GLYCOGEN AND PHOSPHORYLASE DISTRIBUTION THROUGHOUT THE WALLS OF THE HEART AND CONDUCTION SYSTEM

LILLIAN JEDEIKIN

Dept. of Physiology, Albert Einstein College of Medicine, Yeshiva University, New York, N. Y., U.S.A.

WHILE sampling the perfused rabbit heart for glycogen studies, a large variable in glycogen concentration was found that seemed related to the depth of sampling. This led to a detailed study of the glycogen content of the walls of the heart with particular emphasis on the distribution from endocardium to epicardium. Four species were studied: rat, rabbit, dog and ox. The ox heart was included, because it made possible a comparison of the glycogen content of the conduction system with that of the myocardium. A parallel study was made of phosphorylase distribution in the rabbit heart.

Cardiac arrest was produced in the excised heart by plunging it into ice water as rapidly as possible. The rabbits were stunned by a blow on the head, the dogs were anesthetized with sodium pentobarbital using 30 mg/kg, and the ox was treated by the usual slaughter house procedure without anesthesia. The rats were either decapitated or anesthetized with ether or sodium pentobarbital. After cardiac arrest, the right and left ventricles and septum were removed as quickly as possible. In the larger animals, each was divided into three sections, apex, mid and base; in the rat, the walls were not divided at all. Either the entire section or a portion of it was then sliced longitudinally from endocardium to epicardium with a Stadie-Riggs slicer, except for the ox heart which was sectioned with scissors. The order of slicing (whether from endocardium to epicardium or vice versa) made no difference in the results. Each slice was weighed, and when all the weighings were completed, the slices were rapidly placed in 1 ml. of 30% KOH and heated in a boiling water bath for total glycogen determination by the anthrone method of Seifter[1]. Since the primary purpose of this study was to compare the glycogen distribution throughout the heart, the slices were all placed in KOH at the same time so that any loss in glycogen with time would affect all of them equally. Because of the small quantities of glycogen involved, a micro method was devised for precipitation of glycogen based on the micro method of Osterberg[2]. The

glycogen was precipitated by the addition of 3 vol of ethyl alcohol and 0·2 ml. of 2·5% sodium sulfate, followed by heating to the boiling point in a water bath. After cooling, the precipitates were allowed to settle overnight in the cold room and the tubes were centrifuged at 3500 rpm for 15 min in a Clay-Adams centrifuge. The precipitates were dissolved in water and aliquots were taken for glycogen analysis.

