about half of the original glycogen will have disappeared and possible errors due to estimation of traces of glycogen are eliminated. Typical results are shown in Table IV. In two out of the three experiments recorded more than twice as much glycogen disappeared as compared with the lactic acid which was formed, thus indicating that considerable quantities of the intermediate substance must be present during the earlier stages of glycogenolysis.

We have made no attempts to identify the nature of the intermediary substance. In using dissected cat muscles, as in the foregoing experiments, enough material was not available so that determinations of glycogen and lactic acid could be made at a sufficient number of intervals

TABLE IV. Post-mortem changes in cat muscle after standing five minutes at 25° C.

		Initial p.c.	Final (5 mins.) p.c.	Lactic acid increase p.c.	Glycogen decrease p.c.	Balance p.c.
1.	Glycogen	0·950*	0·535		0·440	
		1·000	0·535			
						0·205
	Lactic acid	0·099	0·330	0·235		
		0·090				
2.	Glycogen	0·860	0·465		0·398	
		0·850	0·450			
				0·166		0·232
	Lactic acid	0·050	0·225			
		0·068				
3.	Glycogen	0·680	0·325		0·380	
		0·695	0·290			
						0·209
	Lactic acid	0·082	0·252	0·171		
		0·089	0·262			

* Duplicates.

to permit of accurate time curves of the two processes being compared. We have already published a typical curve for the process in rabbit muscle[1], but since glycogenolysis must have been well on its way before the first estimations were made, little can be learned from the curve with regard to the possible accumulation of the intermediary substance. Neither have we as yet any data to show how closely lactic acid formation may come to glycogen disappearance in mammalian muscle when the post-mortem process is allowed to proceed for longer periods of time than 20 min.

Glycogenolysis in the liver. It is well known that sugar accumulates as glycogen disappears when liver is allowed to stand after death, but the methods for estimation of the sugar have been too inaccurate to make it possible to say whether the two processes run proportionately. In perfused liver it has been found by Seegen(4), Embden(5) and others that more sugar appears in the perfusate than can be accounted for by the glycogen which meanwhile disappears, and Burn and Marks(6) have recently given evidence which shows that the source of this extra sugar may be fat. There can be no doubt that the physiological significance of glycogen is fundamentally a different one in muscle and liver and it seemed of interest to determine, by methods similar to those used for muscle, whether accumulation of sugar and lactic acid during post-mortem glycogenolysis in the liver proceeded differently. This has been carried out for the liver of both the rabbit and the cat, and typical results are shown in Table V. The amounts of glycogen which disap-

[1] *Proc. Soc. exp. Biol. and Med.* 23. p. 659, 1926. The values for lactic acid as given in the curve in the preliminary communication should have been multiplied by 10.

TABLE V. The behaviour of sugar, lactic acid and phosphoric acid during post-mortem glycogenolysis in the liver.

Animal		Immediate	Percentages in minutes after thawing liver powder: 20	30	60	90	115–120	180
Rabbit 1	Glycogen	4·60	—	3·65	—	2·53	—	1·59
	Lactic acid	0·02	—	0·02	—	0·02	—	0·01
	Free sugar	0·12	—	1·04	—	1·72	—	—
Rabbit 2	Glycogen	1·52	—	0·96	—	—	0·71	0·47
	Free sugar	0·11	—	0·44	—	—	0·57	0·93
Rabbit 3	Lactic acid*	0·02	—	0·02	—	0·02	0·02	0·02
Rabbit 4	Lactic acid*	0·03	—	0·01	—	—	0·02	0·01
Rabbit 5	Lactic acid	0·07	0·05	0·05	0·05	—	—	—
	Free sugar	0·26	0·67	0·84	1·13	—	—	—
Cat	Lactic acid	0·04	—	—	0·05	—	0·04	0·03
	Phosphorus	0·04	—	—	0·05	—	0·06	0·06

* The liver stood *in vacuo*.

peared per 100 grm. of liver in the first 30 min. was 0·95 grm. and 0·56 grm. respectively in the two experiments in which it was measured, the rate of disappearance becoming progressively less during each of the subsequent periods. Studies of the exact rate of this process at later stages have already been published by one of us (J. J. R. M.)(7). The absolute amount of glycogen which disappeared in unit time was greater than in muscle, but in striking contrast to muscle, free sugar increased rapidly whereas lactic acid remained practically unchanged. In No. 1, for example, 2·07 grm. of glycogen per 100 grm. of liver disappeared in 90 min. but only 1·60 grm. of sugar accumulated; in No. 2, 1·05 grm. glycogen disappeared and 0·82 grm. sugar appeared. It is possible that much closer agreement between glycogen disappearance and sugar accumulation would be obtained if a more certain method could be devised for determination of the free sugar in tissue. The method employed (see p. 256) is probably suitable enough for comparative studies of the behaviour of the sugar itself, but it is probably not so for the measurement of absolute amounts of sugar.

