Biochemical Analyses of Human Papillary Muscles and Guinea Pig Ventricles in Failure (35151)

MENARD M. GERTLER, ERICH SALUSTE, AND FRANK SPENCER

Department of Rehabilitation Medicine and Surgery, New York University Medical Center, New York, New York 10016

Much of the biochemical evidence relevant to the understanding of heart failure has been studied in laboratory animals. The findings are conflicting because of the variations in animal and in experimental design. The relative ease with which human papillary muscle is available during open heart surgery suggested the possibility that clinical congestive heart failure could be studied with human myocardial tissue. Furthermore, it was thought that the ability to produce an experimental model in the guinea pig for comparison purposes would strengthen the interpretation of the data accumulated in the human material.

The literature suggests that myocardial failure occurs when biochemical energy fails to be converted into mechanical or pumping energy. The fundamental cause of this failure has been studied from diverse viewpoints without complete success. Various authors have published conflicting reports on the role of oxidative phosphorylation (1, 2), reduction in high energy compounds (3, 4), ionic movements (5, 6), contractile protein (7, 8), and protein synthesis (9, 10). Since the mitochondrion is the site for the conversion of energy in the myocardium, studies designed to test the metabolic integrity of these bodies were made in several laboratories. Experiments in this laboratory on failing guinea pig heart mitochondria did not reveal abnormal $P/2e$ ratios unless the mitochondria were challenged with multiple cofactors (11–13). From studies involving the incorporation of inorganic ^{32}P into ATP and ADP, it was concluded that mitochondrial ATP was unaffected by failure (14). These results are essentially in agreement with other authors (2) who suggested that myocardial contractility in experimental heart failure in guinea pigs and cats is not due to an intrinsic defect in mitochondrial function. Normal ATP stores have been found in hypertrophic and failing cat papillary muscles (4). Moreover, it has been reported that in human hearts there is a high degree of mitochondrial structural and functional integrity and normal ATP content in biopsy samples taken from failing left ventricles as compared to nonfailing (hypertrophied) right ventricles from patients with tetralogy of Fallot (15).

Another area of approach merited attention. Starling in 1897 suggested the involvement of the sympathetic nervous system in human heart failure. This lead was followed up and it was learned that myocardial catecholamines are depleted in experimental hypoxia (16), experimentally failing dog hearts (17), and in failing human papillary muscles (18). Recent publications have demonstrated that the action of catecholamines is mediated, at least in part, through the stimulation of adenyl cyclase in adenosine cyclic 3′, 5′-phosphate (CAMP) formation (19, 20). Since CAMP is involved in the conversion of phosphorylase B to A and activation of lipolysis at the cellular level (21, 22), norepinephrine depletion may affect the availability of substrate for oxidative needs and the content of high energy phosphates.

It was this background of information that prompted this investigation which will emphasize: (i) norepinephrine content (catecholamines affect contractility and substrate availability), (ii) glycogen and glycolysis intermediates (especially important in response to oxygen lack and CAMP activated glycogenolysis) and (iii) adenine nucleotide content (they are important for respiratory control and energy generation). Mention is made also on some citric acid cycle interme-

diates and adenyl cyclase activity. Finally, it is hoped that the results will offer a new rationale for the treatment of congestive heart failure.

Methods and Materials. Patients selected for cardiac surgery were classified as to their physiological functional capacity according to the standards approved by the New York Heart Association (23). The patients ranged in age from 9 to 66 years. Twenty-seven were male and 41 were female. No age or sex dependent differences were apparent. The cardiac abnormality in 48 patients was rheumatic mitral stenosis; in 15 patients, aortic stenosis (5 rheumatic and 10 Mönckeberg's disease); and in 5 patients, tetralogy of Fallot in failure. None of the specimens was obtained from patients with normal heart function or failure category I; 9 belonged to category II; 42 to category III; and 17 to category IV. The surgical bypass had been in place from 60 to 90 min before the papillary muscles were removed. The removed portion of the left anterior papillary muscle, weighing circa 500 mg, was immediately dropped into liquid nitrogen, or into petroleum ether–dry ice mixture. The frozen biopsies were kept in small capped vials in dry ice until analyses were performed. Not all assays were performed on each specimen because of the sample size.

