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Myocardial oxygen consumption increased during acetate infusion, but the decrease in myocardial oxygen extraction and the increase in coronary sinus blood oxygen saturation suggest that an active coronary vasodilation which was not a result of the increased cardiac work, occurred. The concentration of hypoxanthine in the coronary sinus and the content of myocardial adenosine increased, which suggests that the increase in coronary blood flow was caused by the vasodilator action of adenosine released from the myocardium, and that adenosine production is not necessarily tied to PO_2.

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Metabolic Control of the Circulation

EFFECTS OF ACETATE AND PYRUVATE

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ABSTRACT Chloralose-anesthetized dogs were infused intravenously with either Tris-acetate or Tris-pyruvate at 0.0375, 0.075, and 0.15 mmol/kg per min successively, each for 20 min. Acetate infusion increased cardiac output, left ventricular *dP/dt* and *dP/dt/P*, and coronary blood flow, while pyruvate infusion did not. Infusions of either substance increased arterial blood and skeletal muscle concentrations of citrate and malate, but only acetate infusion increased the tissue AMP content and decreased the ATP:AMP ratio. The increase in cardiac output produced by acetate was accompanied by an increase in total body oxygen consumption and a decrease in the difference between arterial and mixed venous blood oxygen.

Myocardial oxygen consumption increased during acetate infusion, but the decrease in myocardial oxygen extraction and the increase in coronary sinus blood oxygen saturation suggest that an active coronary vasodilation which was not a result of the increased cardiac work, occurred. The concentration of hypoxanthine in the coronary sinus and the content of myocardial adenosine increased, which suggests that the increase in coronary blood flow was caused by the vasodilator action of adenosine released from the myocardium, and that adenosine production is not necessarily tied to P_{O_2}.

These systemic and coronary hemodynamic changes also occurred when acetate (0.075 mmol/kg per min) was infused into conscious dogs. Acetate infusion also increased blood flow to the gastrointestinal tract, kidneys, intercostal muscle, and diaphragm. These changes were not affected by propranolol pretreatment, but were abolished by pretreatment with fluoroacetate which reduced acetate oxidation.

These results suggest that the circulatory stimulation produced by acetate was not caused by increases in tricarboxylic acid cycle intermediates. Instead, it was probably related to the increased cleavage of ATP to AMP that accompanies activation of acetate to acetyl CoA, and was not mediated via β-adrenergic receptors. It is speculated that hemodynamic changes may occur in patients who undergo hemodialysis with acetate-containing dialysate. Hemodynamic changes of ethanol may also be brought about by acetate, which is one of the intermediates that accumulates during ethanol metabolism.

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INTRODUCTION

Cardiac output is increased in dogs after intra-aortic administration of cyanide, which produces metabolic effects similar to those that occur during hypoxia by inhibiting cytochrome oxidase (1, 2). Furthermore, because cyanide is bound to the tissue and none of it circulates to the heart or the central nervous system, this increase in cardiac output is probably caused by a metabolic factor that originates from the peripheral tissue (1). However, neither cardiac output nor myocardial contractility is affected by selective inhibition of glycolysis by systemic administration of iodoacetate, or of the tricarboxylic acid cycle by fluoroacetate (3). These results suggest that the increases in cardiac output and myocardial contractility that occur during peripheral tissue hypoxia might be caused by the accumulation of tricarboxylic acid cycle intermediates or by factors associated with changes in high energy phosphates. Various tricarboxylic acid cycle metabolites have been shown to exert vasodilator effects although their effects on cardiac performance were not evaluated (4, 5). Rowe (6) showed that intravenous administration of glucose and succinate produced no significant hemodynamic effects; the concentrations of tricarboxylic acid cycle intermediates, however, were not measured in these experiments.

Breakdown of ATP to ADP, AMP, and adenosine, which is increased during hypoxia and exercise (7, 8), may also lead to increases in cardiac output, myocardial contractility, and coronary blood flow. Adenosine is a highly potent vasodilator (9, 10). Adenine nucleotides are also vasodilators (9, 10), but it is probable that they act by being broken down to adenosine. Our recent finding (11) that cardiac output increases after administration of an uncoupling agent, 2,4-dinitrophenol, into vascularly isolated cross-perfused peripheral areas indicates that depletion of ATP in the peripheral tissue may be responsible, at least in part, for the increase in cardiac output that occurs during hypoxia and exercise.

This paper describes the effects of tricarboxylic acid cycle intermediate accumulation and ATP breakdown on the systemic and coronary circulations. Tricarboxylic acid cycle intermediates were increased by intravenous infusions of either acetate or pyruvate. Only acetate infusion increased cardiac output and coronary blood flow. It appears that the hemodynamic responses during acetate infusion can be attributed to ATP utilization, and subsequent formation of AMP (12) and adenosine.

