Anaerobic Rat Heart

EFFECTS OF GLUCOSE AND TRICARBOXYLIC ACID-CYCLE METABOLITES ON METABOLISM AND PHYSIOLOGICAL PERFORMANCE

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1. The ability of tricarboxylic acid-cycle metabolites to influence the physiological performance of the perfused anaerobic rat heart was investigated. Energy expenditure/h [(beats/min) $\times$ 60 $\times$ systolic pressure/g of protein] for various anoxic conditions compared with oxygenated control hearts were: 5mm-glucose, 4.5%; 20mm- or 40mm-glucose, 10%; 20mm-glucose plus fumarate+malate+glutamate, 29%; 20mm-glucose plus oxaloacetate and α -oxoglutarate, 31%. 2. The energy expenditure/lactate production ratio was increased by the tricarboxylic acid-cycle metabolites, indicating that alterations in anaerobic physiological performance did not result from changes in glycolysis. 3. Analysis of tissue constituents provided further indication of an enhanced energy status for fumarate+malate+glutamate- and oxaloacetate+ α -oxoglutarate-perfused hearts; tissue concentrations of both glycogen and ATP were higher than in the 20mm-glucose-perfused groups. 4. A marked increase in the accumulation of succinate in tissues perfused with oxaloacetate+ α -oxoglutarate or fumarate+malate+glutamate provided further evidence that these metabolites were stimulating mitochondrial energy production under anoxia. 5. These studies indicate that mitochondrial ATP production can be stimulated in an isolated mammalian tissue perfused under anaerobiosis with a resulting enhancement of cell function.

The object of this investigation was to determine whether perfusion of anoxic hearts with tricarboxylic acid-cycle metabolites and glucose could stimulate mitochondrial synthesis of ATP and thereby enhance anaerobic cardiac function. Studies of two mitochondrial reactions have demonstrated synthesis of ATP under anoxia. Anaerobic formation of ATP coupled to the fumarate-dependent oxidation of NADH was obtained in ox heart (Sanadi & Fluharty, 1963) as well as in rat heart mitochondrial particles (M. Wilson & J. Cascarano, unpublished work). The second anaerobic ATP-generating reaction involving reduction of oxaloacetate and oxidation of α -oxoglutarate was investigated by Hunter (1949) in rat liver and kidney. Perfusion of anoxic rat hearts with glucose and metabolites such as fumarate, malate and glutamate or oxaloacetate and α -oxoglutarate, which should theoretically stimulate these reactions, resulted in significant increases in heart rate above glucose controls (Cascarano, Chick & Seidman, 1968). Although glucose was found to be an absolute requirement for contractility to occur under anoxia, these findings suggest that mitochondrial reactions could

appreciably enhance ATP production and therefore be of physiological importance in the absence of oxygen. However, these investigators did not assess other aspects of cardiac function contributing to energy expenditure (such as ventricular pressure generated during contraction), nor was there any evaluation of the relative glycolytic and mitochondrial energy contributions.

The present investigation was designed to assess the influence of mitochondrial energy production on cardiac function in the anoxic rat heart. The product of peak aortic pressure and heart rate was selected as the index of cardiac function or of the energy expended by the heart (Katz, 1963; Neely, Liebermeister, Battersby & Morgan, 1967). The first objective was to determine whether the energy expended by the heart could be stimulated under anaerobic conditions by mitochondrial metabolites to a greater extent than that possible with glucose alone. The second objective was to determine whether the mitochondrial metabolites alter the ratio of total energy expenditure to glucose utilization or to lactate output. A constant ratio would suggest that these metabolites were merely stimulating glycolysis whereas an increase

would indicate generation of energy from an extra-glycolytic source. The third objective was to learn whether mitochondrial metabolites serve to increase concentrations of glycogen, ATP, ADP and creatine phosphate. The fourth objective was to determine whether the end product of the anaerobic mitochondrial reactions, succinate, did indeed accumulate in the tissue and perfusion fluid.

