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Metabolic Enzyme Response in the Pressure-Overloaded Heart of Weanling and Adult Rats (41750)

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Abstract. Weanling and adult rats were subjected to left ventricular pressure overload induced by abdominal aortic constriction. At 5 days or 5 weeks postsurgery, the left ventricle (LV) was dissected, weighed, and metabolic marker enzyme activities (μmole/g/min) of tissue homogenates were measured. Enzymes representing glycolytic (phosphofructokinase (PFK)) and mitochondrial (citrate synthase (CS) and malate dehydrogenase (MDH)) metabolisms were evaluated. Five days of pressure overload had detectable, but statistically nonsignificant effects on left ventricles of both weanling and adult rats. Sustained pressure overload (5 weeks) increased LV weight by 52 and 39% in weanling and adult rats, respectively. PFK activity was 24 ± 1 (mean ± SE) in control weanlings and was unaltered in any of the other groups. LDH isoenzyme composition was estimated by substrate inhibition (ratio 0.33/10 m*M* pyruvate). With normal heart development, the LDH ratio increased from 1.89 ± 0.06 to 2.03 ± 0.08. Pressure overload had no influence on the adult LDH ratio. Developmental LDH responses were not observed in weanling LV after 5 weeks of aortic constriction (1.74 ± 0.06). The product of CS activity and LV weight was used to estimate mitochondrial mass in the ventricle. Mitochondria accumulated at a rate of about 5% increase per day over the intervening 5-week period of normal heart growth. Pressure overload for 5 weeks in weanling rats elicited net accumulation of mitochondria at a rate of about 9% increase per day. Mitochondrial accumulation in the adapting adult rat heart amounted to less than 1% increase per day. The results indicate that qualitative and quantitative differences exist between young and adult animals in their heart enzyme adaptive responses to pressure overloading. Divergent metabolic adaptations may contribute to heart functional differences in the enlarged heart of weanlings and adults.

The mammalian heart has come to be recognized as an organ possessing remarkable adaptive capabilities. Imposition of sustained pressure overloading is frequently employed to elicit experimental compensatory heart responses. Thus, cellular and subcellular changes which constitute adult heart adaptation to pressure overload have been described in detail (1, 2). Particular emphasis has been directed toward those metabolic systems intimately related to energy production for heart contraction and other functions. Interest in the relationships between heart metabolism and heart function is especially pertinent because imposition of pressure overload stress and the subsequent development of an enlarged heart is associated with depressed heart contractile function (3–5). In contrast to the impaired contractile function which characterizes the enlarged, adult heart, sustained pressure overloading in young (weanling) rats does not result in aberrant heart contractile performance (6).

The cellular mechanisms associated with heart adaptation in the weanling and adult rat differ dramatically. Adaptive heart growth in the adult rat heart occurs as a primary function of cardiac muscle cell hypertrophy (7–9). Evidence of muscle cell proliferation has not been detected in the adult heart. In hearts of weanling rats, however, cardiac muscle cell proliferation occurs during adaptation in concert with cardiac muscle cell hypertrophy (6, 10). Although these two divergent cellular mechanisms appear to have significant functional ramifications, the subsequent and intervening processes linking muscle cell division with myocardial function remain to be identified.

As an initial step to identify the processes linking cell division with contractile function, the present study sought to establish the response of metabolic marker enzyme activities in the pressure overloaded hearts of weanling and adult rats. Selected marker enzymes representing glycolytic (phosphofructokinase),

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anaerobic (lactate dehydrogenase), and mitochondrial (citrate synthase and malate dehydrogenase) metabolisms were evaluated. The results indicate no significant alteration in glycolytic marker enzyme activity. However, qualitative and quantitative differences exist between young and adult animals in the heart enzymatic adaptive responses representing anaerobic and aerobic metabolisms.

