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THE GLYCOGEN CONTENT OF THE RAT HEART.

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THE function of glycogen in the heart muscle is at present unknown. It is true that under appropriate conditions, glycogen disappears and lactic acid appears in heart muscle [Schenk, 1924; Katz and Long, 1925; Redfield and Medearis, 1926] and that its disappearance during the activity of isolated or heart-lung preparations has been observed [Cruickshank, 1913; Witting, Markowitz and Mann, 1930; McPherson, Essex and Mann, 1931–2; Cruickshank and Shrivastava, 1930]. These observations, superficially at least, would point to its rôle being similar in heart and in skeletal muscle. Other observations give a different indication. Wertheimer [1930], for instance, found for aerobically contracting frog hearts and for ventricular strips that activity did not decrease glycogen nor increase lactic acid. Martini [1931], Wertheimer [1931], Goldenberg and Rothberger [1931] and Clark, Eggleton and Eggleton [1932], in observing hearts poisoned with iodoacetic acid in which no lactic acid is formed, remark that the hearts function well for long periods if oxygen is not excluded. Visscher [1928] and Visscher and Mulder [1930] using heart-lung preparations found that unworked hearts contained 560 mg./100 g. of glycogen, and that hearts after as much as 6 hours' work contained 516 mg./100 g., although in some instances the sugar content of the heart muscle had fallen to zero. They with others [Clark, Gaddie and Stewart, 1929–30, 1931, 1932; Witting, Markowitz and Mann, 1930] have been impressed that when glycogen breakdown did occur it was small when referred to the total energy requirements of the activity. That the aerobically contracting heart takes up lactic acid has been attested by Himwich, Koskoff and Nahum [1928], McGinty and Miller [1932, 1933] and Lovatt Evans *et al.* [1933].

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There is in the literature, however, general agreement that glycogen breaks down and lactic acid appears when oxygen supply to the heart is deficient. Indeed, the newer knowledge of the chemistry of muscular contraction which places carbohydrate breakdown in a place of secondary importance as regards the immediate supply of energy for a single contraction has pointed out the importance of carbohydrate breakdown for continued activity of muscle particularly when oxygen is lacking.

These and other observations have indicated that a study of cardiac glycogen is not without interest, and apart from its relation to cardiac activity might throw some light on the rôle of this substance in skeletal muscle and on carbohydrate in the body generally.

As an approach to the study of cardiac glycogen, and as a foundation for other work now in hand it was decided to determine the level of cardiac glycogen of intact animals (1) under standardized normal conditions, and (2) after subjecting the animals to procedures likely to alter glycogen levels.

This study, the heart being an unpaired organ, must be done by comparison from animal to animal. Wertheimer [1930], and Clark, Gaddie and Stewart [1931] have found large variation in the glycogen content of frogs' hearts without obvious cause. In this laboratory eleven rabbit hearts analysed for glycogen averaged 421 ± 41 mg./100 g., the range being from 267 to 757 mg./100 g.; fifteen cat hearts showed similar irregularity. A perusal of most figures in the literature reveals a like variation.

Obviously, if no better control values than these could be obtained, the search for the factors governing cardiac glycogen would be a difficult if not a hopeless task.

In the hope that this initial difficulty might be obviated the albino rat was chosen as the most convenient standardized animal. In these animals Cori and Cori [1927] have found the glycogen content of gastrocnemii to be quite constant, and they with others have furnished many data on the carbohydrate metabolism of these animals in reference to which the determination of carbohydrate in the heart becomes the more significant.

Method.

Animals were albino rats of well inbred stock of from 120 to 200 g. both sexes being used indiscriminately. They were maintained in good health on a well-standardized diet. Unless otherwise mentioned all animals had fasted 24 hours before being used.

Anæsthesia by sodium amytal (10 mg./100 g.) was used when obtaining samples. The hearts and muscles were removed at a depth of anæthesia uniform from animal to animal.

Removal of gastrocnemius. The essential point in the technique is that the blood supply to the muscle is not interfered with before the muscle is actually removed.