MATERIALS AND METHODS

Hearts were perfused by the technique of Langendorff (1895). Male Sprague-Dawley rats (300–400g) were decapitated 5 min after intravenous injection of sodium heparin (0.2 ml, 1000 U.S.P. units/ml; Invenex, San Francisco, Calif., U.S.A.) and the hearts were rapidly transferred to 0.9% NaCl at 0°C. After the heart had been freed of extraneous tissue it was mounted on a cannula at the aorta, flushed free of remaining blood with 15 ml of perfusate and subsequently perfused in a gassed recirculating system. Hearts were first perfused for 20 min with 25 ml of Krebs–Ringer bicarbonate buffer (Umbreit, Burris & Stauffer, 1964) gassed with O₂+CO₂ (95:5). At the end of this period 25 ml of a metabolite solution was added. For the next 70 min the atmosphere remained the same for aerobic perfusions, but was changed to N₂+CO₂ (95:5) for anaerobic perfusions.

Metabolites were made up in stock solutions as sodium salts at pH 6.9, equivalent in Na⁺ concentration to 0.9% NaCl. Metabolites were incorporated in the Krebs–Ringer bicarbonate buffer for addition to the perfusion medium by substituting metabolite for an equivalent volume of 0.9% NaCl to give the desired concentration. The Na⁺ concentration was constant at 142 m-equiv./l. Final substrate concentrations after addition were: fumarate, 12.5 mM; malate, 10 mM; glutamate, 12.5 mM; oxaloacetate, 14 mM; α -oxoglutarate, 14 mM. Glucose was dissolved in the Krebs–Ringer bicarbonate buffer to give the desired concentration. The final pH of the perfusing solutions was 7.4.

Perfusion was accomplished by maintaining a constant hydrostatic pressure (47.8 mmHg) on the heart. The upper reservoir of the perfusion apparatus maintained a constant fluid height at 65 cm above the heart. Fluid flowed from this reservoir through the aortic cannula to the heart located in a lower chamber. Perfusate draining out of the heart collected in the bottom of the perfusion chamber. A pulsatile pump was used to transfer filtered perfusate back to the upper reservoir. An overflow tube in the upper reservoir maintained the fluid height constant and returned excess of perfusate to the lower chamber. The gases introduced into the heart chamber passed through the overflow tube to the upper reservoir where they were allowed to escape into the atmosphere. Gassing was continuous.

The temperature of the perfusion fluid was maintained at 37 $\pm$ 0.5°C. A small mercury thermometer was inserted in one of the arms of a 4-way connector introduced into the aortic cannula so that perfusing fluid flowed directly over the bulb of the thermometer and provided monitoring of temperature just before the fluid entered the heart. Intra-aortic pressure measurements were made with a Statham P23BB pressure transducer (Honeywell Inc.,

Denver, Colo., U.S.A.) attached to the other arm of the 4-way connector in the aortic cannula and recorded on a Honeywell 1508 visicorder.

With the time of addition of metabolites designated as 0 min, perfusate samples, rate of beating and pressure measurements were taken at 10 min intervals from –1 to +70 min. Perfusate samples were immediately made up to cold 5% (v/v) perchloric acid. The perfusate was assayed for lactate by the method of Hohorst (1965) and for glucose by the method of Huggett & Nixon (1957). Heart rate and aortic systolic pressure were determined from the intra-aortic pressure records. Peak aortic pressure was multiplied by beats/min and used as an index of energy expenditure/min. For total energy expenditure/h these values were averaged, multiplied by 60 and divided by total heart protein.

For determination of tissue constituents experiments were terminated by rapidly freezing hearts with a Wollenberger clamp cooled to –78°C (Wollenberger, Ristau & Schoffa, 1960). The tissue was pulverized in a percussion mortar and then with a mortar and pestle (all at –78°C). The powder was then divided into portions for assay. Glycogen assay was performed by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956), protein by the method of Lowry, Rosebrough, Farr & Randall (1951), ATP and creatine phosphate by the method of Lamprecht & Trautschold (1965), ADP by the method of Adam (1965) and succinate by the method of Busch, Hurlbert & Potter (1952). All tissue, perfusate and energy expenditure analyses are expressed per g of heart protein and are given as the means $\pm$ S.E.M. Statistical analysis of the succinate results was not possible, since this assay was conducted on pooled tissue samples.