Materials and Methods. *Animal selection and treatment.* Weanling (21 days of age) and adult (250–300 g) male, Sprague–Dawley rats were studied. Weanling and adult rats were assigned by random selection to either control or aorta constriction groups. Left ventricular pressure overload was effected by abdominal aortic constriction. Under ether anesthesia, the abdominal aorta was surgically isolated above the renal vessels. The ligature–needle technique described by Beznak (11) was used to constrict the vessel. A blunt 22-gauge needle produced a 30 to 40-mm Hg left ventricular pressure elevation in weanling rats. Identical procedures employing a blunt 20-gauge needle resulted in comparable degrees of pressure overload in adult rats. Control rats for both the weanling and the adult groups underwent sham operations. At either 5 days or 5 weeks postsurgery, the animals were killed by cervical dislocation. The heart was excised and immediately cooled in a beaker placed in ice. Right and left atria were dissected, pooled into a single sample, and weighed. Extraneous tissue, including the great vessels, was then removed. The remaining heart tissue was separated into right ventricular and left ventricular samples and weighed separately. The interventricular septum was included with the left ventricle. Enzyme analyses were performed on left ventricular tissue as described below.

Enzyme determinations in whole-heart homogenate. A 5% (w/v) homogenate of left ventricular tissue was made using a Brinkmann Polytron. Homogenates were prepared in 100 m*M* potassium phosphate buffer, pH 7.4, to which 5 m*M* reduced glutathione had been added. Phosphofructokinase activity was measured using the methods described by Mansour (12). Lactate dehydrogenase activity was determined with pyruvate as enzyme substrate (13, 14). Substrate concentrations were adjusted so that lactate dehydrogenase activities were assayed in the presence of 10, 1, or 0.33 m*M* pyruvate. Citrate synthase enzyme activity was determined using the procedures developed by Srere (15). Nonionic detergent treatment with Triton X-100 fully disrupted mitochondria within the homogenate and assured optimum citrate synthase enzyme activity. Malate dehydrogenase enzyme activity was determined using the procedures of Shonk and Boxer (16). Total malate dehydrogenase enzyme activity was measured in one untreated homogenate sample. An additional homogenate sample was treated with 100% ethanol to inactivate the cytoplasmic form of malate dehydrogenase. From these determinations, it was possible to quantitatively differentiate mitochondrial and cytoplasmic forms of malate dehydrogenase enzyme activities present in whole-heart homogenate.

Preparation and enzyme assays of isolated heart mitochondria. In separate experiments, animals were selected and treated identically as described above for whole-heart homogenate enzyme studies. However, left ventricular tissue was utilized for the preparation of isolated mitochondria. Tissue was homogenized in 250 m*M* sucrose buffered with 10 m*M* Tris, pH 7.4, using a Brinkmann Polytron. Mitochondria were isolated by differential centrifugation as outlined by Aschenbrenner *et al.* (17). Isolated mitochondria were suspended in 100 m*M* phosphate buffer containing 5 m*M* reduced glutathione. Protein measurements (biuret) were made on aliquots of mitochondrial suspensions to determine mitochondrial protein yield and, subsequently, enzyme specific activity (μmole/mg mitochondrial protein/min). Additional samples of mitochondrial suspensions were taken for determining citrate synthase and malate dehydrogenase enzyme activities. Triton X-100 activation and ethanol inactivation procedures were employed with isolated mitochondrial suspensions used to measure citrate synthase activity and malate dehydrogenase activity, respectively.

Statistical analyses. One-way analysis of variance was used throughout these experiments for multigroup comparisons. The Scheffe post-hoc test identified significant group differences. A probability level of 0.05 or less was considered significant for all statistical procedures.

Results. Body weight and heart weight results from weanling and adult rats are given in Table I. Control weanling rats exhibited substantial overall growth during the intervening 5 weeks following initial body weight measurements. Abdominal aortic constriction had no significant influence on body weight of either weanling or adult animals. All heart component parts measured in the present study underwent at least a twofold increase in mass during the 5-week growth period for weanling rats. Heart growth then stabilized in the adult animals such that no further increase in mass of heart components occurred.

