Synthesis of Glycogen Fractions by Heart Homogenates.* (21926)

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Glycogen synthesis, *in vitro,* has been investigated in a number of tissues. Hastings (1) reported synthesis in liver, Stadie and Zapp(2) in diaphragm, Stadie, Haugaard and Perlmutter(3) in heart and Marsh and Miller (4) in the kidney. Interest in the glycogen fractions was renewed by the work of Bloom, Lewis, Schumpert and Shen(5), who described a method for the isolation of 2 types of glycogen through differential solubility in cold trichloroacetic acid (TCA). Cordier and Dessaux(6), utilizing this technic in an investigation of cardiac glycogen disappearance in rats subjected to anoxia, found that the TCA soluble fraction decreased to a greater extent than the TCA insoluble fraction of glycogen. A pronounced species variation of these 2 forms of cardiac glycogen was reported by Merrick and Meyer(7), who found in studies on dogs exposed to conditions of fulminating anoxia that although the TCA soluble glycogen disappeared rapidly from the heart, there appeared to be a temporary increase of the TCA insoluble fraction. The problem of changes in the cardiac glycogen fractions is worthy of more detailed analysis. The present study deals with the changes of concentration of the cardiac glycogens in rat and dog hearts, which have been homogenized in a medium promoting glycogen synthesis, and exposed to either an atmosphere of oxygen or an atmosphere of nitrogen.

Methods. For each of the 10 runs in each series of analyses, either the pooled ventricles from 6 decapitated male albino rats or a portion of the left ventricle from a dog anesthetized with pentobarbital sodium (32.5 mg/kg) was removed, weighed and then frozen between blocks of dry ice. Enough cold (1°C) fluid medium (Krebs Phosphate Ringer's solution or 5.4% glucose solution) was measured to make a 10% weight/volume mixture when homogenized with the heart tissue. The ventricles were ground with the fluid medium in a Ten Broeck Tissue Grinder, the temperature being held at approximately 2-3°C during homogenization. A 3 ml sample of the homogenate was taken for the initial glycogen value. The remainder of the homogenate was transferred to Warburg flasks, 3 ml per flask. The flasks were placed in a Warburg bath set for a temperature of 37.5°C, flushed with O_2 or N_2 and allowed to equilibrate for 30 minutes before oxygen consumption was recorded. One flask of each series of 7 was taken from the bath at succeeding time intervals (as indicated in Table I) and the contents removed for glycogen analysis. The final sample was removed 105 minutes after the initial sample. Extraction of the 2 glycogens was accomplished with essentially the procedure outlined by Bloom, *et al.*(5), that is, the TCA soluble fraction was extracted with cold TCA and separated from the precipitate by centrifugation at 1°C. One modification of the procedure, however, consisted of digesting the precipitate from the TCA extraction with 30% KOH to secure the non-extractable glycogen rather than using a duplicate sample and determining total glycogen by KOH digestion. After hydrolysis in sulfuric acid, the isolated glycogen samples were measured quantitatively by the anthrone procedure of Seifter, Dayton, Novic and Muntwyler(8).

Results and discussion. There was a precipitous drop of both glycogen fractions (Table I) which shows that glycolysis rapidly reduces heart glycogen when the organ has been homogenized. In contrast to the findings of Cordier and Dessaux(6) with the heart of anoxic rats (*in vivo*) where the TCA insoluble fraction was relatively stable, both fractions dropped to about 50% of their initial value within 5 minutes. Although no de-

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TABLE I. Glycogen Content of Heart Homogenates as Mean ± Standard Deviation.

Animal and medium	Glycogen	Time in min. after heart was homogenized 0	5	15	22½	30	60	75	105
Rat	TCA soluble	131 ± 37.7	66 ± 15.6	55 ± 8.8	49 ± 9.6	63 ± 19.3	79 ± 31.6	92 ± 38.5	103 ± 30.7
5.4% glucose	TCA insoluble	140 ± 44.9	74 ± 24.9	33 ± 15.8	23 ± 10.7	20 ± 7.4	16 ± 6.6	16 ± 5.4	22 ± 4.4
100% oxygen	Total	271	140	88	77	83	95	108	125
Rat	TCA soluble	154 ± 51.2	—	—	—	51 ± 22.5	54 ± 29.9	57 ± 38.2	54 ± 18.8
5.4% glucose	TCA insoluble	102 ± 37.7	—	—	—	19 ± 6.4	18 ± 3.7	15 ± 3.7	14 ± 2.6
3% oxygen	Total	256	—	—	—	70	72	72	68
97% nitrogen									
Rat	TCA soluble	86 ± 45.0	—	—	—	52 ± 43.9	53 ± 41.3	46 ± 32.7	34 ± 16.5*
Krebs phosphate	TCA insoluble	109 ± 54.7	—	—	—	25 ± 7.0	25 ± 11.9	23 ± 9.9	21 ± 6.5*
Ringer's	Total	195	—	—	—	77	78	69	55 *
1% glucose									
100% oxygen									
Dog	TCA soluble	471 ± 126.8	405 ± 165.3	244 ± 95.4	166 ± 52.7	125 ± 41.5	55 ± 14.8	45 ± 5.8	45 ± 5.9
5.4% glucose	TCA insoluble	114 ± 28.6	105 ± 16.3	90 ± 16.7	85 ± 20.1	80 ± 20.1	40 ± 16.8	25 ± 9.8	13 ± 2.9
100% oxygen	Total	585	510	334	251	205	95	70	58