METHODS

Adult male dogs that weighed between 18 and 25 kg were starved overnight and anesthetized with α-chloralose (60 mg/kg, i.v., Sigma Chemical Co., St. Louis, Mo.) after induction with halothane (Fluothane, Ayerst Laboratories, New York). The trachea was cannulated with a T tube connected to a Benedict-Roth spirometer filled with 100% oxygen to measure the rate of oxygen consumption. The aorta and the coronary sinus were cannulated with French 8 Cournand catheters, and the pulmonary artery was cannulated with a French 7 Swan-Ganz catheter (Edwards Laboratories, Inc., Santa Ana, Calif.). The aortic catheter was introduced into a femoral artery. Both coronary sinus and Swan-Ganz catheters were inserted under fluoroscopic guidance via external jugular veins. All catheters were connected to Statham P23Db pressure transducers (Statham Instruments, Inc., Oxnard, Calif.), whose signals were fed into a multichannel Brush 480 recorder (Gould, Inc., Instrument Systems Division, Cleveland, Ohio) to measure blood pressures and heart rate.

The left ventricle was cannulated via the left carotid artery with a Millar transducer-tip catheter (Millar Instruments, Inc., Houston, Tex.) for measuring left ventricular end-diastolic and systolic pressures, and the first derivative of left ventricular pressure (dP/dt). The ratio of dP/dt to a developed left ventricular pressure of 50 mm Hg was calculated. This pressure occurred during isovolumic systole and this ratio is referred to as $dP/dt/P$ (*vide supra*) (13).

Cardiac output was determined by an indocyanine green (Cardio-Green, Hynson, Westcott & Dunning, Inc., Baltimore, Md.) dye dilution technique (2), with a Gilford model 140 cardiac output system (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Mean aortic blood pressure was divided by cardiac output to yield the total peripheral vascular resistance.

Coronary blood flow was measured by a 4-aminoantipyrine indicator method (3, 14). Diastolic coronary vascular resistance (CVRd), left ventricular work (LVW), myocardial oxygen consumption ($M\dot{V}O_2$), and mechanical efficiency (ME) were calculated as follows (15):

$$\text{CVRd (dyn-s-cm}^{-5}) = \frac{\text{Adm} - \text{CVm}}{\text{CBF} \times 0.75} \times \text{DFP} \times 1{,}332;$$

$$\text{LVW (kg·m/min)} = \frac{\text{LVsm} \times \dot{Q} \times 1.36}{100};$$

$$M\dot{V}O_2 \text{ (ml/100 g per min)} = \text{CBF} \times (C_aO_2 - C_{cs}O_2) \div 1{,}000;$$

and

$$\text{ME (\%)} = \frac{\text{LVW}}{M\dot{V}O_2 \times 2.06} \times 100;$$

where Adm is aortic mean diastolic pressure, mm Hg; CVm, coronary sinus mean pressure, mm Hg; DFP, coronary diastolic inflow period, s/min; CBF, coronary blood flow, ml/100 g per min; LVsm, left ventricular mean systolic pressure, mm Hg; $\dot{Q}$, cardiac output, liters/min; C_aO_2, aortic oxygen content, ml/liter; and $C_{cs}O_2$, coronary sinus oxygen content, ml/liter.

Arterial and coronary sinus venous blood samples were obtained to measure pH on a Radiometer PHM71 acid base analyzer (Radiometer Co., Copenhagen, Denmark), and to measure oxygen content by gas chromatography (16). Plasma osmolality was calculated from the freezing point depression, with a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Mass.). Blood oxygen capacity was measured by a cyanmethemoglobin method (17). Arterial blood samples were taken for measurements of lactate (18), pyruvate (19), acetate (20), citrate (21), and malate (22). Coronary sinus and arterial blood samples were taken for measurements of adenosine, inosine, and hypoxanthine (23).

The experimental animals were divided into two groups. After a control period of 20 min, one group of seven dogs were infused intravenously with three different Tris-acetate solutions, pH 7.4, at a rate of 1.91 ml/min with a Harvard pump (Harvard Apparatus Co., Inc., Millis, Mass.), each for 20 min. A 3-M Tris-acetate (molarity is quoted with respect to acetate) stock solution was diluted serially with normal saline for each dog; the concentrations, approximately 0.4, 0.8, and 1.6 M, were adjusted to yield infusion rates of 0.0375, 0.075, and 0.15 mmol/kg per min, respectively. A second group of six dogs was infused in the same way with solutions of Tris-pyruvate.