Five days of pressure overload had statistically nonsignificant effects on left ventricular weight in both weanling and adult animals. In contrast, 5 weeks of sustained pressure overload produced significant elevations in atrial and left ventricular weights in both weanling and adult animals. Significant enlargement of the right ventricle did not occur in response to aortic constriction in any experimental group.

Simultaneous alterations in body weight and left ventricular weight exerted profound influences on relative left ventricular mass as assessed by the ratio of left ventricular weight to body weight (LV/BW) (Table I). As natural growth proceeds, body weight increases proportionally more than left ventricular weight resulting in the progressive reduction in LV/BW values for control animals in the present study. Although 5 days of aortic constriction did not significantly increase left ventricular weights of weanling and adult rats, the small reductions in body weight, when coupled with relatively small increases in left ventricular weight were sufficiently additive to produce significant increases in LV/BW values. Body weight alterations were negligible after 5 weeks of aortic constriction in both age groups of animals. Thus, the significant increases in LV/BW directly reflect left ventricular weight responses to long-term pressure overloading.

Protein concentration (mg/g) was 118 ± 6 (mean ± SE) in left ventricular tissue of 5-day control weanlings and was not significantly altered in any of the other groups studied (data not shown). Therefore, conclusions based upon enzyme activities measured in whole homogenates are comparable whether enzyme data are expressed per gram tissue weight or per milligram tissue protein.

Phosphofructokinase enzyme activities in whole heart homogenates are given in Table II. None of these values was significantly altered.

Lactate dehydrogenase enzyme activities in whole-heart homogenates are presented in Table III. At all substrate concentrations measured, an increase in lactate dehydrogenase enzyme activity occurred as normal heart growth and development took place. Relatively greater enhancement of enzyme activity at 0.33 m*M* pyruvate with respect to 10 m*M* pyruvate accounts for the progressive and significant increase in 0.33/10 enzyme activity ratio that was observed as a function of normal heart development (Table III). Different 0.33/

TABLE I. BODY WEIGHTS AND HEART WEIGHTS OF WEANLING AND ADULT RATS

	Body wt (g)	Atria (mg)	RV (mg)	LV (mg)	LV/BW (mg/gm)
Weanling					
5-Day control (10)	91 ± 11	22 ± 2	72 ± 7	259 ± 28	2.89 ± 0.06
5-Day AC (10)	87 ± 9	27 ± 3	71 ± 6	295 ± 23	3.49 ± 0.20*
5-Week control (10)	284 ± 7	43 ± 3	156 ± 6	609 ± 18	2.15 ± 0.06
5-Week AC (10)	279 ± 13	75 ± 6*	182 ± 8	926 ± 56*	3.36 ± 0.21*
Adult					
5-Day control (10)	316 ± 5	49 ± 4	169 ± 4	667 ± 24	2.07 ± 0.05
5-Day AC (10)	284 ± 9	46 ± 3	146 ± 8	725 ± 23	2.63 ± 0.06*
5-Week control (10)	340 ± 9	39 ± 3	139 ± 13	663 ± 19	1.95 ± 0.02
5-Week AC (10)	349 ± 12	62 ± 7*	178 ± 14	920 ± 54*	2.61 ± 0.11*

Note. Values shown are means ± SE. Numbers of animals given in parentheses. AC, aorta constricted; RV, right ventricle; LV, left ventricle.

* $P < 0.05$ vs comparable control.

TABLE II. PHOSPHOFRUCTOKINASE ENZYME ACTIVITY IN WHOLE-HEART HOMOGENATES OF WEANLING AND ADULT RATS

	Phosphofructokinase (μmole/g/min)
Weanling	
5-Day control (10)	24.4 ± 1.2
5-Day AC (10)	24.0 ± 1.6
5-Week control (10)	26.1 ± 2.0
5-Week AC (10)	23.3 ± 1.7
Adult	
5-Day control (10)	27.8 ± 1.3
5-Day AC (10)	22.9 ± 1.5
5-Week control (10)	22.6 ± 1.1
5-Week AC (10)	22.3 ± 1.4

Note. Values shown are means ± SE. Numbers of animals given in parentheses. AC, aorta constricted.

