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Cardiac Phosphorylase in Different Species and after Reserpine Administration.* (30399)

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Several species of animals have been used in studies of cardiac phosphorylase activity (1), and there appears to be some difference in enzyme activity from one species to another. In addition, differences in activity of phosphorylase within the various layers of the heart have been demonstrated. For example, Jedeikin(2) has shown that phosphorylase activity increased progressively from the epicardium to the endocardium in dog and rabbit hearts.

The present investigation is concerned with the phosphorylase activity in hearts from 3 species, *i.e.*, rabbit, rat and frog. The studies also include measurements of the enzyme in the ventricles and atria from the 3 species as well as studies of the effect of reserpine administration on ventricular and atrial phosphorylase.

Methods. The animals were male New Zealand white rabbits (1.8-2.0 kg), male albino rats of the Wistar strain (200-250 g) and male and female frogs (*Rana pipiens,* 30-50 g).

Rats and rabbits were killed by a blow on the head and frogs were pithed. After opening the thorax, the heart was quickly removed, cooled in an empty beaker kept in crushed ice. Portions of tissue were dissected as described below, weighed on a torsion balance and extracts prepared immediately by grinding the tissue in ice-cold mortars containing a solution of 0.001 M $EDTANa_2$-0.02 M NaF (3 ml/100 mg tissue wet weight). In the experiments with rabbit hearts and with some of the rat hearts, the left and right atria and the middle portions of the left and right ventricles were used for phosphorylase assay; in other experiments with rat hearts (Table III), enzyme activity was determined in the whole atria and the apical portions of the ventricle. When frog hearts were used, the atrium was separated from the ventricles and each of these portions extracted for enzyme assay. When reserpine was administered, the drug was given in a single intraperitoneal injection, unless otherwise indicated, at doses of 2.5 or 5.0 mg/kg. The animals were sacrificed at stated intervals following drug administration.

Phosphorylase determination. Heart extracts were centrifuged in the cold room and the supernatant fluid was further diluted 3 times with EDTA-NaF solution so that the final concentration of tissue was 6.54 mg/ml. The extracts were kept in an ice bath and analyzed for enzyme activity within 4 hours of their preparation.

Phosphorylase activity was determined in the direction of glycogen synthesis ($\text{glucose-1-P} + \text{glycogen}_{(n)} = \text{Pi} + \text{glycogen}_{(n+1)}$) according to the method of Cori and Illingworth(3) as follows: 0.5 ml extract (3.27 μg tissue) was incubated at 30° for 5 minutes with 0.5 ml substrate mixture and the forma-

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TABLE I. Phosphorylase Activity in Ventricles and Atria of Frog Heart.

	Ventricles	Atria	Significance of difference
Phosphorylase *a*, units/g ± SEM	584 ± 32	169 ± 19	P <.001
Total phosphorylase, units/g ± SEM	655 ± 40	230 ± 23	P <.001
% Phosphorylase *a*	89.2 ± 1.7	73.5 ± 2.5	

The results are the means of 12 experiments.

TABLE II. Phosphorylase Activity in Different Areas of Rat and Rabbit Heart.

	Left ventricle	Left atrium	Significance of difference
RAT HEART (N = 8)			
Phosphorylase *a*, units/g ± SEM	723 ± 38	459 ± 34	P <.001
Total phosphorylase, units/g ± SEM	1051 ± 107	840 ± 53	P <.050
% Phosphorylase *a*	68.8 ± 2.5	54.6 ± 1.7	P <.001
RABBIT HEART (N = 6)			
Phosphorylase *a*, units/g ± SEM	517 ± 43	245 ± 22	P <.001
Total phosphorylase, units/g ± SEM	920 ± 51	573 ± 44	P <.001
% Phosphorylase *a*	56.2 ± 3.0	42.8 ± 3.6	P <.025

tion of inorganic phosphate determined(4). The substrate used for measuring total phosphorylase (*a* + *b*) consisted of 0.032 M glucose-1-phosphate, 2% glycogen and 0.002 M 5′-adenylic acid (AMP). In the determination of phosphorylase *a*, AMP was omitted from the substrate. The results were calculated as units of phosphorylase activity per assay as described by Cori *et al*(5) and converted to units per mg of tissue. To obtain the activity per gram of tissue, the unit of activity per ml was divided by the weight of tissue (6.54 mg) and multiplied by 1000. Phosphorylase *a* was expressed as percent of total phosphorylase.

