Differences in Efficiency of Energy Transfer in Mitochondrial Systems Derived from Normal and Failing Hearts.*† (26253)

MENARD M. GERTLER
Research Division, Dept. of Physical Medicine and Rehabilitation, New York Univ. Medical Center, New York

In contrast to the voluminous literature on the problem of congestive heart failure from both the clinical and pathophysiological viewpoint, there is a dearth of information on the basic mechanism of congestive heart failure from the viewpoint of bioenergetics. A method for producing congestive heart failure in animals has recently become available and has afforded the opportunity to study one important type of congestive heart failure, *i.e.*, decreased output failure, backward failure, or increased forward resistance failure, etc., with employment of biochemical technics(1,2).

Many theories which have been espoused to explain the genesis of congestive heart failure are adequately reviewed elsewhere(1,3). Starling's Law of the heart is utilized in nearly all instances except those where there is failure in energy production, *e.g.*, beriberi; energy transport, *e.g.*, thyrotoxicosis and energy utilization, *e.g.*, marked coronary artery disease with fibrosis.

The data presented herein are an attempt to demonstrate some metabolic differences in mitochondrial systems derived from normal guinea pig hearts and hearts from guinea pigs in experimental congestive heart failure. Also, further information was sought on bioenergetics as related to Starling's Law of the heart(4).

Experiments were designed to obtain further information on these two important questions from the biochemical viewpoint. Heart mitochondrial systems were obtained from guinea pigs in which congestive heart failure was produced experimentally. The effects of adding various cofactors known to affect the metabolism of the heart mitochondrial system were noted on the $Q^N_{O_2}$‡ values and P/O ratios in heart mitochondrial systems prepared from normal guinea pigs and experimental guinea pigs.

Methods and materials. Hartley Albino male guinea pigs weighing circa 400 g were divided into 2 groups. In one group (usually 6-8 animals) congestive heart failure was produced by restricting the aortic outflow *via* an intrathoracic incision(2). The remaining group of 6-8 animals served as a control. Heart mitochondrial systems were prepared by a method of Maley and Plaut(6). The mitochondria prepared showed no oxidation without substrate and there was no demonstrable ATPase activity(7).

In the oxidation and phosphorylation studies, each Warburg vessel contained the following medium (variations from this composition are indicated in the text and tables): (a) Main compartment: phosphate buffer pH 7.4 (0.1 M) 50.00 μM, magnesium chloride (0.1 M) 10.00 μM, ATP (0.1 M) 6.00 μM, cytochrome C (0.003 M) 0.03 μM, mitochondria 0.80 ml (equivalent to 0.5 mg of nitrogen), substrate α-ketoglutarate (0.2 M) 20.00 μM, sucrose (0.25 M) to a final volume of 3.0 ml. (b) Center well: 5N potassium hydroxide 0.20 ml. (c) Side-arm: hexo-

* This work was supported by Nat. Inst. Health grant.

† The following symbols will be used throughout this paper:

CoA	—	Coenzyme A
DPN	—	Diphosphopyridine nucleotide (oxidized)
TPN	—	Triphosphopyridine nucleotide (oxidized)
TCA	—	Trichloroacetic acid
ATP	—	Adenosine triphosphate
ATPase	—	Adenosine triphosphatase
C.H.F.	—	Congestive heart failure
$Q^N_{O_2}$	—	μl of oxygen consumed per hr per mg of mitochondrial nitrogen
P/O	—	$\frac{\text{Micromoles of orthophosphate consumed}}{\text{Microatoms of oxygen utilized}}$
ΔP	—	Micromoles of orthophosphate consumed by the system
ΔO	—	Microatoms of oxygen utilized by the system

‡ The abbreviations are listed separately.

kinase (400 Cori units) in 2% glucose.