Systemic hemodynamics were measured at 5-min intervals during the preinfusion control phase and throughout the period of infusion with acetate or pyruvate. Averages were obtained from triplicate measurements of each hemodynamic variable during the control period. The values from each dog were then averaged and are reported as control values. Coronary hemodynamic measurements were made during the control period, and 15–20 min after starting each dose of acetate or pyruvate.

Blood samples for metabolite measurements were obtained at 10-min intervals throughout the experiments. Samples of quadriceps muscle were obtained with a rapid-freezing technique during the control period and at the end of the last infusion. These samples were used to measure the contents of ATP, creatine phosphate (24), ADP, and AMP (25). At the end of the experiment, left ventricular muscle samples were obtained through left thoracotomy, while the animals were artificially ventilated, to determine the contents of adenine nucleotides, purine nucleosides, and hypoxanthine.

Arterial pH increased during acetate infusion. To study the effects of this pH change on systemic hemodynamics, five chloralose-anesthetized dogs were infused intravenously with 0.5 M Tris–HCl, pH 10, for 20 min. Systemic hemodynamic measurements were taken at 5-min intervals, and arterial pH at 10-min intervals.

Acetate infusions caused circulatory stimulation, whereas

pyruvate infusions did not. Experiments were then conducted to study the effects of acetate on systemic hemodynamics and regional blood flows in conscious dogs. The animals, sedated with morphine sulfate (1 mg/kg subcutaneously), were instrumented for measurements of hemodynamics as previously described for the anesthetized dogs, with the exception that their vessels were cannulated under local anesthesia with lidocaine (Xylocaine, Astra Pharmaceutical Products, Inc., Worcester, Mass.). Organ blood flows were measured by the radioactive microsphere method (26), as recently detailed (3). The animals were infused with acetate (0.075 mmol/kg per min) for 20 min. Systemic hemodynamic measurements were made at 5-min intervals, and organ blood flows were measured before and at the end of the acetate infusion.

To determine whether the effects of acetate are mediated via β-adrenergic receptors, acetate (0.075 mmol/kg per min) was administered to conscious animals that had been given propranolol (0.3 mg/kg, i.v.) 30 min previously. Cardiac β-adrenergic receptor blockade was verified in each dog by measuring heart rate response to intravenous injections of isoproterenol (27). The dose required to increase the heart rate 25 beats/min was determined by interpolation on the curve which related dose of isoproterenol to increment in heart rate. Acetate (0.075 mmol/kg per min) was also administered to conscious dogs that had been given fluoroacetate (2 mg/kg, i.v.) 30 min previously. Systemic hemodynamic measurements were made in both groups of dogs during the 20-min acetate infusion, as in normal conscious dogs. In addition, organ blood flows were measured in propranolol-pretreated dogs before and at the end of the acetate infusion.

Experimental results were subjected to analysis of variance for repeated measures (28), and the statistical significance of differences between the control and experimental values was determined by Dunnett's test (29). Correlation and linear regression were computed. Student's t test for paired comparisons was used to determine the statistical significance of a difference between two means in the same group of animals. The changes are considered statistically significant if P values are less than 0.05. Values given are mean±SE.

RESULTS

Hemodynamic effects of acetate and pyruvate infusions in anesthetized dogs. Fig. 1 shows that cardiac output rose stepwise as both the concentration of acetate infused and plasma acetate increased. Mean aortic blood pressure remained unchanged throughout the experiment except at the end when a slight decrease in blood pressure was noted. The increase in cardiac output was associated with an increase in total body oxygen consumption and a decrease in oxygen difference between arterial blood and mixed venous blood (Fig. 2). Furthermore, it was accompanied by increases in both heart rate and stroke volume (Fig. 3). Arterial blood pH increased significantly from 7.38±0.01 during the control period to 7.44±0.02 during the period when the highest concentration of acetate was infused.

Left ventricular end-diastolic pressure did not change during acetate infusion, but left ventricular *dP/dt* and *dP/dt/P* increased significantly (Table I). Total peripheral vascular resistance fell, and pulmonary arterial blood pressure increased.

Table II shows that coronary blood flow increased markedly during acetate infusion. Coronary sinus oxygen saturation increased, while myocardial oxygen extraction decreased. Concomitantly, diastolic coronary vascular resistance fell. Myocardial oxygen consumption and left ventricular work increased during infusion of acetate, but mechanical efficiency did not change. Coronary sinus blood pH increased from 7.31±0.01 to 7.41±0.01 ($P < 0.05$).