Removal of heart. The rat having been tied with its back to the board a "buttonhole" cut was made through the skin and abdominal muscles in the mid-line immediately below the xiphisternum. The xiphisternum being held by forceps two diverging longitudinal cuts were made through the thoracic wall and skin, the diaphragm consequently being cut in two places. On lifting this chest flap a piece of diaphragm remains attached, and the heart is drawn forward slightly by its attachments to the anterior mediastinum. One cut suffices to sever the strip of diaphragm and these attachments, and the disposition of the parietal pericardium is such that with the same cut the sac is opened and the heart being held lightly with a pair of toothed dissecting forceps was removed at its base with one snip. The auricles were not cut through but remained intact with the ventricles. The whole operation takes from 3 to 5 sec. The essential of the method, as will be shown, is speed.

Glycogen determination. The method used is a modification of the cold KOH method of Cori [1932]. The heart or gastrocnemius immediately on removal was dropped into a tared 50 c.c. graduated pyrex centrifuge tube containing 2 c.c. of cold 30 p.c. KOH, which was then reweighed. The tube was heated with shaking over a small flame until the tissue was digested. Water was then added to bring the volume of fluid to 6 c.c. The tube was then reheated to boiling so that the sides of the tube were washed down. It was then placed on a steam bath for half an hour. On removal, water was added to bring the volume to 10 c.c.; 20 c.c. of absolute alcohol were then added and the whole stirred by aeration. The tube was covered and let stand overnight. In the morning it was centrifuged and the liquid decanted and discarded. A drop of phenol red was added and then 11 c.c. of 2·5 p.c. HCl blown in from a pipette so as to break up the button of glycogen in the bottom of the tube. Hydrolysis was carried on for 4 hours on the steam bath, the tubes being covered to prevent evaporation. At the end of this time the hydrolysate was neutralized to the phenol red already in the tube and diluted with water to suitable volume. A 5 c.c. aliquot was taken and analysed for glucose by Somogyi's modification [1926] of the Shaffer-Hartman method. Glycogen is expressed as glucose in mg./100 g. of tissue. The deviation

expressed is the standard error of the mean. As the amount of non-fermentable reducing substance in the final hydrolysate was found to be small and relatively constant, no correction was made for it.

Animals fasted 24 hours (controls).

Preliminary determinations leading to the establishment of satisfactory control values were as follows: (*a*) animals were decapitated at one stroke, and with as little delay as possible tied down, and the hearts removed according to the technique described. The hearts of twenty-two animals so treated were found to average 342 ± 19 mg./100 g. of glycogen. The extreme values were 184 and 477; the standard deviation of the series was 85. Age, weight, sex and amount of struggling seemed to bear no relation to high or low glycogen values. It was thought, however, when the greatest delay occurred between decapitation and the securing of the heart, that the values were low. Consequently (*b*) animals were first tied down, then decapitated and the heart straightway removed. Three such animals gave values of 474, 520 and 541 mg./100 g. It was supposed then that anoxæmia may have been the cause of the previous low values. To test this (*c*) seven animals were asphyxiated with coal gas and the hearts promptly removed; the average content of glycogen was found to be 25 mg./100 g., the highest value being 61 mg./100 g. (*d*) With the idea of avoiding terminal anoxæmia, and so that gastrocnemii might be removed before securing the hearts amytal anæsthesia was next tried. Fourteen hearts thus secured averaged 488 ± 15 mg./100 g.; the gastrocnemii from the same animals contained 424 ± 11 mg./100 g. of glycogen. The extreme values for hearts were 407 and 630; the standard deviation of the series was 54 and therefore showed an increased regularity as compared to the first series of twenty animals. (*e*) Six hearts were removed by the usual technique except that 30 sec. was allowed to elapse between the opening of the chest and the removal of the heart. The mean value for these hearts was 287 mg./100 g. The low values obtained in these animals emphasized the necessity of speed in the removal of the heart.

Method adopted. Using the method of (*d*) above and with particular care to avoid delay in removing the hearts, twenty hearts were found to contain 508 ± 12 mg./100 g.; the glycogen content of the gastrocnemii from these animals was 479 ± 8 mg./100 g. The extreme values for the hearts were 444 and 675, and the standard deviation of the series was ± 54 mg. These were the most satisfactory values yet obtained and this was the method adopted. An additional thirty-two animals were done

from time to time; grouped with the above twenty they averaged for hearts 497 ± 8 and for gastrocnemii 525 ± 7 mg./100 g. These last given values have been used as controls on the work which follows.