10 enzyme activity ratios for lactate dehydrogenase indicate altered isoenzyme composition. Progressive increase in this ratio from 1.89 ± 0.06 at 26 days of age, to 1.96 ± 0.05 at 8 weeks of age, to 2.16 ± 0.06 at approximately 10 weeks of age suggest significant variance in heart lactate dehydrogenase isoenzyme composition. Pressure overloading for 5 days had no significant influence on the 0.33/10 enzyme activity ratios of either weanling or adult rat hearts. Five weeks of pressure overload was also without significant effect in adult animals. However, 5 weeks of pressure overloading in weanling rat hearts elicited a significantly lower 0.33/10 enzyme activity ratio when compared to age-matched control values.

The results from analyses of isolated mitochondria prepared from left ventricles of weanling and adult rats are given in Table IV. Although minor differences in mitochondrial protein yield were present, no systematic or significant alteration in this measurement was observed in any of the groups studied.

The specific activity of citrate synthase enzyme measured in purified mitochondrial preparations was not significantly altered in any of the groups studied. The mitochondrial form of malate dehydrogenase enzyme was specifically measured in purified mitochondrial suspensions because suspensions were treated with ethanol to assure inactivation of any potential contaminating cytoplasmic form of the enzyme. Under these controlled conditions, significant differences in malate dehydrogenase specific activity could not be detected in any of the groups. Therefore, neither normal growth nor imposed pressure overload influenced mitochondrial protein yield, citrate synthase specific enzyme activity, or malate dehydrogenase enzyme specific activity.

Because the specific activity of citrate synthase measured in isolated mitochondria did not vary significantly, the citrate synthase enzyme results for whole-heart homogenate given in Table V provide estimates of total mitochondrial mass and relative mitochondrial mass per gram left ventricular tissue as indicated by their respective values (μmole/

TABLE III. LACTATE DEHYDROGENASE ENZYME ACTIVITY IN WHOLE-HEART HOMOGENATES OF WEANLING AND ADULT RATS

	10 m*M* Pyruvate	1 m*M* Pyruvate	0.33 m*M* Pyruvate	0.33/10
Weanling				
5-Day control (10)	242 ± 12	496 ± 24	454 ± 20	1.89 ± 0.06
5-Day AC (10)	240 ± 11	486 ± 24	447 ± 23	1.87 ± 0.06
5-Week control (10)	266 ± 6	559 ± 9	517 ± 8	1.96 ± 0.05
5-Week AC (10)	310 ± 10*	588 ± 18	534 ± 14	1.74 ± 0.06*
Adult				
5-Day control (10)	237 ± 5	532 ± 13	509 ± 11	2.16 ± 0.06
5-Day AC (10)	255 ± 7	548 ± 12	529 ± 9	2.10 ± 0.05
5-Week control (10)	268 ± 9	566 ± 12	538 ± 14	2.03 ± 0.08
5-Week AC (10)	286 ± 15	631 ± 8	601 ± 9	2.13 ± 0.10

Note. Values shown are means ± SE. Numbers of animals given in parentheses. All enzyme activities are μmole/g/min. AC, aorta constricted.

* $P < 0.05$ vs comparable control.

TABLE IV. ENZYME ACTIVITIES IN MITOCHONDRIA ISOLATED FROM HEARTS OF WEANLING AND ADULT RATS

	Protein yield (mg/gm)	Citrate synthase (μmole/mg/min)	Malate dehydrogenase (μmole/mg/min)
Weanling			
5-Day control (6)	9.0 ± 0.7	2.37 ± 0.05	14.44 ± 0.92
5-Day AC (6)	6.3 ± 0.8	2.48 ± 0.11	15.41 ± 1.00
5-Week control (10)	6.3 ± 0.4	2.39 ± 0.10	11.58 ± 1.00
5-Week AC (12)	7.0 ± 0.6	2.51 ± 0.13	13.69 ± 1.61
Adult			
5-Day control (11)	6.4 ± 0.5	2.24 ± 0.14	11.60 ± 1.40
5-Day AC (9)	5.9 ± 0.5	2.32 ± 0.23	15.60 ± 1.60
5-Week control (6)	5.7 ± 0.4	2.55 ± 0.14	10.59 ± 0.78
5-Week AC (10)	6.5 ± 0.2	2.58 ± 0.14	11.47 ± 0.41