Hexokinase was prepared by the Colowick and Darrow§ method and purification accomplished to the Bentonite step. The medium components were added to flasks chilled in an ice-bath. The freshly prepared suspension of mitochondria (containing circa 0.8 mg of nitrogen per flask) was added to the main compartments just prior to attaching the flasks to the manometers. After an equilibration period of 12 minutes at 30°C, the hexokinase-glucose mixture was added to the main compartment, the zero time flask was removed, and manometric readings of the incubated samples were made, in sequence at one minute intervals. Each sample was run for 15 minutes and occasionally for 20 minutes. At termination of the experiment each flask was quickly removed from the bath, placed in ice, and the reaction was stopped immediately with 2.0 ml of ice-cold 20% trichloracetic acid. The coagulated protein was removed by centrifugation. The orthophosphate content of the protein-free filtrate was determined by the method of Lowry and Lopez(8). All analyses and experimental flasks were done in duplicate and occasionally in triplicate to assure reliable results. Nitrogen was determined by a modification of Johnson's technic(9).

Results. Table I summarizes the results obtained by adding, either in combination or individually, various cofactors such as CoA, DPN and TPN to mitochondrial preparations from both normal and failure guinea pigs. $Q^N_{O_2}$ values and P/O ratios showed little difference between heart mitochondrial systems derived from the normal and experimental guinea pigs, *i.e.,* 1170 and 1032, and 2.90 and 2.91 respectively. Addition of the individual cofactors revealed certain differences in $Q^N_{O_2}$ value and P/O ratio. In the mitochondrial systems derived from both the normal and failure animals, there was a stimulation of respiration with addition of CoA and DPN added separately, but P/O ratio was affected only slightly. Addition of CoA and DPN together and CoA and TPN together stimulated respiration to an even greater degree but did not lower the P/O ratio appreciably. TPN alone did not stimulate the respiration and appeared to decrease the efficiency of oxidative phosphorylation in both groups. Whether this is an artifact or real cannot be answered at this juncture.

The effect of adding all cofactors, *i.e.,* CoA, DPN and TPN to both mitochondrial systems had a similar effect in both groups, *i.e.,* stimulation of respiration and reduction in efficiency of oxidative phosphorylation. These results are emphasized in Table I.

$Q^N_{O_2}$ values increase under this treatment in both the normal and failure mitochondrial

TABLE I. Effect of Adding Various Cofactors Either Singly, or in Combination, on Respiration and Efficiency of Phosphorylation by Cardiac Mitochondrial System Prepared from Normal and Failure Guinea Pigs Employing α-Ketoglutarate as Substrate.*

	Normal†		Failure‡	
	$Q^N_{O_2}$	P/O	$Q^N_{O_2}$	P/O
Complete system	1170	2.90	1032	2.91
Idem + CoA + DPN + TPN	1480§	2.32‖	1524¶	1.95**
" + CoA	1385	2.88	1256	2.73
" + DPN	1163	2.80	1032	2.95
" + TPN	380	1.25	305	1.07
" + CoA + DPN	1403	2.95	1595	2.46
" + CoA + TPN	1096	2.63	1069	2.64

* For each experiment 6-8 guinea pig hearts were employed. Figure represents avg of 3 exp.

† Values represent avg of 4 exp.

‡ " " " " 5 " .

§ Represents increase of 26.5% over normal values in complete system only. "t" test = 6.03; p = >.001.

‖ Represents a decrease of 20.0% as compared with normal values in complete system only. "t" test = 3.5; p = >.01.

¶ Represents increase of 47.5% over normal values in complete system only. "t" test = 20.4; p = >.0001.

** Represents decrease of 33.0% as compared with normal values in complete system only. "t" test = 6.2; p = >.001.

Test of significance employed is based upon Snedecor(12).

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{(s.e._1)^2(N_1-1) + (s.e._2)^2(N_2-1)}{N_1+N_2-2} \times \frac{1}{N_1} + \frac{1}{N_2}}}$$

$\overline{X}$ = mean value; N = total No. in sample; s.e. = stand. error of mean value.

§ The author wishes to thank Dr. Colowick and Dr. Darrow for permitting the author to use the method prior to publication.

