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Effect of Cardiac Drugs on the Phosphorylated Intermediates of the Rat.*

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Reports[1-3] have appeared in the literature on the effects of cardiac drugs on glycogen metabolism of laboratory animals. Several studies on the effect of cardiac drugs on the concentration of certain acid-soluble phosphorus compounds have been reported. These findings have been summarized in a recent review[4] on the biochemistry and pharmacology of the heart. Wollenberger[5] concluded from his studies on dog heart-lung preparations that the primary action of cardiac glycosides must be concerned with a phase of myocardial contraction other than the generation of utilizable chemical energy. On a series of perfusion studies of the isolated hearts of cats, rabbits, and rats, with digitoxin, Sjoerdsma *et al.*[6] reported that the appearance of creatinine in the perfusion fluid is not altered. However, no systematic analysis of the phosphorylated intermediates of glycolysis has been reported. The purpose of the present investigation was to measure the concentration of a number of phosphorylated intermediates of glycolysis under various conditions of treatment with cardiac drugs.

Experimental. Normal Sprague-Dawley rats of approximately 250 g were digitalized intraperitoneally by the daily administration of 12 mg/kg of crystalline digitoxin in propylene glycol for 3 days. Control animals received equivalent amounts of the solvent throughout the digitalization period. Following a fasting period of 24 to 36 hours, the animals were anesthetized by the intraperitoneal injection of 45 mg/kg of sodium pentobarbital, and decapitated immediately after light anesthesia. The tissues were quickly blotted on filter paper and frozen between slabs of dry ice, weighed, and the phosphorus fractions then measured according to the methods outlined by LePage and Umbreit.[7] Glycogen in normal and digitalized rat heart was determined on separate samples of tissues by the method of Good *et al.*[8] The method of Folin and Malmros[9] was used for measuring the glucose after hydrolysis of glycogen.

None of the digitalized animals elicited any symptoms of over-digitalization, such as tremors or convulsions. The data presented in Table I indicate that digitoxin produced no marked alteration in the distribution of the acid-soluble phosphorylated intermediates of the rat heart. Although reports have appeared in the literature on changes in the phosphocreatine content of the heart under the influence of cardiac drugs, no such effect was noticed, under the conditions of the experiment.

Results presented in Table II indicate that there was no marked difference in heart glycogen values, but that there was an increased amount of liver glycogen in the digitalized animals as compared with the controls, there

* This work was supported by grants from the Life Insurance Medical Research Fund. The digitoxin used in these experiments was kindly supplied by Dr. K. K. Chen of the Lilly Research Laboratories, Indianapolis, Ind.

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TABLE I.
Distribution of Acid-Soluble Phosphorus Compounds in the Heart of Normal and Digitalized Rats.

Phosphorylated Intermediates*	Normal	Digitalized
A. Analysis on T.C.A. Extracts:		
1. "True" Inorganic Phosphorus	993	1100
2. Phosphocreatine Phosphorus	262	293
3. Phosphopyruvic	1450	1242
4. Total Phosphorus	3110	3184

	Barium-Soluble		Barium-Insoluble	
	Normal	Drug	Normal	Drug
B. Analysis of Fractions:				
1. Inorganic Phosphorus	239	245	1232	1326
2. ATP 7 min Hydrolyzable			229	200
3. Total Phosphorus	877	1180	1693	2155
4. Fructose	17	18		
5. Fructose 1-6 Diphosphate			102	97

* Expressed as micromoles/100 g.

TABLE II.
Effect of Digitoxin on Liver and Heart Glycogen of Rat Given 12 mg/kg Digitoxin in Propylene Glycol for 3 Days, Fasted 24-36 Hours, and Anesthetized with 45 mg/kg Sodium Pentobarbital.

	Control			Digitalized		
Tissue	Rat No.	% glycogen	Mean	Rat No.	% glycogen	Mean
Heart	1	0.34		4	0.30	
	2	0.27	0.27	5	0.29	0.30
	3	0.21		6	0.31	
Liver	1	1.96		4	2.06	
	2	1.88	1.85	5	2.32	2.39
	3	1.71		6	2.81	

being 2.39% glycogen in the former and 1.85% in the latter. These values suggested the possibility that digitoxin may exert a protective action on liver glycogen by decreasing the rate of glycogenolysis and experiments were, therefore, conducted to measure this possibility. Samples of liver tissue from both digitalized and normal animals were allowed to stand at room temperatures for periods of 2, 5, and 8 minutes prior to freezing with dry ice. Results indicated that there was no difference in the rate of breakdown of glycogen in the excised liver tissue in normal and digitoxin treated rats. It should be noted that these and subsequent experiments, in which the liver has been removed from its normal environment, do not take into consideration the possible influence of epinephrine or insulin on glycolysis or glycogenesis *in situ*.

Inasmuch as Yorimitsu[10] had demonstrated that digitalis preparations promoted resynthesis of glycogen from lactic acid, experiments were conducted to ascertain the effect of digitoxin on the synthesis of liver glycogen from glucose in rat liver, using the method as outlined by DuBois *et al.*[11] Normal Sprague-Dawley rats of approximately 250 g were digitalized intraperitoneally with 5 mg/kg of crystalline digitoxin in propylene glycol for 3 days. Controls received equivalent amounts of the solvent during the digitalization. The experiments were begun following a fasting period of 24 to 36 hours. Data given in Table III show that the initial glycogen values were higher in the digitalized animals, and there was an indication of an increased amount of glycogenesis from injected glucose

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TABLE III.