Note. Values are means ± SE. Number of animals given in parentheses. AC, aorta constricted.

min and μmole/g/min). Mitochondrial mass underwent approximately a threefold increase during the normal heart developmental period encompassing 26 days postbirth (31 μmole/min) until 8 weeks postbirth (88 μmole/min). At that juncture and beyond, the left ventricular citrate synthase values (μmole/min) stabilized as shown by no further alteration in 5-day control and 5-week control adult values.

Five days of sustained pressure overload resulting from abdominal aortic constriction had no significant influence on either total mitochondrial mass or mitochondrial mass per gram tissue assessed by citrate synthase enzyme measurements. In contrast, 5 weeks of aortic constriction-induced pressure overload results in significant and substantial increases in total mitochondrial mass amounting to approximately 45 and 30% in weanling and adult rat hearts, respectively.

TABLE V. CITRATE SYNTHASE ENZYME ACTIVITY IN WHOLE-HEART HOMOGENATES OF WEANLING AND ADULT RATS

	Citrate synthase	
	(μmole/min)[a]	(μmole/g/min)
Weanling		
5-Day control (10)	31 ± 2	112 ± 9
5-Day AC (10)	36 ± 4	90 ± 9
5-Week control (10)	88 ± 5	133 ± 6
5-Week AC (10)	128 ± 18*	119 ± 5*
Adult		
5-Day control (10)	81 ± 3	120 ± 2
5-Day AC (10)	84 ± 3	126 ± 1
5-Week control (10)	78 ± 2	118 ± 3
5-Week AC (10)	101 ± 5*	109 ± 6

Notes. Values are means ± SE. Numbers of animals given in parentheses. AC, aorta constricted.

[a] μmole/min = μmole/g/min × left ventricular weight.

* $P < 0.05$ vs comparable control.

Malate dehydrogenase enzyme assays in whole homogenate have the capability for determining both the cytoplasmic and mitochondrial forms of the enzyme. The total quantity of malate dehydrogenase enzyme, i.e., sum of cytoplasmic and mitochondrial forms, increased roughly in proportion to the overall increase in left ventricular mass in view of the "total activity" results presented in Table VI. Cytoplasmic malate dehydrogenase constituted approximately 45% of the total enzyme activity in the left ventricle of 26-day-old rats. During normal heart growth and development, the relative percentage of cytoplasmic malate dehydrogenase was 49 ± 3%, 46 ± 3%, and 46 ± 2% for 5-week weanling control, 5-day adult control, and 5-week adult control groups, respectively (Table VI). Five days or 5 weeks of pressure overloading had no influence on the relative contribution of cytoplasmic enzyme.

As was the case for citrate synthase enzyme activities measured in whole-heart homogenates, the malate dehydrogenase enzyme activity values given in Table VI provide estimates of total mitochondrial mass (μmole/min) and mitochondrial mass per gram left ventricular tissue (μmole/g/min). In addition, the relative proportion of enzyme activity present in the mitochondrial form is also given in Table VI. Malate dehydrogenase results in whole homogenate show that mitochondrial mass increased approximately 2-fold as a