The frozen samples were weighed in a porcelain crucible which was precooled with dry ice and placed into a Carver cylinder[1] and pulverized by several hammer strikes. The pulverized tissue was carefully ground in a mortar to a fine powder. At this stage the coarse particles of connective tissue were separated manually from the fine muscle powder by repeated shaking of the mortar against a hard surface. Glycogen analyses were performed according to Seifter *et al.* (24) on three portions ranging from 25 to 50 mg. The rest of the muscle powder was transferred to an all glass Potter-Elvehjem type tissue homogenizer and the weight of the tissue was noted. A prechilled 6% (w/v) solution of perchloric acid was added in the amount of 3.2 ml/g of tissue powder. The tissue was homogenized. The homogenate was centrifuged at 4° and 1000*g* for 5 min. The clear supernatant was neutralized with 5 *M* potassium carbonate against bromocresol green indicator and kept at 0° until used. The subsequent assays were performed immediately after neutralization on suitable aliquots. Norepinephrine and epinephrine were assayed as a unit after the method of Crout (25). Small amounts of ^{3}H-DL-norepinephrine (sp act, 1 Ci/mmole) were used for recovery corrections after aluminum oxide absorption and eluation. Glucose-6-phosphate and ATP were assayed according to Lamprecht and Trautschold (26a); pyruvate, ADP and AMP according to Adam (26b); lactate and malate according to Hohorst (26c); and glutamate according to Bernt and Bergmeyer (26d). All analyses were pretested and recovery was found to be 92 to 104%. Adenyl cyclase was estimated in separate fresh tissue samples essentially according to Sutherland *et al.* (19) and employing ^{14}C-labeled ATP as substrate. The reaction was stopped by 3-min incubation in boiling water. A suitable aliquot from clear supernatant was chromatographed in isopropanol:concentrated ammonia:water (70:10:20). Radioactive areas were located by strip scanning; and final radioactivity measurements were made by scintillation counting directly on the paper of the area containing CAMP. Activity is expressed in units used by Sutherland *et al.* (19).

Experimental heart failure was produced in guinea pigs according to Gertler (27). Surgical manipulations were performed under sodium pentobarbital anesthesia using 0.25 ml of 60 mg/ml solution injected intraperitoneally per animal. The restriction of blood flow by about 33% through the ascending aorta causes an enlargement of the left ventricle, left atrium, and right cardiac chambers; and passive venous congestion of lungs, liver, spleen, and kidneys (27). The animals were sacrificed after 10 days and the increase of heart and spleen weight by at least 75% over control values was used as an estimate of the degree of failure. The tissues and assays of metabolites were performed as described above for human papillary muscles. The entire heart was used.

Results. Norepinephrine, glycogen, and glucose-6-phosphate: Table IA represents

[1] Arthur A. Thomas Co. Cat. No. 8479-A.

TABLE IA. Human Papillary Muscle Content of Norepinephrine, Glycogen, and Glucose-6-phosphate in Various Degrees of Failure.

Compound	Cat. II			Cat. III			Cat. IV		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Norepinephrine (μg/g)	3	0.37	±0.04	6	0.24	±0.20	9	0.16	±0.07[a]
Glycogen (mg/g)	6	5.69	±1.10	23	7.39	±2.65	14	8.89	±3.28[b]
Glucose-6-phosphate (μmoles/g)	6	0.30	±0.24	22	0.19	±0.18	15	0.09	±0.12[b]

[a] Comparison between categories II and IV, $p < .001$; [b] $p < .05$.

B. Guinea Pig Heart Content of Norepinephrine, Glycogen, and Glucose-6-phosphate in Controls and Failure.

Compound	Control hearts			Failing hearts		
	N	Mean	SD	*N*	Mean	SD
Norepinephrine (μg/g)	5	1.22	±0.32	6	0.34	±0.08[a]
Glycogen (mg/g)	6	3.42	±0.95	6	6.56	±1.56[b]
Glucose-6-phosphate (μmoles/g)	6	0.30	±0.08	6	0.37	±0.05[c]

[a] Comparison between controls and failure hearts, $p < .001$; [b] $p < .01$; [c] $p < .10$.

the levels of norepinephrine, glucose-6-phosphate and glycogen in human papillary muscles. It is noted that there is a fall in the former two and a rise in the latter with increasing degree of failure. The data show significant decreases in glucose-6-phosphate and norepinephrine from category II to category IV ($p < .001$ and $< .05$, respectively) and a significant increase in glycogen from category II to category IV ($p < .05$).