TABLE II. This Table Summarizes $\triangle P$, $\triangle O$, and $Q^N_{O_2}$ Values and P/O Ratio in Both Normal and Experimental Failure Animals with and without Addition of Cofactors CoA, DPN and TPN. Amount of energy derived from the mitochondrial systems is also given.

	$\triangle P$	$\triangle O$	P/O	$Q^N_{O_2}$	Calories, energy $\sim P^*$
Normal	27.08	9.29	2.90	1170	306×10^4
Normal + cofactors	26.98	11.70	2.32	1480	306×10^4
Failure	21.12	7.15	2.91	1032	268×10^4
Failure + cofactors	21.14	10.82	1.95	1524	265×10^4

* Calculated on basis of $Q^N_{O_2}$ value.

$Q^N_{O_2}$ value

11.2 (for conversion of molecular oxygen to atomic oxygen)

$\times$ 10,000 (assuming each terminal bond of phosphorus is equivalent to 10,000 calories)

$\times$ Oxidative phosphorylation (P/O ratio)

=

Sample calculation:

$$\frac{1170}{11.2} \times 10{,}000 \times \frac{2.90}{1} = 306 \times 10{,}000 =$$

systems, but P/O ratios decrease in both instances. $Q^N_{O_2}$ values increase to a larger degree in the failure systems, 47.5% *vs.* 26.5%, while P/O ratio falls to a great degree, 33.0% *vs.* 20.0% in the same system.

In the studies with mitochondrial systems derived from normal and failure animals, the calculated energy derived does not change with addition of cofactors. This is of particular interest in the case of the experiments with mitochondrial systems derived from failure hearts where there is a considerable drop in the P/O ratio but the inordinate rise in $Q^N_{O_2}$ value in the same system compensates for the loss in efficiency of phosphorylation (Table II).

Discussion. The syndrome of congestive heart failure produced experimentally in the guinea pig is very similar to that observed in humans with chronic aortic stenosis. This experimental type of congestive heart failure differs from that produced by Alexander *et al.* (10), and Barger(11), in that in the former experiments the abdominal aorta is compressed while in the latter the tricuspid valve is avulsed and the pulmonary artery compressed. In the former the failure produced shows more hypervolemia while the latter is more of chronic right heart failure.

These data pose an interesting question which may have some relationship to Starling's Law of the heart(4). One of the most interesting observations in the phenomenon of Starling's Law of the heart is that cardiac oxygen consumption continues to increase during the increased systolic ejection concomitant with cardiac efficiency and continues to increase in spite of the decreased systolic ejection and subsequent decrease in cardiac efficiency(5). Several questions arise from these observations: (a) Why does oxygen consumption continue to increase despite the decreased cardiac efficiency? (b) What is the relationship between oxygen consumption and source of energy required by the heart to continue ejecting the required blood?

A possible explanation for the increased oxygen utilization in spite of decreased cardiac efficiency as observed by Evans *et al.* (5) may be apparent from the experiments presented herein. It is possible to compare certain aspects of bioenergetics when heart mitochondrial systems are prepared from animals in congestive heart failure and compared with similar systems derived from normal animals, (a) under conditions which may closely parallel those observed *in vivo* and (b) under conditions of biochemical stress or stimulation. With the substrate α-ketoglutarate, $Q^N_{O_2}$ values and P/O ratios are virtually the same in both groups (Table I). Under the influence of the combination of various cofactors, *e.g.*, CoA, DPN and TPN, $Q^N_{O_2}$ value of the heart mitochondrial systems prepared from animals in congestive heart failure rises to 1524 while that of the heart mitochondrial system prepared from the normal group rises to 1480. The most important difference exists, however, in the P/O ratios. The P/O ratio in the experimental group decreases to 1.95 while that in the normal group decreased slightly to 2.32.