TABLE VI. MALATE DEHYDROGENASE ENZYME ACTIVITY IN WHOLE-HEART HOMOGENATES OF WEANLING AND ADULT RATS

	Malate dehydrogenase					
	Total activity	Cytoplasmic activity		Mitochondrial activity		
	(μmole/g/min)	(μmole/g/min)	(% total)	(μmole/min)[a]	(μmole/g/min)	(% total)
Weanling						
5-Day control (10)	1487 ± 53	677 ± 47	45 ± 3	244 ± 19	810 ± 48	55 ± 3
5-Day AC (10)	1317 ± 115	618 ± 89	45 ± 4	266 ± 18	700 ± 52	55 ± 4
5-Week control (10)	1676 ± 96	836 ± 101	49 ± 3	549 ± 40	840 ± 39	51 ± 3
5-Week AC (10)	1509 ± 72	658 ± 58	43 ± 3	827 ± 48*	851 ± 50	57 ± 3
Adult						
5-Day control (10)	1423 ± 158	682 ± 91	46 ± 3	499 ± 60	741 ± 81	53 ± 3
5-Day AC (10)	1621 ± 129	750 ± 115	42 ± 4	634 ± 53	863 ± 53	58 ± 4
5-Week control (10)	1721 ± 89	812 ± 70	46 ± 2	711 ± 37	908 ± 36	54 ± 2
5-Week AC (10)	1715 ± 129	938 ± 73	51 ± 3	800 ± 44	853 ± 46	49 ± 3

Note. Values shown are means ± SE. Numbers of animals given in parentheses. AC, aorta constricted.

[a] μmole/min = μmole/g/min × left ventricular weight.

* $P < 0.05$ vs comparable control.

function of normal heart growth from 26 days postbirth to 8 weeks postbirth. In contrast to the results obtained for citrate synthase, mitochondrial malate dehydrogenase results suggest that an additional 1.5-fold increase in mitochondrial mass occurs during the intervening 5-week growth period from 8 to 10 weeks of age (5-day adult control) until 13 to 15 weeks of age (5-week adult control). Five days of pressure overloading resulted in statistically nonsignificant increases in mitocondrial mass in weanling and adult rats, respectively. More prolonged pressure overloading (5 weeks) resulted in a statistically significant increase in total mitochondrial mass for weanling animals amounting to approximately 50%. Mitochondrial mass accumulated in proportion to the magnitude of accumulated left ventricular tissue as indicated by the enzyme specific activity results for 5-week aorta constricted weanling rats. Although 5 weeks of pressure overload in adult rats brought about a small increase in mitochondrial mass, amounting to approximately 12–15%, left ventricular tissue accumulated to a relatively greater degree, thus resulting in a 5–10% reduction in enzyme activity per gram left ventricular tissue.

The relative percentage of whole-heart homogenate malate dehydrogenase enzyme activity present as the mitochondrial form was approximately 55% in all control groups studied (Table VI). Five days or five weeks of pressure overloading exerted no significant effect on the percentage of mitochondrial enzyme in weanling or adult rat hearts.

Discussion. The present studies were conducted to examine the response of metabolic marker enzyme activities in the hearts of pressure overloaded weanling and adult rats. Pressure overloading stimulated left ventricular tissue accumulation in both the young and adult animal; however, a significant potential difference in adaptive heart growth becomes readily apparent when left ventricular weight responses are examined. The left ventricle of 26-day-old rats was undergoing rapid enlargement as a function of normal, developmental growth. A 2.5-fold increase in left ventricular weight took place during the 5-week period of normal growth encompassing 26 days of age until 8 weeks of age. This magnitude of heart growth, in and of itself, is remarkable. Nevertheless, imposition of pressure overloading in weanling rat hearts induced heart adaptive growth over and above the normal heart growth. The summation of these two processes in aorta constricted weanling rats, i.e., normal growth plus adaptive growth, produced a 52% increase in left ventricular weight when compared with age-matched controls. Although pressure overloading in adult rats

increased left ventricular weight by approximately 1.5-fold, the magnitude of the weight increase (39%) was substantially less than that observed in the weanling animal. Thus, induction of adaptive heart growth by pressure overload produces differences in relative magnitude depending upon the existing status of myocardial growth at the time an adaptive stimulus is encountered. Rapidly enlarging hearts of weanling rats are more responsive in terms of adaptive growth than the less rapidly enlarging hearts of adult rats (17).

Significant alterations in phosphotofructokinase enzyme activity were not detected in these experiments; therefore, it appears that glycolytic potential is well established in the young mammalian heart and this level of metabolic potential is maintained during both normal growth (19) and adaptive growth in young and adult rats.