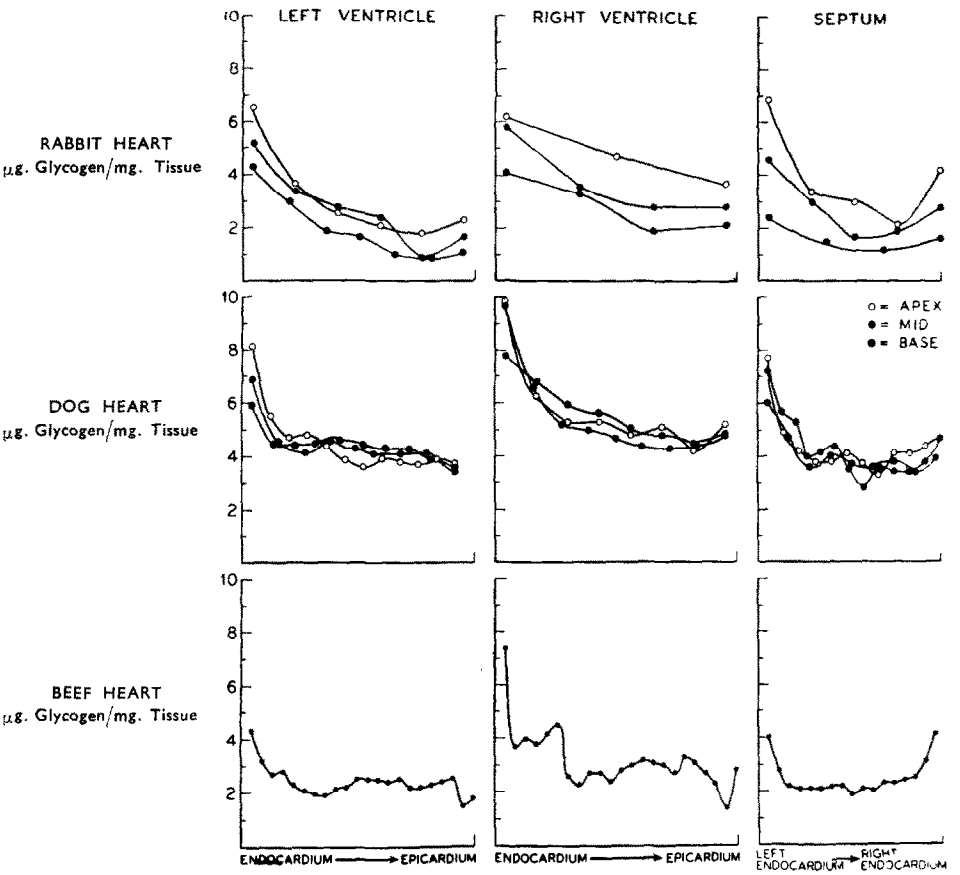

FIG. 1

Fig. 1 shows the results of the study of glycogen distribution in the walls of three of the species studied. The values shown are an average of five experiments for rabbit and dog and three experiments for the beef heart. The walls of the rabbit heart were divided into apex, mid-portion and base before being sliced for analysis. A definite gradient of decrease was observed in going from endocardium to epicardium in the right and left ventricles of the rabbit, with as much as a 6-fold difference in concentration being observed. In the septum where values are from endocardium of left heart to endocardium of right heart, there is a dip with a rise as the endocardial surfaces are approach-

TABLE I

Rabbit heart left ventricle
(μg. Glycogen/mg. Tissue)
(Endocardium → epicardium)

	Apex						Mid						Base					
Control	6·5	3·7	2·6	2·1	1·8	2·3	5·2	3·4	2·8	2·4	0·9	1·7	4·3	3·0	1·9	1·7	1·0	0·9	1·1
Perfusion Time 3-15 min.	4·2	2·6	1·6	1·5	1·8		4·0	2·2	2·0	1·3	0·4	1·2	3·2	2·1	2·2	1·8	0·9	0·6	1·3
	3·7	2·0	1·1	0·7	0·7		2·2	1·7	1·2	0·6	0·5	0·7	1·3	0·6	0·4	0·3	0·3	0·3	0·7
	3·0	1·4	1·2	0·7	0·4	1·0	2·0	0·9	0·7	0·4	0·5	1·2	1·5	0·8	0·6	0·4	0·2	0·2	0·6
	2·6	0·8	0·8	0·6	0·4	0·8	2·0	1·0	1·0	0·4	0·4	0·6	1·5	0·6	0·3	0·3	0·3	0·2	0·7
	1·6	0·3	0·2	0·1	0·2	0·3	1·6	1·1	1·2	0·2	0·1	0·6	1·3	0·6	0·7	0·3	0·1	0·5	0·3

ed. The gradient is evident throughout the walls. There is also a gradient of decrease from apex to mid-portion to base in all three walls.

The pattern of distribution of glycogen for the dog heart was quite similar to that for the rabbit heart. The concentration of glycogen was higher throughout, but the relative diminution in concentration at the epicardial layers as well as the dip in the septum were similar in both animals. In neither species, were there significant differences in concentration among right and left ventricles and septum.