One interpretation of these data is as follows. In the normal guinea pig heart, the inordinate increase in rate of respiration, as

evidenced by the increases $Q^N_{O_2}$ value does not change the efficiency of phosphorylation. Thus, amount of work may be increased, efficiency remains constant, and total calories produced are the same (Table II). In the heart mitochondrial systems derived from the experimental animals, there is an even greater increase in respiration rate, but there is less available energy per atom of oxygen because the efficiency of phosphorylation is decreased. However, in spite of this apparent loss of efficiency, total amount of energy obtained is the same (Table II).

It thus may be possible to interpret Starling's Law in biochemical terms. The energy which the heart (whether normal or in failure) requires to perform its normal cardiac output is provided by the coupling of oxidation and phosphorylation. However, when there is an uncoupling or loss in efficiency of rate of phosphorylation from oxidative mechanisms, as is the case of mitochondria derived from failing guinea pig hearts under the influence of cofactors, greater oxidation will be required to produce the equivalent amount of energy for the usual amount of cardiac work (Table II). Thus it may be reasonable to suggest a relationship between the physiologic observation that oxygen consumption increases in the failing heart, *viz.* Starling's Law, with the observation of the increased $Q^N_{O_2}$ value of mitochondria derived from failing hearts under the influence of cofactors.

Summary and conclusions. 1. Oxidation rates employing α-ketoglutarate as substrate and efficiency of phosphorylation coupled with oxidation were studied in heart mitochondrial systems prepared from normal guinea pig hearts and hearts from guinea pigs in congestive heart failure. 2. $Q^N_{O_2}$ values were 1170 and 1032 for the normal guinea pig hearts and experimental guinea pig heart mitochondrial systems for α-ketoglutarate oxidation respectively. P/O ratios were 2.95 and 2.91 respectively. 3. Addition of cofactors (CoA, DPN and TPN), increased $Q^N_{O_2}$ values from 1170 to 1480 in the normal heart mitochondrial systems and from 1032 to 1524 in the mitochondrial systems derived from animals in experimental congestive heart failure. P/O ratios decreased slightly in the normal systems (2.95 and 2.32) while the P/O ratio decreased from 2.91 to 1.95 in the animals in congestive heart failure. 4. The increase in energy production parallels the overall increase in ~ phosphorus during addition of cofactors in both normal animals and failure animals. Although the efficiency of phosphorylation in the mitochondrial system derived from failing hearts is markedly decreased during the influence of the cofactors, energy production is maintained by the inordinate increase in respiration. The possible relationship of this observation to basic physiologic mechanisms is suggested.

1. Burch, G. E., *A Primer of Congestive Heart Failure,* C. C Thomas, Springfield, Ill., VIII, 126.

2. Gertler, M. M., Thesis: Some Biochemical Aspects of Experimentally Produced Congestive Heart Failure. April, 1958, N.Y.U. Post-Grad. Med. School.

3. Harrison, T. R., *Principles of Internal Medicine,* 2nd ed., XXII, 1703, v86, p117, 1954.

4. Patterson. S. W., Piper, H., Starling, E. H., *J. Physiol.,* 1914, v48, 465.

5. Evans, C. L., *ibid.,* 1918-19, v52, 6.

6. Maley, G. F., Plaut, G. W. E., *J. Biol. Chem.,* 1953, v205, 297.

7. Lardy, H., Copenhaver, J. H., *Nature,* 1954, v174, 231.

8. Lowry, O. H., Lopez, J. A., *J. Biol. Chem.,* 1946, v162, 421.

9. Johnson, M. J., *ibid.,* 1941, v137, 575.

10. Alexander, N., Hinshaw, L. B., Drury, D. R., *Circulation Res.,* 1957, v4, 375.

11. Barger, A. C., Richardson, G. S., Roe, B. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 113.

12. Snedecor, George W., *Statistical Methods,* 5th ed. Conts. 13, pp. 534, Iowa State College Press, Ames, Iowa, 1956.

Received September 6, 1960. P.S.E.B.M., 1961, v106.