In contrast to the phosphofructokinase evaluations, apparent lactate dehydrogenase isoenzyme composition systematically altered in the present studies. Young animals in general, and hearts of young animals in particular, are known to be more tolerant of hypoxia than their adult counterparts (20, 21). Our lactate dehydrogenase enzyme activity results obtained in the presence of different substrate concentrations are consistent with a superior anaerobic metabolic capacity in left ventricular tissue of young rats. Anaerobic capacity is progressively reduced as normal heart growth and development transpire (22, 23). The fully developed heart of adult animals seems to have less dependency upon potential anaerobic metabolism, presumably due to the progressive enhancement of an aerobic reserve capacity (19, 24, 25). Once the adult heart has reduced its anaerobic reserve capacity, pressure overloading does not reestablish the lactate dehydrogenase enzyme activity pattern characteristic of the younger heart so long as extensive myocardial fibrosis is not induced (26, 27). Pressure overloading in weanling animals appears to arrest the normal lactate dehydrogenase developmental responses.

From the whole-homogenate results of the present study, we agree with the conclusion of Baldwin *et al.* (19) that enzyme activities associated with aerobic metabolism are augmented in normal heart growth. It is also interesting to note that mitochondrial numbers accumulate to a greater extent than left ventricular tissue increased during normal heart growth. These "excess" mitochondria per gram left ventricular tissue may represent the initiation of an aerobic metabolic reserve capacity to offset declining anaerobic reserves (24).

Because citrate synthase enzyme specific activity was unaltered in isolated mitochondria from all heart tissue examined in our experiments, the product of whole homogenate enzyme specific activity (μmole/g/min) and left ventricular weight (g) provided an estimate (μmole/min) of existing mitochondrial numbers. Mitochondrial numbers increased approximately 3-fold as normal heart development occurred. Imposition of pressure overload for the same 5-week period in weanling rats elicited adaptive mitochondrial accumulation at approximately twice the normal accumulation rate. Pressure overload in adult rat heart resulted in a substantial net accumulation of mitochondria (approximately 25%) over a 5-week period. However, the rate of mitochondrial accumulation in the adapting adult heart amounted to less than 1% increase per day.

The relative partitioning of malate dehydrogenase enzyme within the heart cell should provide information regarding enhancement of enzyme synthesis as well as enzyme compartmentalization during adaptive heart growth. Malate dehydrogenase "total activity" was maintained or slightly enhanced during normal growth and adaptive growth in weanling and adult rat hearts. Therefore, it appears that enzymatic synthesis, or more correctly net accumulation of enzyme, it appropriately enhanced to maintain the proper proportion of enzyme within the tissue. If it is assumed that malate dehydrogenase enzyme is synthesized primarily within the cytoplasmic portion of the cell via the nuclear genome (29, 30), then the mitochondrial form of malate dehydrogenase ensues by subsequent incorporation of preformed enzyme protein into the mitochondrial matrix (31, 32). Cytoplasmic and mitochondrial forms of malate dehydrogenas enzyme comprise about 45 and 55%, respectively, of total enzyme activity in the groups of rats studied. Normal heart growth and pressure overloading are without influence on relative enzyme partitioning. Thus, incor-

poration of enzyme into the mitochondrial matrix is not influenced during normal and adaptive heart growth.

The results of the present experiments show that adaptation-inducing stresses imposed upon hearts of weanling and adult rats yield qualitatively and quantitatively different responses when selected enzymes representing various metabolic systems are examined. It may be that reduction of aerobic metabolic reserve occurs in hearts of both weanling and adult animals exposed to pressure overloading. In contrast to the heart of weanling animals, adult hearts do not compensate for "dilution" of mitochondrial number per gram tissue via alteration of lactate dehydrogenase isoenzyme composition. Although this proposed metabolic situation may contribute substantially to the limitation of functional reserves associated with adult heart adaptation to pressure overload, the control of underlying synthetic mechanisms remains to be determined.

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