Guinea pig failing ventricles showed a similar trend in norepinephrine and in glycogen levels but not in glucose-6-phosphate (Table IB).

TABLE IIA. Human Papillary Muscle Content of Adenine Nucleotides in Failure.[a]

Compound	Cat. II			Cat. III			Cat. IV		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
ATP (μmoles/g)	6	3.20	±1.44	24	2.50	±0.87	14	2.49	±0.72
ADP (μmoles/g)	5	0.90	±0.49	22	1.05	±0.44	9	1.11	±0.31
AMP (μmoles/g)	4	0.23	±0.08	21	0.27	±0.14	10	0.32	±0.27
ATP/ADP	5	3.84	±2.02	22	2.71	±1.39	9	2.47	±0.88

[a] Differences between categories II and IV are not significant at .05 level.

B. Guinea Pig Heart Content of Adenine Nucleotides in Controls and Failure.

Compound	Control hearts			Failing hearts		
	N	Mean	SD	*N*	Mean	SD
ATP (μmoles/g)	6	3.67	±0.32	6	1.77	±0.08[a]
ADP (μmoles/g)	6	1.45	±0.16	6	1.21	±0.05[b]
AMP (μmoles/g)	6	0.41	±0.06	6	0.39	±0.02[a]
ATP/ADP	6	2.55	±0.31	6	1.47	±0.09

[a] Comparison between control and failure hearts, $p < .001$; [b] $p < .01$.

TABLE III. Adenylate Cyclase Activity in Normal and Failing Guinea Pig Heart Homogenates.[a]

No.	Activity (μmoles/100 g of tissue/15 min) (19)	
	Control hearts	Failing hearts
1	17.6	19.9
2	16.1	19.5
3	17.0	18.5
4	17.0	16.8
Av	16.92	18.68
SD	0.62	1.38
SE	0.31	0.69

[a] Df = 6; $t = 2.312$; difference not significant at the .05 level.

ATP, ADP, and AMP. The content of ATP, ADP, and AMP did not vary to any significant degree in the human papillary muscles (Table IIA). There was, however, a significant drop in ATP ($p < .001$) and in ADP ($p < .01$) between the failure and normal guinea pig ventricles (Table IIB). The ATP/ADP ratio did not vary significantly among the three categories of human papillary muscle but did show a significant decrease ($p < .001$) between normal and failing guinea pig ventricles.

Adenyl cyclase activity. The studies on this question are summarized in Table III. These data show that there is virtually no arithmetical difference between the normal and failing guinea pig hearts in the levels of adenyl cyclase. This is not in agreement with Sobel and co-workers' observation (28). An attempt to explain these differences is made in the discussion.

Glycolysis and citric acid cycle intermediates. These data are summarized in Table IVA and B for human papillary muscles and guinea pig hearts. Note that in spite of the arithmetical differences seen in the tables, only lactate concentration was significantly higher in failing guinea pig hearts.

Discussion. The study of human papillary muscles in various degrees of failure has yielded information concerning changes in various biochemical parameters in human heart failure. These findings are compared with the results obtained from experimentally produced failing guinea pig hearts. The most important and significant observation in both papillary muscles and guinea pig hearts is a gradual decrease in the norepinephrine content consonant with an increase in glycogen level with increasing severity of failure. The values for norepinephrine content in papillary muscles reported herein are close to those reported earlier (18, 29, 30). Their patients were not classified according to de-

TABLE IVA. Human Papillary Muscle Content of Glycolysis and Citric Acid Cycle Intermediates in Failure.[a]

Compound (μmoles/g)	Cat. II			Cat. III			Cat. IV		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Pyruvate	5	0.14	±0.10	22	0.23	±0.18	10	0.19	±0.22
Lactate	3	13.58	±2.06	22	10.18	±4.93	8	12.11	±8.08
Malate	2	2.28	±1.03	6	2.35	±2.72	3	5.28	±3.78
Glutamate	4	4.80	±2.56	14	3.97	±2.17	6	4.90	±5.02

[a] Differences not significant at the .05 level.