Fasting and feeding. Determinations were made of the glycogen in heart and gastrocnemius of ten rats that had not fasted and of ten that had fasted 48 hours. Table I shows the results. The increase of cardiac

TABLE I. Effect of fasting and feeding.

	No. of animals	Glycogen mg./100 g.	
		Heart	Gastrocn.
48-hour fasted	10	578 ± 14	455 ± 9
24-hour fasted (controls)	52	497 ± 8	525 ± 7
24-hour fasted + maximum glucose absorption for 4 hours	17	449 ± 11	690 ± 11
Fed animals	10	341 ± 15	574 ± 20

glycogen with fasting contrasts with the decrease of this substance in gastrocnemius. The glycogen of hearts of animals that had not fasted is 69 p.c. of the control value; similar determinations in two other colonies of rats have given values of 75 and 61 p.c. for this ratio.

Glucose in 50 p.c. solution was given by stomach tube to seventeen rats, each animal receiving 1 g. of glucose for each 100 g. of body weight; according to Cori [1925] this is in excess of the amount necessary to provide for maximum absorption for 4 hours. At the end of 4 hours the animals were anæsthetized and the hearts and gastrocnemii taken. The results of the analyses are shown in Table I. The increase in glycogen in the gastrocnemii was marked, but the value for hearts is below the control level.

In addition, two fed animals were given like amounts of glucose every 4 hours and taken at the end of 11 hours. The glycogen values in these animals were for hearts 384 mg./100 g. and for gastrocnemii 892 mg./100 g., showing again that the glucose although increasing glycogen in skeletal muscle does not alter cardiac glycogen significantly.

Insulin. Animals were given insulin subcutaneously in amounts varying between 8 and 15 units per 100 g. of body weight and the hearts, gastrocnemii and blood obtained at the end of from 3 to 11 hours; for the longer periods the dose of insulin was repeated. At the same time as the insulin was given glucose in 50 p.c. solution was given by stomach tube in excess of the total quantity capable of being absorbed during the period of observation as calculated from the absorption values of Cori [1925]. Of the twenty-nine animals receiving insulin thirteen showed the hypoglycæmic signs of weakness and prostration and were taken for determina-

tion of glycogen and blood sugar at the end of the hourly period in which they showed such signs. The blood sugar in none of these was found to be over 50 mg./100 c.c. The other sixteen showed no hypoglycæmic signs and the lowest terminal blood sugar was 62 mg./100 c.c.

The results shown in Table II indicate that insulin favoured the deposition of cardiac glycogen in the sixteen animals showing no hypoglycæmic signs, but that the hypoglycæmic animals had low cardiac

TABLE II. Effect of glucose and insulin. Insulin subcutaneously. Glucose by mouth in excess.

	No. of animals	Glycogen mg./100 g.		Blood sugar mg./100 c.c.	
		Heart	Gastrocn.	Mean	Range
Animals showing hypoglycæmic signs	13	319	793	45	40–48
No hypoglycæmic signs	16	611	790	112	62–167

glycogen. In both groups of animals the glycogen of gastrocnemii is much increased over the control value showing that relative to the heart the gastrocnemius glycogen was but little affected by hypoglycæmia, and this in spite of the fact that six of the thirteen hypoglycæmic animals exhibited slight tremors.

The values for cardiac glycogen were quite irregular; of animals receiving a uniform dose of insulin, some developed hypoglycæmia and others showed no signs of it. It was felt that the irregularities in absorption of both insulin and glucose were probably the cause of the irregular results, and further investigation of the effect of insulin is therefore being done by means of intravenous perfusion.

Adrenaline. Rats were injected subcutaneously with 0·02 mg. of adrenaline per 100 g. body weight; this was given in 1/40,000 solution. The hearts and gastrocnemii were taken ½, 3 and 7 hours later. A subcutaneous dose of 0·25 mg. and a slowly injected intravenous dose of 0·001 mg./100 g. body weight were also tried. The results as given in Table III indicate that cardiac glycogen unlike glycogen of skeletal muscles is not lowered by adrenaline.

TABLE III. Effect of adrenaline.