Drugs. 1-Epinephrine bitartrate was generously supplied by Sterling-Winthrop Research Institute, Rensselear, N. Y.; reserpine by Smith Kline and French Laboratories, Philadelphia, Pa.

Results. Phosphorylase activity in poikilothermic and homothermic animals. Results of experiments in which phosphorylase was measured in frog hearts are presented in Table I. In these hearts from poikilothermic animals, both total phosphorylase and phosphorylase *a* were markedly lower in atria than in ventricles. The major portion of the phosphorylase was in the *a* form, probably the result of the sympathetic stimulation caused by pithing of the frogs.

Table II contains results of experiments with hearts from homothermic animals, rats and rabbits. The levels of phosphorylase in myocardial tissue from rats or rabbits were higher than the corresponding values in frog heart. In addition, total phosphorylase activity per gram of tissue was generally higher in rat than in rabbit myocardium. As with frog hearts, total phosphorylase and phosphorylase *a* in ventricles were considerably higher in atria. Because there were no significant differences between the enzyme levels in the right and left sides of the heart, only data obtained from left ventricles and atria are presented. Most of the enzyme was present in the *a* form with the consistent finding that phosphorylase *a* in % of total phosphorylase was higher in ventricles than in atria.

Effect of reserpine on phosphorylase activity in rat and rabbit heart. It has been demonstrated previously that in rats chronic administration of reserpine prevented the pronounced rise in cardiac phosphorylase *a* activity caused by decapitation (Hess *et al*(6); Lacroix *et al*(7)). However, there is little information concerning the time course of the effects of reserpine on heart phosphorylase.

Following administration of reserpine to rats or rabbits, the animals were killed by a blow on the head at various time intervals and phosphorylase determinations were made on extracts of ventricle and atria. The results of these experiments are recorded in

TABLE III. Activity of Cardiac Phosphorylase *a* Following Administration of Reserpine.

Animal	Time after reserpine*	N	% Phosphorylase *a*			
			Ventricle	P†	Atrium	P†
Rat	Control	19	72.5 ± 2.07		69.0 ± 3.15	
	15–30 min	12	80.1 ± 3.08	<.05	88.5 ± 1.86	<.001
	1–2 hr	12	74.7 ± 2.56	N.S.	70.7 ± 3.15	N.S.
	3 "	6	77.2 ± 3.31	N.S.	73.0 ± 5.06	N.S.
	24 "	9	54.8 ± 1.23	<.001	60.3 ± 2.69	N.S.
Rabbit	Control	9	51.4 ± 1.18		37.4 ± .72	
	30 min	6	59.9 ± 2.26	<.005	62.0 ± .52	<.001
	48 hr	7	30.9 ± 2.65	<.001	24.5 ± 2.70	<.001

* Rats were given a single i.p. injection of reserpine, 5 mg/kg. With rabbits the initial dose was 2.5 mg/kg. In the 48-hr group 2 additional injections of 1.25 mg/kg were given at 24 and 46 hr.

† Significance of difference from control. N.S. = not significant.

Table III. Since there was no significant effect of reserpine on total phosphorylase, enzyme activity in this Table is expressed as phosphorylase *a* in % of total phosphorylase.

In agreement with earlier experiments with decapitated rats(6,7), the major proportion of phosphorylase was present in the *a* form, and the % phosphorylase *a* was considerably higher than that found normally *in vivo*(1). This is probably a consequence of the sympathetic stimulation associated with the sacrifice of the animals. Anoxia of the myocardium has been shown to lead to conversion of phosphorylase *b* to phosphorylase *a* (8,9), and this factor may also have played a role in causing the high activity in phosphorylase *a* seen in the control animals. As in the experiments in Table II, phosphorylase *a* in % of total phosphorylase was considerably lower in rabbit than in rat heart.