B. Guinea Pig Heart Content of Pyruvate and Lactate in Controls and Failure.

Compound (μmoles/g)	Control hearts			Failing hearts		
	N	Mean	SD	N	Mean	SD
Pyruvate	6	0.13	±0.03	6	0.12	±0.01
Lactate	6	7.22	±1.00	6	10.41	±0.64[a]

[a] Comparison between control and failure hearts, $p < .001$.

gree of failure, but they found a significant correlation between the ventricular norepinephrine concentration and the maximum active tension developed by the muscle. These observations support the view that catecholamines influence both the contractile force and metabolism of normal and failing heart. The relationship between cardiac performance and glycogen content has not been clearly established. Elevated cardiac glycogen has been reported in rats with hypertrophy of the heart produced by daily periods of swimming (31). Even surgical sympathetic denervation of the heart produces depletion of myocardial stores of catecholamines with simultaneous increase in glycogen (32, 33). Accordingly, when there is a decrease in norepinephrine, it is reasonable to suggest that the activation of adenyl cyclase would be impaired and consequently there would be a diminished amount of CAMP available for the activation of phosphorylase B to A with consequent failure in glycogenolysis with glycogen deposition.

There was a significant decrease in glucose-6-phosphate ($p < .001$) between category II and category IV in the human papillary muscle studies. This trend was not observed in the guinea pig hearts. The explanation for this is not readily apparent.

With our assay system for adenyl cyclase, there is virtually no difference in failing and normal guinea pig hearts. This observation is at a variance with Sobel and his group (28). However, it should be pointed out that the method of producing experimental heart failure is essentially the same for both groups (27), but the assay systems for adenylate cyclase activity are different. The values reported herein are based on activity in whole heart homogenates while Sobel and coworkers' conclusions are based on data obtained from purified particle fractions. By using ^{14}C-labeled ATP as substrate it was possible to detect very small amounts of CAMP generated. This permitted the use of 10 to 50 mg of tissue/assay and linearity within a 15-min incubation period. The ratio of tissue to final assay volume was in the range of 1 part tissue to 50 to 250 parts of medium, thus effectively eliminating the somewhat variable substrate contribution from endogenous ATP. Endogenous norepinephrine content may affect seriously the fraction of active adenyl cyclase. Even relatively low amounts of residual norepinephrine in failing guinea pig hearts may be enough to activate adenylate cyclase maximally during the homogenization procedure. The assays reported herein were performed in the presence of 0.01 *M* sodium fluoride. According to Sutherland and co-workers (19) fluoride at a concentration of 0.01 *M* doubles or triples adenyl cyclase activity and may largely or entirely erase the hormone effect. At the onset of this work, it was found that without sodium fluoride, adenylate cyclase activity was low and highly variable. It is reasonable to suggest that our results concerning adenylate cyclase represent the maximal adenyl cyclase activity available, which is not diminished in failing whole guinea pig heart homogenates. Although our observations at present do not permit any direct conclusions, Sobel and co-workers' observation of a decreased adenyl cyclase activity in failing guinea pig heart particles supports the possibility of a defect in norepinephrine-adenyl-cyclase-CAMP system in heart failure. This is supported by the observations reported herein that there is a rise in glycogen content and a decrease in glucose-6-phosphate as failure increases.

Comparing the content of ATP, ADP, and AMP in various degrees of failure in human papillary muscles we found a decrease in ATP content consonant with an increase in ADP content. None of the changes was significant. AMP and the sum of ATP + ADP + AMP remained nearly unaffected in papillary muscles. Chidsey and his colleagues (15) and Brunwald and his group (34) also found virtually no difference in the ATP content of the papillary muscles removed from six humans who were not in congestive heart failure [3.6 ± 0.6 (SE of the mean) μmoles/g] as compared to left ventricular papillary muscles removed from seven patients who were in moderate to severe congestive heart failure secondary to rheumatic heart disease [3.2 ± 0.5 μmoles/g]. They concluded that there was no evidence that a defi-

ciency in high energy phosphate stores is responsible for the onset of heart failure. Using their nonfailing control values of 3.6 ± 0.6 μmoles/g for ATP as base line and comparing the values against categories III and IV reported here, a slight but nonsignificant ($p > .05$) decrease of ATP content might be present. No comparable values for ADP and AMP are available in literature for human papillary muscles in failure.