Effect of Digitoxin on Synthesis of Liver Glycogen in Rats Given 5 mg/kg Digitoxin in Propylene Glycol for 3 days; Fasted 24 Hours; No Further Injection of Drug Given During Glycogenesis; No Convulsions or Tremors.

	Control				Digitalized			
Glucose schedule	Exp. No.	Rat No.	% glycogen	Mean	Exp. No.	Rat No.	% glycogen	Mean
No Glucose	I	1	0.59		I	9	0.95	
		2	0.63	0.61		10	0.85	0.90
	II	17	0.19		II	25	1.02	
		18	0.41	0.30		26	0.99	1.00
0.15 g/hr for 2 hrs before death	I	3	1.22		I	11	2.36	
		4	1.27	1.24		12	2.52	2.44
	II	19	0.48		II	27	1.86	
		20	0.61	0.54		28	1.43	1.64
0.15 g/hr for 4 hrs before death	I	5	2.09		I	13	2.81	
		6	2.03	2.06		14	2.71	2.76
	II	21	1.03		II	29	1.96	
		22	1.18	1.10		30	2.80	2.38
0.15 g/hr for 6 hrs before death	I	7	2.05		I	15	2.86	
		8	2.13	2.09		16	2.84	2.85
	II	23	1.06		II	31	2.86	
		24	1.32	1.19		32	2.43	2.64

TABLE IV.

Effect of Digitoxin on Synthesis of Liver Glycogen in Over-Digitalized Rats Given 4 mg/kg Digitoxin in Propylene Glycol for 3 days; Fasted 24 Hours; Additional Injection of 5 mg/kg Digitoxin Given at 0 Hour.

	Control			Digitalized		
Glucose schedule	Rat No.	% glycogen	Mean	Rat No.	% glycogen	Mean
No glucose	34	0.31		40*	0.21	
	35	0.29	0.30	41*	0.17	0.19
0.15 g/hr for 2 hrs before death	36	1.01		42†	0.04	—
	37	0.86	0.93	43*	0.29	—
0.15 g/hr for 4 hrs before death	38	1.83		44†	Died	—
	39	1.73	1.78	45†	0.02	—

* Occasional tremors.
† Tremors and convulsions.

in digitalized animals. There was no additional injection of the drug during the 6 hour period of glucose injection. None of the digitalized animals showed any tremors, convulsions, or other toxic symptoms. Difference in normal values of Experiment I and II are probably due to differences in amount of previously ingested food, prior to fasting.

The results given in Table IV show the effect of digitoxin on glycogen synthesis by the liver after administration of glucose to fasted over-digitalized rats. A group of six rats which had been digitalized for 3 days by the daily administration of 4 mg/kg of crystalline digitoxin in propylene glycol was given an additional injection of 5 mg/kg of digitoxin in propylene glycol intraperitoneally at the start of the glucose injection period. The controls received an equivalent amount of the solvent. Both groups were fasted for 24 hours. It can be seen from the data that there was a decreased amount of

liver glycogen in overdigitalized animals, the per cent of glycogen decreasing somewhat as the animals approached death. These results seem to indicate that increased glycogenesis occurred in the liver of rats given non-toxic doses of digitoxin, while toxic doses of the drug resulted in a decrease in the amount of liver glycogen in rats in which symptoms of over-digitalization are manifested.

Summary. There was no marked difference in the distribution of the acid-soluble phosphorylated intermediates of glycolysis in the heart muscle of normal and digitoxin-poisoned rats. Small differences in the glycogen values of heart values were noted, but there was an increased amount of liver glycogen in the digitalized animals as compared with the controls as indicated by an average of 2.39% of glycogen in the former and 1.85% in the liver of the latter. Neither was there any difference in the rate of breakdown of glycogen in the excised liver tissue in normal and digitoxin treated rats. Increased glycogenesis occurred in the liver of rats given non-toxic doses of digitoxin while toxic doses of the drug resulted in a decrease in the amount of liver glycogen in rats in which symptoms of over-digitalization were manifested.

The author wishes to express his appreciation to Dr. K. P. DuBois and to Dr. E. M. K. Geiling of the Department of Pharmacology for their interest and helpful advice in this problem.

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Effect of Fibrinolysin upon Oxytocin, Vasopressin and Hypertensinogen.

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Plasma fibrinolysin has been considered a tryptase. Its proteolytic effect may be observed not only in the dissolution of fibrin but also in the digestion of fibrinogen, gelatin and casein, which is shown by the production of non-protein nitrogen when it acts upon these substrates (Tagnon *et al.*;[1] Kaplan *et al.*;[2] Christiensen,[3] Ferguson,[4] Shinowara,[5]). Nevertheless fibrinolysin does not inactivate hypertensin (Croxatto and Badia,[6]) a substrate which is attacked by all proteolytic enzymes tested up to now, including crystallized pancreatic trypsin (Croxatto[7]).

As fibrinolysin may appear in the bloodstream in its active form, an experimental study was carried out by observing its effect upon the hormones of the neurohypophysis-oxytocin and vasopressin- and upon hypertensinogen, in a similar way to that used before for trypsin. This also has the advantage of permitting a more detailed comparison between the effects of fibrinolysin and pancreatic trypsin. It has already been established that the latter is capable of inactivating hypertensin and vasopressin but not oxytocin (Croxatto[7]).

Hypertensinogen is attacked by trypsin but without the formation of hypertensin. Moreover after this enzyme has been used the substrate is incapable of producing hypertensin even when renin is added.

The results published in this paper show that fibrinolysin, just as trypsin, destroys the pressor effect of vasopressin but does not, under the same conditions, alter the effect of

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