Shortly after injection of reserpine (15-30 minutes), there was a significant increase over the control in phosphorylase *a* activity in ventricles and atria of both species of animals. In the rat, there was a decline to control values one to 3 hours after drug administration and by 24 hours there was a significant decrease in phosphorylase *a* activity in the ventricles. The activity of the enzyme in the rat atrium tended to diminish, although the decrease from the control after 24 hours was not statistically significant. The values for phosphorylase *a* in ventricles and atria were markedly depressed in rabbits which had received an initial injection of reserpine of 2.5 mg/kg 48 hours before the experiment followed by two injections of 1.25 mg/kg 24 and 2 hours prior to sacrifice.

Discussion. The results of these experiments show that there is an appreciable difference in the level of activity of the phosphorylase enzymes in hearts from poikilothermic and homothermic animals. This is consistent with the low metabolic rate of cold-blooded animals. When cardiac phosphorylase of the 2 warm-blooded animals is compared, it is seen that the rabbit has a lower enzyme activity per gram of tissue. One would expect this from other studies indicating that metabolism per weight of tissue is inversely proportional to the size of the animals.

Total phosphorylase activity in atria was markedly lower than in ventricles in all 3 species. A biochemical difference between ventricles and atria in several animals, including frog and rabbit, was also reported by Davies *et al*(10). These investigators found that the total adenine nucleotide concentration in ventricles was appreciably higher than in atria. Since both the adenine nucleotides and the phosphorylase enzymes are important in the reactions involved in muscle contraction, their presence in larger amounts in ventricles than in atria is probably related to the greater work performed by the ventricles.

Paasonen and Krayer(11) injected 5 mg/kg of reserpine intraperitoneally into rats and measured the norepinephrine content of the rat heart at different intervals after drug administration. It was found that after 30 minutes norepinephrine concentration in the heart had decreased to half of the control value. After 2 or 4 hours, norepinephrine

content was extremely low but still measurable. At the end of 24 hours no norepinephrine could be determined in the heart. The alterations in cardiac phosphorylase *a* activity reported here show some correlation with the changes in norepinephrine concentration described by Paasonen and Krayer(11). During the period immediately following reserpine administration, when the heart is being depleted of norepinephrine, a stimulation of phosphorylase *a* occurs. This is followed by a return of enzyme activity to control values after one or 3 hours, and after 24 hours, when the heart is depleted of norepinephrine, there is a significant decrease in phosphorylase *a* activity. These results suggest that the norepinephrine stored in the heart plays a role in the regulation of the activity of cardiac phosphorylase *a*.

Summary. Total phosphorylase was higher in rat and rabbit hearts than in frog hearts and greater in ventricles than in atria of all 3 species. Reserpine administration caused an initial increase in cardiac phosphorylase *a* followed by a marked diminution.

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Inhibition of Erythropoietin Formation in Hypoxic Rats by Hexose Analogues.* (30400)

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Hypoxemia or anemia are known to stimulate erythropoietin formation whereas hyperbaric oxygen(1) and hypertransfusion polycythemia(2) depress erythropoiesis, probably through a depression of erythropoietin formation. The latter thus appears to be inversely proportional to the oxygen tension in erythropoietin producing tissue. Metabolic pathways and the mechanism which accelerates synthesis of the active hormone in response to low pO_2 have remained obscure. The present study examines the role of glycolysis in erythropoietin formation. It was prompted by reports on erythropoietin formation in several types of tumors(3). Moreover, the kidney, which produces most of the body's erythropoietin, exhibits in its medulla a high glycolytic metabolism. The exact site of erythropoietin formation in the kidney is unknown, but it seemed worthwhile to explore whether glycolysis perhaps represents the common link in erythropoietin formation in these diverse tissues. Erythropoietin serum levels were measured in hypoxic rats with or without administration of D-glucosamine (GA) or 2-desoxy-D-glucose (2-DG). These hexose analogues depress the rate of glycolysis through competitive inhibition of hexokinase(4). They reportedly do not affect cellular respiration, and their administration should thus curtail glycolysis without inducing alterations in the pO_2 of erythropoietin producing tissue.

Methods. Female Sprague-Dawley rats (200 to 225 g) in groups of 7 were fasted for 16 hours before experiments. Group (1) re-

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