The results as they stand are quite sufficient to show, in so far as can be judged from the immediate post-mortem process, that the fate of glycogen in the liver is an entirely different one from that in muscle. Once it has become deposited in muscle, glycogen may be considered to have entered into an irreversible reaction, in that its only breakdown pathway is by way of lactic acid; and it is impossible for it again to form glucose unless in cases in which the oxygen supply is inadequate for its oxidative synthesis into glycogen, when some will enter the blood and

be re-formed into glycogen (glucose) in the liver. In support of this view may be cited the fact, first observed by Mann and Magath and confirmed by Soskin, that the blood sugar does not become increased when epinephrin is injected into animals deprived of the liver, or when such animals are asphyxiated, even although abundance of glycogen is still present in the muscles.

THE EFFECT OF INSULIN ON THE FREE SUGAR OF THE MUSCLES AND LIVER.

Although of a somewhat different nature we wish to include in this paper certain observations which we have made pertaining to the behaviour of free sugar in the liver and muscles following the injection of insulin. Observations of a similar nature have already been published both from this laboratory and by Cori and Cori(8), but certain objections can be raised to the results.

The standard white rat was the animal chosen in the present observations. After fasting for 24 hours it was decapitated and the liver and muscles immediately excised and frozen in liquid air. The hard frozen tissue was then ground to a dust-fine powder, of which weighed quantities were thoroughly shaken with ice cold alcohol (70 p.c.) and then rapidly centrifuged. These extractions were repeated until no reducing substance could be detected in the evaporated alcoholic extract. The reducing power of a solution of the residue of the combined alcoholic extracts was found to be very similar for different rats (about 0·540 p.c.) and was very close to the value reported as free sugar by Cori and Cori. But of course this cannot all be free sugar, for if it were so then the liver cell would contain more than five times as much free sugar as the blood, indeed much more than this if we allow for the blood present in the liver. After trial of various methods for the removal of the interfering substances (tungstic acid, phosphotungstic acid, etc.) we finally decided to use neutral lead acetate and alumina cream, the actual proportions used being as follows: the final residue from 2 grm. of liver powder was dissolved in 6 c.c. of water in a centrifuge tube and after centrifuging 2 c.c. were pipetted off and mixed in another centrifuge tube with 0·2 c.c. of a saturated solution of neutral lead acetate and 0·4 c.c. alumina cream. After standing, a drop of the lead acetate solution was added to make certain that precipitation was complete. Excess of lead was then removed from the solution by shaking with a trace of potassium oxalate, the volume was made up to 5 c.c., centrifuged and 2 c.c. of the supernatant fluid used for sugar determination. It will be seen from

Table VI that a large amount of reducing substance is removed from the alcoholic extracts by the above treatment, and we cannot be certain that

TABLE VI. Free sugar in liver of normal starved rats.

	Direct determination		After lead acetate and alumina cream	
Date	1st extract	Total	1st extract	Total
Nov. 6	0·272	0·530	0·048	0·085
	0·273	0·549	0·046	0·077
Nov. 12	0·266	0·533	0·089	0·131
	0·275	0·579	0·074	0·084
Nov. 17	0·279	0·584	0·066	0·072
	0·248	0·581	0·060	0·065
Nov. 19	0·244	0·460	0·089	0·138
	0·247	0·475	0·066	0·076
Averages:	0·263	0·538	0·067	0·079

All animals starved for a period of 18–24 hours previous to experiment.

all of the reducing substance which remains is glucose. Although, as certain of the duplicates show, this method cannot be considered as quantitative our results are much more likely to represent the true glucose concentration of the liver than are the much higher ones of other workers, since they correspond much more closely to the values for blood sugar. Thus, the average percentage of glucose in the blood of a rat starved 24 hours may be taken as 0·100 and that of our liver observations is 0·067. Actually the percentage in the liver cells must be considerably less than this, since much blood is contained in the viscus when it is frozen. It is impossible to calculate what the actual percentage in the liver cell may be, because we do not know the amount of blood. The reagents used do not precipitate any glucose, as can readily be shown by adding a measured quantity to the solution of the alcoholic extract and then precipitating with lead acetate and alumina cream when all the added glucose is recovered.