The materials used were obtained from the following sources: glucose, Baker and Adamson, Morristown, N.J., U.S.A.; Dowex AG1-X8, (200–400 mesh), Bio-Rad Laboratories, Richmond, Calif., U.S.A.; oxaloacetic acid, α -oxoglutaric acid, malic acid, fumaric acid, glutamic acid, sodium phosphoenolpyruvate, ATP, ADP, NAD⁺, NADH, NADP⁺, lactate dehydrogenase, glucose oxidase, peroxidase, myokinase, glucose 6-phosphate dehydrogenase, hexokinase, creatine phosphokinase and *o*-dianisidine, Calbiochem, Los Angeles, Calif., U.S.A.; bovine serum albumin, Pentex Inc., Kankakee, Ill., U.S.A.; pyruvate kinase, Sigma Chemical Co., St Louis, Mo., U.S.A.

RESULTS

Influence of metabolites on cardiac function, glucose uptake and lactate output. These experiments were undertaken to determine whether perfusion with various metabolites could enhance anaerobic cardiac function and influence the relationship of function to lactate output. This was done by examining the influence of the perfusion medium on heart rate, aortic pressure generated during systole, energy expenditure/min, glucose utilized and lactate produced. The 20 min preliminary period (in which hearts were perfused with oxygenated substrate-free medium) was used to establish whether cardiac performance had attained certain minimal criteria of rate and stability so that

possible damage from handling would not be confused with the effects of anoxia.

Anaerobic perfusions were then conducted with 0, 5, 20 and 40 mM-glucose to establish optimum glucose perfusion concentrations. Addition of glucose was essential for stimulating cardiac function under anoxia. Substrate free medium (0 mM-glucose) resulted in complete cessation of physiological activity; most hearts stopped beating by +20 min and none were beating at +40 min (Table 1). The pressure indicated at +50 and +70 min for this group in Table 2 is simply the hydrostatic pressure imposed on the heart by the conditions of the experiment. Heart rate, peak

aortic pressure and energy expenditure (+10 to +70 min; Tables 1, 2 and 3) were all stimulated by the presence of glucose. Increasing the glucose concentration from 5 to 20 mM increased total energy expenditure, glucose uptake and lactate output (Table 4). No further increases were evident at 40 mM-glucose. All subsequent perfusions conducted with tricarboxylic acid-cycle metabolites included 20 mM-glucose.

To obtain an index of myocardial performance under optimum conditions hearts were perfused with 20 mM-glucose *in vitro* in the presence of oxygen. The 20 min preliminary perfusion appeared to be adequate for stabilizing the preparation since the

Table 1. *Rate of beating in perfused rat hearts subjected to anoxia (70 min) after aerobiosis (20 min)*

Perfusions were conducted with 25 ml of Krebs-Ringer bicarbonate buffer gassed with O₂ + CO₂ (95:5) from -20 to 0 min. At 0 min the gassing mixture was changed to N₂ + CO₂ (95:5) and an additional 25 ml of Krebs-Ringer bicarbonate buffer containing the metabolites was added. The final concentrations of glucose in the perfusion medium are indicated below. In addition, one group of hearts was perfused with 12.5 mM-fumarate + 10 mM-malate + 12.5 mM-glutamate and 20 mM-glucose (FMG) and another group with 14 mM-oxaloacetate + 14 mM- α -oxoglutarate and 20 mM-glucose (OO) (the abbreviations FMG and OO have the same significance in Tables 2-5). Anaerobic perfusions lasted from 0 to +70 min. Mean heart rate $\pm$ s.e.m. is expressed as beats/min at 37°C with the numbers of hearts perfused indicated in parentheses.

Concn. of glucose (mM)	Perfusion time	Heart rate (beats/min)				
		... -1 min	+10 min	+30 min	+50 min	+70 min
Substrate and N ₂ + CO ₂ (95:5) introduced at 0 min						
0	(5)	244 ± 28.90	89.2 ± 6.53	0.67 ± 0.67	0	0
5	(6)	232 ± 28.60	16.5 ± 4.17	13.00 ± 5.69	7.19 ± 2.94	2.46 ± 1.35
20	(6)	269 ± 16.90	27.9 ± 10.40	24.30 ± 6.12	24.00 ± 8.05	18.80 ± 9.16
40	(6)	189 ± 13.90	24.8 ± 8.41	17.70 ± 5.38	15.30 ± 3.29	7.01 ± 2.17
20+OO	(6)	264 ± 17.90	114.0 ± 5.51	83.20 ± 5.28	86.10 ± 11.00	75.10 ± 14.90
20+FMG	(6)	268 ± 7.38	91.9 ± 13.00	84.40 ± 12.10	78.10 ± 8.58	62.00 ± 5.96
Substrate introduced at 0 min; O ₂ + CO ₂ (95:5) throughout						
20	(6)	280 ± 9.52	279.0 ± 7.93	288.00 ± 6.20	269.00 ± 7.07	266.00 ± 6.58