Adrenaline mg./100 g. body weight	No. of animals	Time after injection hours	Glycogen mg./100 g.	
			Heart	Gastrocn.
0·02 subcut.	4	½	475	332
0·02 ,,	16	3	509 ± 12	239 ± 9
0·02 ,,	7	7	549 ± 13	299 ± 24
0·25 ,,	2	2	593	152
0·001 intrav.	3	½	475	—

Exercise. (*a*) Three rats were allowed to swim in a tank of water until at the end of about half an hour they became exhausted. The hearts and gastrocnemii were then removed.

(*b*) Under anæsthesia one electrode was placed under the skin of the back over the lumbar vertebræ and the other applied to the tail. Electrical stimuli from the secondary of an induction coil at the rate of 60 per minute were applied for 10 min., at the end of which time the hearts and gastrocnemii were removed. The whole body could not be exercised in this way because it interfered with efficient breathing by the animal and produced cyanosis, and, as will be recorded later, anoxæmia rapidly lowers cardiac glycogen.

Table IV records the glycogen values found in these three naturally

TABLE IV. Effect of exercise.

	Glycogen mg./100 g.	
	Heart	Gastrocn.
Swimming	537	393
	511	329
	590	357
Electrical stimulation	531	379
	513	413
	492	351
Average	529	371

and three artificially exercised animals. These experiments, like those with adrenaline, were done as a means of determining in the intact animal the effect of increased work or increased contraction rate on the glycogen content of the heart. Here, with exercise, as previously with adrenaline, no decrease in cardiac glycogen was found despite the marked lowering of the glycogen in gastrocnemii.

Acid-base change of the blood. In some of the experiments here presented and in others contemplated the $H_2CO_3/NaHCO_3$ and other buffer ratios in the blood are altered. To discover whether such changes might have a marked effect upon cardiac glycogen, two groups of experiments were done. In one, animals were placed for 3 hours in a glass chamber through which streamed a gas mixture containing 92 p.c. O_2 and 8 p.c. CO_2. To each of another group 5 c.c. of a 4 p.c. solution of $NaHCO_3$ were given by stomach tube, and the hearts and gastrocnemii removed 4 hours later. The change in *p*H of the blood as a result of these procedures, calculated from solubility values for CO_2 and from the Henderson-Hasselbalch equation is in excess of 0·1 of a *p*H unit on either side of normal. The results as given in Table V show that the glycogen in

TABLE V. Effect of acid-base change.

	No. of animals	Glycogen mg./100 g. Heart	Glycogen mg./100 g. Gastrocn.	Plasma CO_2 capacity vol. p.c.
Breathing 8 p.c. CO_2 for 3 hours	8	458±8	532±9	—
$NaHCO_3$ by mouth	6	466±10	517±14	83±2
Controls	6	—	—	58±1

gastrocnemii remained unaltered and that the cardiac glycogen was only slightly reduced by each of the procedures.

Anoxæmia. (*a*) Four rats were anæsthetized with amytal and one gastrocnemius removed from each; the trachea was then clamped and 2 min. later the other gastrocnemius and the heart removed. The average reduction in the glycogen of gastrocnemii was only 50 mg./100 g., whereas the cardiac glycogen was lowered to 83 mg./100 g.

As already mentioned, seven animals asphyxiated in coal gas were found to have but 25 mg./100 g. of glycogen in the heart.

These results furnish adequate proof of the extreme and rapid lowering of cardiac glycogen during anoxæmia.

(*b*) Lesser degrees of oxygen want were tried. Animals were placed in a glass chamber through which was passed a mixture of nitrogen and oxygen; the oxygen varied in different groups of experiments from 12·1 to 5·3 p.c. Care was taken that the temperature and humidity of the air in the chamber were approximately that of the room air. The current of gas was such that the CO_2 content of the outflowing gas did not exceed 0·1 p.c., nor the oxygen content fall by more than that amount. All the gas mixtures caused the animals to be hypernœic; cyanosis was obvious in the lowest oxygen tensions. At the end of 3 hours the animals were removed from the chamber, promptly anæsthetized and the hearts and gastrocnemii taken. The animals were moderately prostrated in the

TABLE VI. Effect of low oxygen tension in inspired air.