The behavior of adenine nucleotide in failing guinea pig hearts is somewhat different. Both, ATP and ADP were depleted in failing hearts to a highly significant degree. AMP remained unchanged. There was a significant ($p < .001$) net loss of 2.16 μmoles/g adenine nucleotides, mainly derived from the ATP pool and accounting to 39% of total adenine nucleotides present in control hearts. The exact nature of this loss is not known. Increased availability of AMP to sarcoplasmic adenylic deaminase may be one possibility. The explanation for the different behavior of adenine nucleotides in experimental heart failure in guinea pigs may lie in the fact that the degree of failure observed in the guinea pigs is far greater than category IV failure observed in humans. Increased lactate concentration in failing guinea pig hearts indicates the presence of anoxemia, known to affect cardiac ATP stores (3).

Chance and Williams (35) as well as Plaut and Aogaichi (36) have presented evidence which favors the concept that the ATP/ADP ratio is an indication of respiratory control in the mitochondria. The higher the ratio the more anaerobic the cell respiration. In these results the ratio decreased significantly in guinea pig hearts ($p < .001$) and decreased almost significantly in human papillary muscles as the failure worsened (Table IIA, B). The theoretical concept as well as the observed decrease in ATP/ADP ratio suggests a shift from anaerobiosis to a greater need of oxygen in failing heart because of the increased requirement of oxidative energy. This is in keeping with previous published observations by our group whereby it was shown that in mitochondria derived from failing guinea pig hearts the $\overset{N}{Q}_{O_2}$ values increased almost twofold to generate ATP (12, 13).

There were no significant differences in the levels of various intermediates such as pyruvate, lactate, malate, and glutamate in the various failure categories. This is somewhat at variance with animal experiments which stated that the lactate increased during experimental failure. In the failing guinea pig hearts the lactate levels increased significantly ($p < .001$). The question arises whether the rise in guinea pig heart lactate was due to failure or anoxemia which is known to cause an increase in cardiac lactate (37). Since none of the metabolites in this group was significantly increased or decreased in failing human papillary muscles it is reasonable to suggest that the mechanisms of glycolysis and citric acid pathways are essentially undamaged in human cardiac failure. This concept is further supported from work in this laboratory (12, 13) and by Chidsey and coworkers (15) that electron transport and coupled phosphorylation were normal in mitochondria isolated from congestive failing guinea pig hearts and from failing human hearts. In addition, there was no real reduction of the content of high energy phosphate compounds in human hearts. The theoretical implications from this communication suggests that congestive heart failure is a result of gradual deterioration of metabolic processes which eventually lead to a failure of myocardial contractility; but according to Chidsey *et al.*, (18) there is no evidence to suggest that the contractile process itself is so damaged in the failing heart that its function cannot be increased by stimulation with catecholamines. These data support the thesis that the increase of available CAMP would be beneficial to the restoration of congestive heart failure to normal function. This has been tested by giving intravenous glucagon (38), epinephrine and other sympathomimetic substances (39), each of which increases adenyl cyclase activity so that ATP may be converted to CAMP. As an extension of this work CAMP has been employed directly by intravenous infusion in cases of intractable human congestive heart failure. The success has been gratifying and the treatment is being pursued and extended (40).

Summary. Studies were made on (a) hu-

man papillary muscles derived during open heart surgery in various degrees of failure, and (b) experimentally produced failing guinea pig hearts. There was a significant decrease in norepinephrine from 1.22 to 0.34 μg/g in the experimental guinea pig hearts as compared to normal. There was also a significant decrease in norepinephrine from 0.37 to 0.16 μg/g in human papillary muscle as the degree of failure increased. Glucose-6-phosphate decreased significantly from 0.30 to 0.09 μmoles/g in the human papillary muscles with increasing degree of failure. Similarly there was a significant rise in glycogen from 3.42 to 6.56 mg/g in the guinea pig hearts and from 5.69 to 8.89 mg/g in the papillary muscles. The differences in glycolysis and citric acid cycle intermediates were not significant. The ATP/ADP ratio decreased from 3.84 in grade II failure to 2.47 in grade IV failure. This ratio is a reflection of the degree of aerobiosis in mitochondria metabolism and indicates in failure a shift to more aerobiosis. The values on guinea pig heart adenyl cyclase activity were not statistically different, being 16.9 and 18.7 μmoles/100 g of tissue/15 min.

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