Table 2. *Aortic systolic pressures generated by perfused hearts subjected to anoxia (70 min) after aerobiosis (20 min)*

Experimental conditions were identical with those described in Table 1. Systolic pressure was determined as described in the Materials and Methods section. Mean systolic pressure $\pm$ s.e.m. is expressed as mmHg at 37°C with the numbers of hearts perfused indicated in parentheses.

Concn. of glucose (mM)	Perfusion time	Aortic systolic pressure (mmHg)				
		... -1 min	+10 min	+30 min	+50 min	+70 min
Substrate and N ₂ + CO ₂ (95:5) introduced at 0 min						
0	(5)	70.2 ± 3.44	49.7 ± 0.22	48.0 ± 0.20	47.8 ± 0.00	47.8 ± 0.00
5	(6)	74.0 ± 5.11	83.2 ± 13.40	101.0 ± 12.10	88.4 ± 8.48	68.5 ± 11.60
20	(6)	75.7 ± 3.73	99.2 ± 15.70	105.0 ± 22.30	70.2 ± 8.40	57.5 ± 3.31
40	(6)	75.6 ± 3.32	109.0 ± 15.40	108.0 ± 11.70	114.0 ± 14.70	107.0 ± 10.30
20+OO	(6)	76.9 ± 2.21	63.0 ± 1.87	69.3 ± 1.09	68.8 ± 1.08	68.4 ± 1.13
20+FMG	(6)	70.6 ± 3.02	60.1 ± 2.00	62.4 ± 2.59	61.7 ± 2.59	60.9 ± 2.55
Substrate introduced at 0 min; O ₂ + CO ₂ (95:5) throughout						
20	(6)	69.5 ± 1.51	69.6 ± 2.55	70.5 ± 2.65	65.7 ± 2.28	61.7 ± 1.68

Table 3. *Energy expenditure in perfused rat hearts subjected to anoxia (70 min) after aerobiosis (20 min)*

Experimental conditions were identical with those described in Table 1. Energy expenditure/min was determined by multiplying beats/min by systolic pressure in mmHg. Mean values $\pm$ S.E.M. are given with the numbers of hearts perfused indicated in parentheses.

Concn. of glucose (mM)	Perfusion time	Energy expenditure/min				
		... -1 min	+10 min	+30 min	+50 min	+70 min
Substrate and N ₂ + CO ₂ (95:5) introduced at 0 min						
0	(5)	91.0 ± 17.1	23.60 ± 3.13	0.09 ± 0.106	0	0
5	(6)	106.0 ± 16.8	8.75 ± 2.71	6.67 ± 2.67	4.28 ± 2.22	1.57 ± 1.20
20	(6)	139.0 ± 16.1	15.40 ± 5.04	12.20 ± 2.04	11.10 ± 3.78	8.54 ± 4.47
40	(6)	87.3 ± 12.8	12.70 ± 4.17	10.20 ± 2.87	10.10 ± 3.54	4.77 ± 3.13
20 + 0O	(6)	128.0 ± 17.5	45.10 ± 5.26	36.10 ± 3.67	36.20 ± 4.63	31.00 ± 6.85
20 + FMG	(6)	132.0 ± 9.2	37.40 ± 3.46	35.40 ± 2.88	32.80 ± 2.19	25.80 ± 1.49
Substrate introduced at 0 min; O ₂ + CO ₂ (95:5) throughout						
20	(6)	121.0 ± 10.4	120.00 ± 5.51	126.00 ± 8.14	110.00 ± 7.72	102.00 ± 8.65