Inspired air for 3 hours oxygen p.c.	No. of animals	Glycogen mg./100 g. Heart	Glycogen mg./100 g. Gastrocn.
12·1–9·2	11	507±23	541±18
7·4	4	440	508
6·2	7	339±30	520±33
5·8	8	221±15	497±18
5·3	4	218	532

lowest oxygen tensions but recovered promptly on removal, and were active. Table VI shows the glycogen values for these animals. The unchanged level of glycogen in gastrocnemii contrasts with the marked fall

in cardiac glycogen, which begins to be evident at 7·4 p.c. oxygen and is well marked at 6·2 and 5·8 p.c.

(*c*) To determine approximately how low cardiac glycogen can fall before heart failure occurs, animals were placed in the chamber in groups of four and subjected to atmospheres containing 4–5 p.c. of oxygen. When two of the four had died, the other two were taken. Eleven hearts of animals surviving in this manner for an average of 2¼ hours contained 192 ± 17 mg./100 g. of glycogen. It is to be noted that this value is not much lower than that for animals placed in 5·8 p.c. O_2 for 3 hours. The hearts of the animals which did not survive were also taken, but taken while respirations still persisted. This was possible because of the premonitory signs of cardiac failure, viz. (1) unconsciousness, (2) suddenly deepening cyanosis, and (3) gasping respiratory movements. If this succession of events was watched it was possible to remove the animals from the chamber at the onset of the gasping, and to have secured the heart before respiration ceased. On opening the chests of these animals the hearts were found to be dilated widely, the ventricles not contracting and the lungs pale. The hearts of ten such animals contained 85 ± 4 mg./100 g. of glycogen. It will be recalled that this is approximately the same value as was obtained 2 min. after clamping the trachea. This value for non-survivors is approximately 100 mg./100 g. lower than that for survivors. However, three of the values obtained for survivors, that is to say for hearts which were still contracting forcibly, were quite low, viz. 100, 102 and 128 mg./100 g. It is not meant to imply a causal relation between low glycogen and cardiac failure, but if a certain level of glycogen is necessary for efficient contraction of the anoxæmic rat heart it does not lie much above 100 mg./100 g. Experiments now in hand indicate, that if the blood is kept on the alkaline side of normal the glycogen of anoxæmic hearts may fall much lower before arrest occurs.

(*d*) The average cardiac glycogen of four animals after 1 hour in 4 p.c. oxygen was 312 mg./100 g.; this, compared to the values for 3 hours in 5·8 p.c. oxygen, and for 2¼ hours in 4–5 p.c. oxygen, suggests that the reduction of cardiac glycogen during exposure to low oxygen tension does not occur suddenly but is progressive with time.

Recovery of cardiac glycogen. A series of animals were placed in 5·8 p.c. oxygen for 3 hours, then removed to room air for varying intervals at the end of which the hearts and gastrocnemii were taken; Table VII gives the results and shows that within 1 hour the recovery of cardiac glycogen is practically complete, the greater part of it occurring within three-quarters of an hour and slowly thereafter.

TABLE VII. Recovery of cardiac glycogen after 3 hours in 5·8 p.c. oxygen.

Recovery period hours	No. of animals	Glycogen mg./100 g.	
		Heart	Gastrocn.
0	8	221 ± 15	497 ± 18
¼	5	400 ± 8	511 ± 16
1	4	485 ± 6	494 ± 5
1½	4	484 ± 12	540 ± 5
3	4	547 ± 6	560 ± 20

DISCUSSION.

It is considered important that satisfactorily constant values have been found for cardiac glycogen in animals used as controls, the variation being of the same order as that found for glycogen in gastrocnemii. The absence of such regularity in controls has lessened the significance of many values recorded in the literature. Rapid removal of the heart, and the avoidance of anoxæmia before removal are thought to be necessary points in the technique. Lawrence and McCance [1931], however, have recorded a mean glycogen value of 570 mg./100 g. for rat hearts removed after stunning. In this connection it should be stated that subsequent work in other laboratories with other colonies of rats has yielded both higher and lower values for cardiac glycogen, but each series had no higher standard deviation than the present one.

The low cardiac glycogen of fed animals as compared to 24-hour fasted animals has been a repeated finding. No explanation is offered. That it may be due to the specific dynamic action of the protein in the diet was not borne out by experiments in which raw meat was fed to 24-hour fasted animals. Lawrence and McCance did not find such a difference between fed and fasted animals. They did, however, find in agreement with the present results that after 48 hours' fasting cardiac glycogen was well maintained.