* 90 min. samples. Each value represents the mean of 10 determinations.

terminations were made during the first half hour in the series utilizing Krebs Ringer's or low oxygen in 5.4% glucose, the 30-minute values suggest that they follow the same glycolytic pattern. A plateau independent of initial glycogen concentration is reached at 30 minutes. The TCA soluble glycogen, except when synthesis occurs, stabilizes at approximately 50 mg per 100 g of tissue while that part not extracted by cold TCA drops to 20 mg per 100 g of tissue.

This behavior of rat heart glycogen contrasts strongly with the situation in the dog where the TCA insoluble glycogen is found to decrease gradually over the 105 minute period (Table I). The TCA soluble glycogen of the dog heart, although disappearing rapidly, does not reach a stable level until 75 minutes after the first sample is taken.

Synthesis of glycogen by the rat heart was achieved in oxygenated 5.4% glucose confirming the experience of Stadie, Haugaard and Perlmutter(3). The most marked synthesis was in the TCA extractable portion, which after declining to 54 mg per 100 g of tissue, then began to rise and continued until a value of 103 mg per 100 g of tissue was reached at the end of 105 minutes. This represents synthesis at the rate of 0.594 mg of glycogen per 100 g of heart tissue per minute. The TCA insoluble glycogen, however, continued to decline for 75 minutes and then rose from 16 to 22 mg per 100 g of heart tissue at 105 minutes, a difference significant to the 98% confidence level. The insoluble fraction is resynthesized at roughly one-third the rate that the TCA soluble glycogen is formed.

Nothing in the data from rat heart homogenates suggests that the TCA soluble glycogen undergoes glycolysis in preference to the TCA insoluble fraction, although this is the implication from the work of Cordier and Dessaux(6) on the intact animal. However, this does seem to be true of the homogenized dog heart. The homogenization of the tissue may well alter metabolic pathways by destroying the normal anatomy of the cell.

The resynthesis of glycogen, under the conditions of the experiment, starts with the formation of the TCA soluble moiety. Whether or not the TCA insoluble fraction is

formed directly from the glucose, or represents the binding of the soluble form to proteins or some other molecule which is insoluble in cold TCA has yet to be determined.

The dog heart homogenate did not synthesize either glycogen fraction under the same conditions in which the rat heart showed glycogenic activity. Neither form of glycogen of rat heart was synthesized in Krebs Ringer's, though adequate glucose was present, nor in 5.4% glucose when oxygen tension was reduced to 21.5 mm Hg.

Glycogen synthesis was well correlated with oxygen consumption of the tissue homogenates. In the one medium (oxygenated 5.4% glucose) which promoted glycogenesis, oxygen consumption was 303 μl-O_2 per g of wet tissue per hour compared to the utilization of 145 μl-O_2 per g of wet tissue per hour in the unoxygenated sugar medium and the 131 μl-O_2 per g of wet tissue per hour in the oxygenated Krebs Ringer's. The dog heart suspension exhibited the lowest oxygen uptake, using only 114 μl-O_2 per g of wet tissue per hour.

Summary. Glycolysis reduces both glycogen fractions of the rat heart at about the same rate for the first 5 minutes, regardless of the medium used for suspension of the tissue. In Krebs Phosphate Ringer's solution and in unoxygenated 5.4% glucose, the 2 glycogens drop to a low, but stable level. The glycogen synthesized in the sugar medium is primarily the TCA soluble glycogen. The TCA soluble glycogen begins synthesis at the end of 22.5 minutes and synthesis of the TCA insoluble fraction begins 50 minutes later. A distinct species difference was observed in the metabolism of cardiac glycogen in the rat and dog, inasmuch as the TCA insoluble fraction was relatively more stable in the dog.

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Clotting Factor X. Physiologic and Physico-Chemical Properties. (21927)

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The use of serum from various pathological conditions in the thromboplastin generation test(1,2) led to the conclusion that besides the serum factors already known (Factor VII = SPCA, proconvertin and Factor IX = Christmas factor, PTC)* another clotting factor exists in serum for which the designation Factor X(3-5) was proposed. Matching experiments with serum from hemophilia B (deficient in Factor IX) proved to be especially demonstrative.

Method. A modification of the thromboplastin generation test developed in our laboratory was employed(2). The 4 components incubated to generate blood thromboplastin are: Platelet suspension prepared according to Flückiger *et al.*(6). calcium chloride, oxalated plasma adsorbed on barium sulfate (source of antihemophilic globulin) and serum. The first 3 components are kept

* The following numbering of clotting factors is proposed: Fibrinogen (F.I), Prothrombin (F.II), Thromboplastin (F.III), Calcium (F.IV), Labile factor, Proaccelerin (F.V & VI), SPCA Proconvertin (F.VII), antihemophilic Globulin (F.VIII), P.T.C., Christmas Factor (F.IX).