Table 4. *Glucose uptake, lactate output and energy expenditure/h in perfused rat hearts subjected to anoxia (70 min) after aerobiosis (20 min)*

Experimental conditions were identical with those described in Tables 1, 2 and 3. Analytical procedures are described in the Materials and Methods section. The values listed below are cumulative results obtained from +10 to +70 min. The time period 0 to +10 min was not included to allow for equilibration of metabolites and exchange of gases. Glucose uptake and lactate output are expressed as μ mol/h per g of protein. Values of total energy expenditure are averages of energy expenditure/min (Table 3) multiplied by 60 and divided by total heart protein. These results are given as means $\pm$ S.E.M. with the numbers of hearts perfused indicated in parentheses. The other columns represent ratios of these means.

Concn. of glucose (mM)	Glucose uptake	Lactate output	10 ⁻⁵ $\times$ Total energy expenditure	Lactate output/ glucose uptake	Energy expenditure/ μ mol of glucose	Energy expenditure/ μ mol of lactate
Substrate and N ₂ + CO ₂ (95:5) introduced at 0 min						
0	(5) 0	32 $\pm$ 4	—	—	—	—
5	(6) 759 $\pm$ 40.0	1515 $\pm$ 107	3.09 $\pm$ 0.89	2.00	490	206
20	(6) 1045 $\pm$ 112.0	2094 $\pm$ 230	7.09 $\pm$ 2.12	2.02	695	318
40	(6) 1066 $\pm$ 132.0	1963 $\pm$ 213	5.76 $\pm$ 1.49	1.84	561	293
20+00	(6) 1410 $\pm$ 121.0	2832 $\pm$ 242	21.93 $\pm$ 1.79	2.01	1634	817
20+FMG	(6) 1676 $\pm$ 42.0	2501 $\pm$ 82	19.98 $\pm$ 1.70	1.49	1193	795
Substrate introduced at 0 min; O ₂ + CO ₂ (95:5) throughout						
20	(6) 186 $\pm$ 31.0	66.6 $\pm$ 15	69.14 $\pm$ 4.12	—	42000	165000

functional performance of hearts was not significantly changed from -1 to +70 min when experiments were continued at 0 min with an oxygenated 20 mM-glucose medium (Tables 1, 2 and 3). For aerobic hearts glucose consumption was 18%, lactate output 3.2% and total energy expenditure 975.0% of that seen in similar hearts perfused under anoxia (Table 4). The ratio of energy expenditure/ μ mol of glucose was approx. 60-fold greater in the aerobic group, apparently reflecting oxidation of endogenous metabolites in addition to a more efficient utilization of glucose.

Anaerobic perfusions were conducted next with 20 mM glucose combined with (a) 12.5 mM-fumarate + 10 mM-malate + 12.5 mM-glutamate or (b)

14 mM-oxaloacetate + 14 mM- α -oxoglutarate, to examine the possible involvement of mitochondria in anaerobic energy production. Cardiac performance (Tables 1, 2 and 3) was substantially improved above the anaerobic 20 mM-glucose control in both fumarate + malate + glutamate- and oxaloacetate + α -oxoglutarate-perfused hearts; the total energy expenditure was approx. threefold greater (Table 4). In addition, increases were obtained for both glucose uptake and lactate output (Table 4). However, these increases were small in comparison with the stimulation obtained in the total energy expenditure. The ratio of energy expenditure/ μ mol of lactate was 2.5-fold greater than the 20 mM-glucose control for both fumarate + malate + glutamate-

Table 5. *Tissue glycogen, ATP, ADP, creatine phosphate and succinate concentrations in perfused rat hearts subjected to anoxia (40 min) after aerobiosis (20 min)*

Experimental conditions were identical with those described in Table 1 except that these studies were terminated at +40 min by freeze-clamping hearts with a Wollenberger clamp cooled to -78°C . Analytical procedures are described in the Materials and Methods section. Results are given as means $\pm$ s.e.m. with the numbers of hearts perfused indicated in parentheses. All values are expressed as $\mu\text{mol/g}$ of protein.