In the beef heart, the AV node and bundle of His could be dissected out from the right side of the septum and studied independently of the myocardium. Because of the great thickness of the walls of this heart, a small section was removed from the central part of each wall, and this was then cut with scissors from endocardium to epicardium. As was observed in the other species, the maximum values for right and left ventricles occurred in the endocardium. In the beef heart there was a rather abrupt drop at the endocardium after which the glycogen values continued at a low level, with minor fluctuations, to the epicardium. In the septum, both endocardial layers had a steep rise in concentration with a wide zone of constancy in between. The highest concentration of glycogen was found in the conduction system, particularly in the bundle of His. It was at least three times greater than that of any part of the myocardium. The values obtained for the AV node were not so high as for the bundle, but were consistently higher than those of the myocardium. The concentration in the AV node was in the order 6·5 μg/mg of tissue. These results correlated with the studies on the distribution of the conduction system in the beef heart by Cardwell and Abramson[3] indicate that the higher glycogen values are associated with the distribution of the conduction system and the lower concentrations may represent truer myocardial values.

Because of the low glycogen values observed in the rat heart when the animals were decapitated, the effects of ether and sodium pentobarbital anesthesia were studied. The results are summarized in Fig. 2. Both types of anesthesia raised the glycogen values significantly. In this species, a smaller gradient was observed, which was generally less than two-fold. Whereas the left ventricle resembled that of other species, the right ventricle was more like the septum, with a central dip and a rise at both walls.

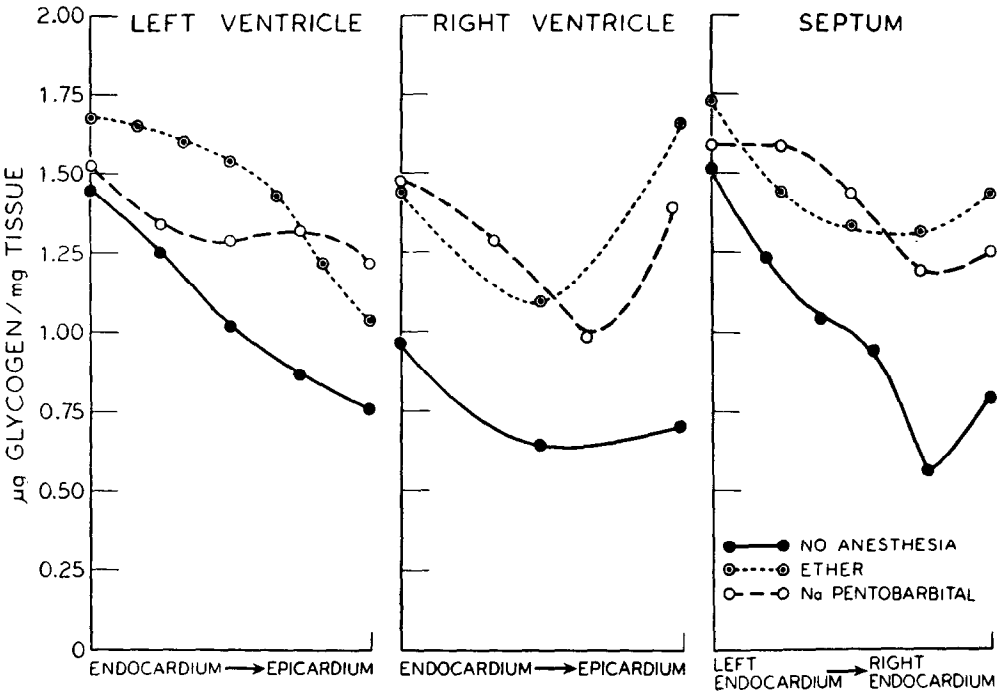

FIG. 2. Effect of anesthesia on glycogen in rat heart tissue. Each point represents the average of 3 experiments.

In order to test whether the glycogen of any part of the heart was preferentially utilized over any other during contraction, perfusion studies were carried out on rabbit hearts using the Langendorff preparation[4]. Oxygenated Feigen's solution[5] at 38° C. was used as the perfusate medium, except that glucose was omitted to ensure glycogen utilization. Table I shows the results observed after perfusion times of 3–15 min. Similar analyses were carried out on the right ventricle and septum, but since the results were all similar in pattern, only the values for the left ventricle are presented. It is apparent that when hearts contract without an external energy source, the glycogen of the myocardium acts as the source of energy. The data show that the glycogen is diminished throughout the heart with a gradient similar in pattern

to that of the freshly excised heart. Although the gradient does appear to be steeper than for the freshly excised heart, it would be difficult to draw the conclusion from these experiments that the glycogen from any section of the heart was preferentially utilized in comparison with any other.