When insulin was injected into the rats in amounts sufficient to bring the blood sugar to about the convulsive level, Table VII, the free liver sugar was markedly reduced although very little change could be detected in the reducing power of the extract before treating it with lead acetate. That insulin reduces the free sugar of the liver has already been observed by Cori and Cori, the method of extraction being one, however, in which there was probably some hydrolysis of glycogen (extraction with ice-cold 2 p.c. HCl and subsequent treatment of the extract with mercuric chloride). Insulin therefore reduces both the free sugar and the glycogen of the liver when it is given to fasted rats

TABLE VII. Free sugar in liver of starved insulin-treated rats.

Date	Direct determination: 1st extract	Direct determination: Total	After lead acetate and alumina cream: 1st extract	After lead acetate and alumina cream: Total	Blood sugar p.c.	Remarks
Nov. 24	0·255	0·540	0·039	0·039	—	Starved 24 hrs.
	0·255	0·585	0·039	0·038	—	½ unit insulin.
Nov. 26	0·264	0·487	0·010	0·010	0·040	Starved 24 hrs.
	0·263	0·508	0·011	0·011	—	1 unit insulin.
Dec. 1	0·294	0·517	0·010	0·010	0·030	Starved 24 hrs.
	0·276	0·512	0·010	0·010	—	1 unit insulin.
	—	—	—	0·049	0·070	Starved 24 hrs. 1 unit insulin.
	—	—	0·055	0·055	—	Animal recovering.
Averages:	—	0·524	0·031	0·031	—	—

Note. All animals starved for a period of 18–24 hrs. previous to experiment.

(cf. Barbour, Chaikoff, Macleod and Orr) (9), and it is possible that the mechanism by which it does so is inhibition of gluconeogenesis, although it is at present impossible to obtain direct evidence that this occurs in normal animals. It is perhaps significant in this connection that the greatest reduction in free sugar occurred in the two rats of our series in which the blood sugar became most markedly depressed.

The results for muscle, using exactly the same methods as for liver, are also shown in Table VIII, and it can be seen, in confirmation again of

TABLE VIII. Free sugar in rat muscle.

Date	Total Direct	Total after lead	Blood sugar p.c.	Remarks
Jan. 7	0·206*	0·045	—	Rat starved 24 hrs.
	0·199	0·038	—	
Jan. 12	0·245	0·051	—	Rat starved 24 hrs.
	0·260	0·052	—	
Jan. 14	0·250	0·051	—	Rat starved 24 hrs.
	0·238	0·030	—	
Jan. 19	0·190	0·055	0·065	Rat starved 24 hrs.
	0·230	0·055	—	1 unit insulin, but no hypoglycaemic symptoms.
Jan. 21	0·415	0·039	0·055	2 unit insulin, definite
	0·420	0·048	—	hypoglycaemic symptoms.
Jan. 26	—	0·042	0·058	2 unit insulin, definite
	—	0·039	—	hypoglycaemic symptoms.

* Duplicates.

Cori and Cori, that insulin does not cause the free sugar to become lowered. These workers used for the muscles a somewhat different method for determination of the free sugar from that which they used

for the liver (boiling water extraction and removal of interfering substances with colloidal iron and Lloyd's alkaloidal reagent) and their average results are higher than ours (0·079 p.c. as compared with 0·045 p.c.). The failure of insulin to lower the concentration of free sugar of muscle is very difficult to explain, but it is significant that the result harmonises with the absence of any change in that of glycogen, under similar conditions. It has been customary to assume that glucose disappears from the blood following insulin because of its more rapid diffusion into tissues (muscles) in which its concentration has become lowered (glucatonia). The results of both Cori and Cori and ourselves do not lend support to this view.

Conclusions.

1. In mammalian muscle which is frozen and ground up in liquid air immediately after its removal from a living animal (decapitated cat) and then allowed to stand at room temperature, glycogen almost entirely disappears within 20–30 min.

2. Lactic acid does not appear at the same rate as glycogen disappears, indicating the presence of intermediary products.

3. There is only a slight increase in free sugar and inorganic phosphorus while glycogenolysis is proceeding.

4. Entirely different results are obtained when the liver is studied by the same methods, for in this case free sugar accumulates almost in proportion as glycogen disappears, and lactic acid does not increase.

5. Insulin causes the free sugar of the liver to be decreased but has no effect on that of the muscles.

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