Concn. of glucose (mM)	Tissue concentration ($\mu\text{mol/g}$ of protein)					
	Glycogen	ATP	ADP	Creatine phosphate	Succinate	
					Tissue	Perfusate
Substrate and N ₂ + CO ₂ (95:5) introduced at 0 min						
0 (5)	18.5 ± 1.30	0.13 ± 0.10	2.54 ± 0.60	0.03 ± 0.02	3.57	0.0
20 (5)	39.5 ± 6.10	6.97 ± 1.30	13.60 ± 0.90	0.00 ± 0.00	4.47	2.58
20 + 0O (5)	46.4 ± 7.10	10.20 ± 0.70	22.60 ± 1.80	0.00 ± 0.00	24.80	30.90
20 + FMG (5)	56.9 ± 2.60	9.45 ± 0.70	17.80 ± 2.90	0.20 ± 0.10	89.70	—
Perfusions terminated at 0 min						
0 (6)	40.1 ± 4.40	17.60 ± 1.50	14.40 ± 1.80	18.90 ± 2.00	0.0	0.0

and oxaloacetate + α -oxoglutarate-perfused hearts.

Analysis of glycogen, high-energy phosphates and succinate. Both heart rate and energy expenditure were found to be less stable after +40 min of anoxic perfusion under some of the experimental conditions. In addition, glucose uptake and lactate output were not linear after +40 min for all of the conditions studied. For this reason, experiments were terminated at +40 min for analysis of glycogen, high-energy phosphates and succinate. Experiments were conducted as above except that hearts were freeze-clamped at +40 min to prevent rapid deterioration of glycogen and high-energy phosphates. In addition, the controls for this group were hearts perfused under oxygen for 20 min and freeze-clamped at zero time. Six hearts were perfused for each experimental condition.

Tissue glycogen at +40 min in 0 mm-glucose hearts was less than half that in the 0 min aerobic controls (Table 5). Glycogen was not exhausted even though all hearts in this group had ceased beating by +40 min. The presence of glucose in the perfusion fluid (20 mm-glucose group) served to maintain glycogen at concentrations comparable with those found in the 0 min controls. Of note is the fact that net glycogen synthesis had occurred in the fumarate + malate + glutamate-perfused group; glycogen concentrations were significantly elevated above the 0 min control values.

The concentrations of ATP detected in each group roughly parallel energy expenditure, the 0 mm-glucose group having the lowest ATP (Table 5) and energy expenditure (Table 3), with increases occurring in both these parameters proceeding from 20 mm-glucose- to fumarate + malate + glutamate- or oxaloacetate + α -oxoglutarate-perfused hearts and finally to 0 min controls. The results for

ADP show no definitive trend other than a depressed concentration in 0 mm-glucose hearts. Creatine phosphate was essentially undetectable in all hearts except the aerobic group.

Succinate was not detectable in either the medium or tissue of the 0 min aerobic controls (Table 5). In hearts perfused with fumarate + malate + glutamate tissue succinate was 20-fold higher than in hearts perfused with 20 mm-glucose. The perfusion fluid of the fumarate + malate + glutamate group could not be properly assessed owing to the interference of the large quantity of malate. Tissue succinate plus that present in the medium for the oxaloacetate + α -oxoglutarate-perfused group was eightfold greater than values determined for the 20 mm-glucose-perfused hearts. It should be noted that succinic acid was found to accumulate in both 0 and 20 mm-glucose-perfused groups.

DISCUSSION

Many investigators have attempted to relate some measure of cardiac function to the total expenditure of energy by the heart, i.e. oxygen consumption. Cardiac output represents a minor portion of the total energy expended, since the greatest utilization of energy is involved in the development of tension in the walls of the heart and not in the ejection of fluid from the heart (Burton, 1965). It would appear that maximal tension generated in the walls of the heart would be directly reflected by peak intraventricular pressure or by peak intra-aortic pressure during ejection of fluid from the heart. Sarnoff *et al.* (1958) have shown that stroke oxygen consumption is linearly dependent on blood pressure. Katz (1963) reported a good correspondence between oxygen consumption

and the product of blood pressure and heart rate. Examination of the results obtained by Neely *et al.* (1967) reveals that the product of peak aortic pressure and heart rate is directly proportional to oxygen consumption in Langendorff-perfused hearts. On this basis the product of peak aortic pressure and heart rate was selected to approximate the energy expenditure of the heart.