Total phosphorylase and phosphorylase "a" concentrations were assayed across the walls of the rabbit heart in an attempt to correlate glycogen distribution with that of the enzymes partaking in glycogen metabolism. The assay for phosphorylase was carried out in the direction

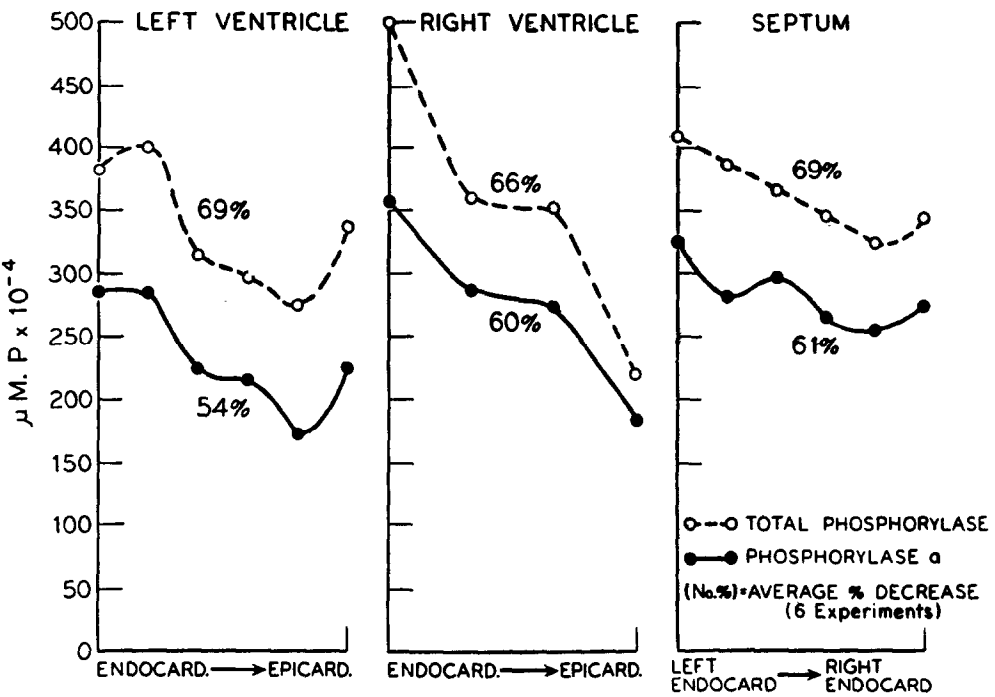

FIG. 3. Phosphorylase distribution throughout rabbit heart wall (μ M.P. released/Mg. tissue after 5 min. of incubation at 30° C).

of glycogen synthesis by the determination of phosphate relased from glucose-1-phosphate using the method of Cori and Illingsworth[6]. The walls of the heart were removed and sliced in the same manner as decribed previously, except that the entire wall was sliced. Because of the fragility of the enzyme only one wall was studied per animal. Immediately after the slices were weighed, they were placed in cold 30 ml porcelain mortars containing 1 ml of 0·001 M versene buffer at pH 7 and 0·02 M NaF. Additional buffer solution was added to bring the final volume to 3 ml/100 mg tissue as suggested by Kukovetz, *et al.*[7]. All the tissues were ground thoroughly and at the same time. Centrifugation and subsequent incubation with adenylate for total phosphorylase activity

and without adenylate for phosphorylase "a" were carried out for 5 min at 30°C as described by Cori and Illingsworth. Fig. 3 shows the μM of phosphate released per mg of tissue across each wall in representative experiments. The per cent decrease in an average of six experiments is presented with each curve. The gradients for total phosphorylase and for phosphorylase "a" are in the same direction as those for glycogen distribution. The per cent change was of smaller magnitude, a somewhat less than two-fold drop being observed across the wall.

We are extending this comparative study to include other enzymes.

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