The findings in the intact animal (1) that glucose feeding to previously fasted animals does not increase cardiac glycogen, but (2) that when insulin is also given the cardiac glycogen is increased unless (3) hypoglycæmia occurs in which case cardiac glycogen is lowered, are in agreement with those of Cruickshank and Startup [1933] who show in the heart-lung preparation that hyperglycæmia increases cardiac glycogen but little unless insulin is also given, and that in hypoglycæmia (below 50 mg./100 g.) cardiac glycogen is drawn upon. Fisher and Lackey [1925] also have recorded lowering of cardiac glycogen in insulin hypoglycæmia. On the contrary, Dudley and Marrian [1923] found that

insulin hypoglycæmia, although producing definite glycogenolysis in liver or skeletal muscle, increased the glycogen of the heart.

The experimental results with adrenaline fit in well with observations recorded in the literature. Junkersdorf and Hanisch [1927] find cardiac glycogen increased after adrenaline. Lawrence and McCance [1931] find it unaltered. Geiger and Schmidt [1928] found that adrenaline, although decreasing the already low skeletal muscle glycogen, raised the already high cardiac glycogen of phlorrhizinized dogs. It is not surprising then to find that exercise does not lower cardiac glycogen.

There is general agreement in the literature that oxygen lack causes a marked breakdown of carbohydrate in the heart. This finding has been made by Clark, Gaddie and Stewart [1932], Visscher and Mulder [1930], McGinty [1931], McGinty and Miller [1932], Schenk [1924], and Wertheimer [1930, 1932].

The currently accepted theory of the chemistry of muscular contraction indicates that carbohydrate breakdown is of importance to continued anaerobic activity [Hill, 1932]. Recent investigations have borne this out for cold-blooded hearts [Clark, Eggleton and Eggleton, 1932; Clark, Gaddie and Stewart, 1932].

The present experiments on cardiac glycogen in anoxæmia merely establish the fall that occurs under standardized conditions and serve as a basis for further work aiming to discover if increased stores of glycogen, by providing for greater breakdown, are capable of prolonging the activity of anoxæmic mammalian hearts.

If glycogen does not disappear from aerobically acting hearts, as indicated by work already referred to in the introduction, as well as by a number of experiments here recorded, three possibilities are open. (1) It may not be broken down during ordinary activity but be reserved for anoxæmic emergencies. Favouring this view is the finding that hearts poisoned with iodoacetic acid continue to function well so long as oxygen is not excluded. (2) Glycogen may be broken down with each systole, but during diastole (an imposed periodic "recovery period") be quickly resynthesized. (3) The heart may be provided from without by carbohydrate to form glycogen as rapidly as it is used. Against this explanation is the fact that glycogen has been found relatively undiminished during activity of isolated preparations.

That the heart can replace rapidly glycogen lost during anoxæmia has been shown in the present experiments. It is interesting to note that not only in these experiments on recovery from anoxæmia but also in the experiments with adrenaline and exercise, that there was a tendency to

slightly higher than normal glycogen values. In all three of these conditions blood lactic acid is raised and the possibility presents itself that this lactic acid contributes to the recovery of cardiac glycogen, it having already been shown that the heart is capable of taking up considerable quantities of lactic acid [McGinty and Miller, 1932, 1933; Lovatt Evans *et al.*, 1933].

SUMMARY.

A method is outlined for obtaining satisfactorily uniform values for cardiac glycogen in the albino rat fasting for 24 hours.

Fed rats have less cardiac glycogen than fasting rats. There is no decrease in cardiac glycogen between the 24th and 48th hour of fasting.

Feeding glucose does not increase cardiac glycogen. If insulin is injected at the same time as glucose is fed, cardiac glycogen is increased unless marked hypoglycæmia occurs, in which case cardiac glycogen is lowered.

Cardiac glycogen in the intact fasting rat has been found essentially unchanged by exercise, the injection of adrenaline, and by changes in the acid-base reaction of the blood.

Cardiac glycogen is rapidly lowered by anoxæmia but is quickly restored to its original level when anoxæmia is relieved.

The suggestion is offered that cardiac glycogen is not decreased in ordinary aerobic activity but is reserved for anoxæmic emergencies.

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