Anoxia stimulated both glucose uptake and lactate output. In addition, glucose uptake was apparently non-maximal when hearts were perfused with 5mM- but not with 20mM-glucose. The results obtained on anoxic hearts perfused with 0, 5, 20 and 40mM-glucose with regard to glucose uptake, lactate output, glycogen, ATP and creatine phosphate are qualitatively comparable with those obtained by other investigators (Cornblath, Randle, Parmeggiani & Morgan, 1963; Morgan, Henderson, Regen & Park, 1961; Morgan, Neely, Brineaux & Park, 1965; Williamson, 1966). Not only was glucose utilization increased by higher concentrations of glucose, but total energy expenditure was also elevated (Table 4). In addition, an increased energy expenditure/ μ mol of lactate was obtained with both 20 and 40mM-glucose, suggesting the possibility that extraglycolytic energy production was stimulated at these higher glucose concentrations. This is supported in part by the twofold increase obtained in the production of succinate in 20mM-glucose-perfused hearts as compared with 0mM-glucose-perfused controls (Table 5).

In addition to the well-recognized contribution of glycolysis to anaerobic energy production, there is experimental evidence implicating the importance of mitochondria in this regard. Sanadi & Fluharty (1963) obtained coupled phosphorylation during the fumarate-dependent oxidation of NADH in anaerobic ox heart mitochondrial particles. This reaction has also been obtained in mitochondrial particles of heart, liver and gastrocnemius of the rat (M. Wilson & J. Cascarano, unpublished work). A second energy-generating reaction has been reported by Hunter (1949, 1951); substrate-level phosphorylation in anaerobic liver and kidney mitochondria was found to accompany reduction of oxaloacetate and oxidation of α -oxoglutarate. Succinate was found to accumulate in both reactions. It is not surprising therefore that use of metabolites that should theoretically stimulate these two reactions (fumarate + malate + glutamate or oxaloacetate + α -oxoglutarate), enhanced the physiological performance of the isolated anoxic heart in the present investigation.

Even though tricarboxylic acid-cycle metabolites stimulated glucose uptake and lactate output in this study, the energy expenditure/ μ mol of lactate was increased 2.5-fold over the anoxic 20mM-glucose control value (Table 4). This tends to rule

out the hypothesis that these metabolites were simply stimulating glycolysis. Further qualitative evidence implicating mitochondrial activity is the increase in succinate found in both fumarate + malate + glutamate- and oxaloacetate + α -oxoglutarate-perfused hearts. Other evidence for increased energy status is a net increase in glycogen for fumarate + malate + glutamate-perfused hearts above the 0min oxygenated control. In addition, ATP concentrations for both fumarate + malate + glutamate- and oxaloacetate + α -oxoglutarate-perfused hearts are higher than in the anaerobic 20mM-glucose-perfused control.

Succinate has been found to accumulate in anoxic mammalian tissues, a further indication that some anaerobic mitochondrial activity does occur in mammalian cells. It was first detected in anaerobic guinea-pig brain slices incubated in glucose + α -oxoglutarate or glucose + pyruvate (Weil-Malherbe, 1937). In addition, infusion of tritiated lactate or fumarate into intact rat liver perfused under conditions of limited oxygen partial pressure has resulted in the formation of tritiated succinate (Hoberman & Prosky, 1967).

There is some evidence that these mitochondrial reactions may be of physiological importance to the intact mammal. It was shown by Chick, Weiner, Cascarano & Zweifach (1968) that increased survival of rabbits resulted during haemorrhagic shock when tricarboxylic acid-cycle metabolites were infused into the animal. Survival was increased from 30 to 70% when infusion fluid was changed from saline or glucose to either fumarate or a combination of oxaloacetate and α -oxoglutarate.

The present investigation provides evidence for the involvement of mitochondrial energy production in perfused anaerobic hearts. The total energy expenditure of anoxic hearts was increased threefold above those perfused with glucose alone. Further, the ratio of energy expenditure/ μ mol of lactate was increased 2.5-fold, suggesting a substantial contribution of energy from extraglycolytic sources. In addition, a pronounced increase in the production of succinate was obtained. It does appear that mitochondrial reactions can be stimulated to generate sufficient energy under anaerobiosis to augment appreciably the physiological performance of the isolated rat heart.

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