In contrast to acetate infusion, pyruvate infusion produced no hemodynamic changes in either systemic or

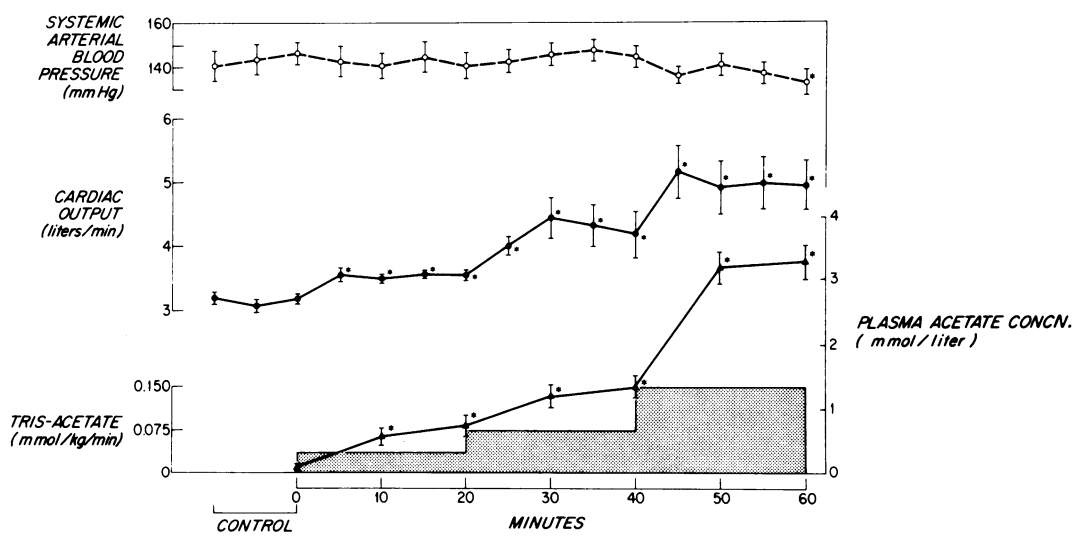

FIGURE 1 Changes in mean aortic blood pressure, cardiac output, and plasma acetate concentration during acetate infusions at rates of 0.0375, 0.075, and 0.15 mmol/kg/min each for 20 min (shaded areas) in seven chloralose-anesthetized dogs. Asterisks (*) indicate values that are statistically different from the zero-time value (control) at $P < 0.05$, as determined by Dunnett's test (29).

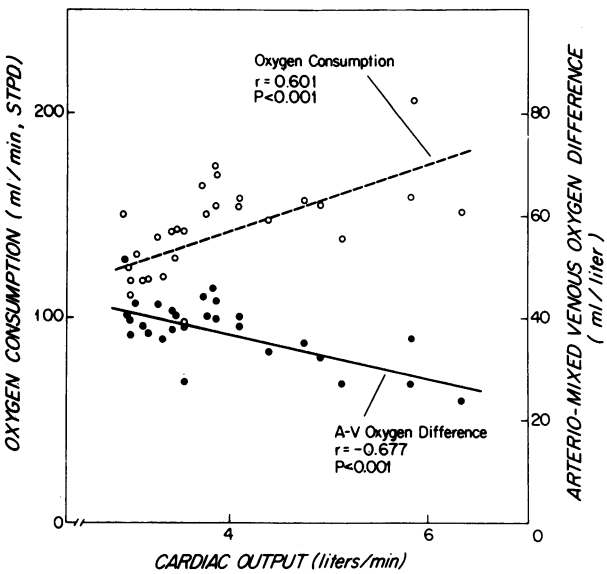

FIGURE 2 Positive correlation between cardiac output and total body oxygen consumption (open circles) and negative correlation between cardiac output and arterio-mixed venous blood oxygen difference (closed circles) during acetate infusion. r is correlation coefficient, and the solid line is the least-squares linear fit through the data. STPD, standard temperature and pressure, dry.

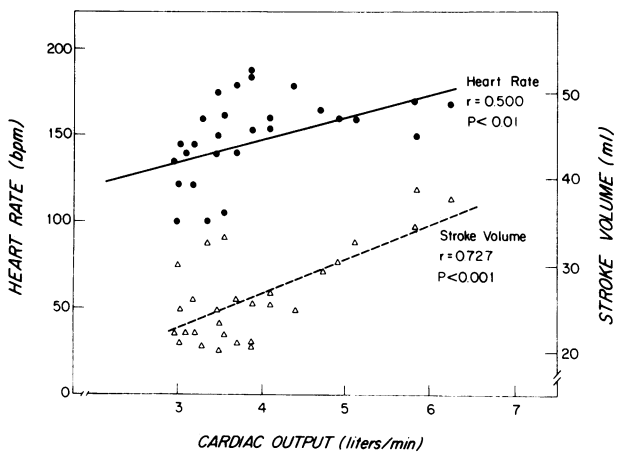

FIGURE 3 Correlations of cardiac output with heart rate (circles) and stroke volume (triangles) in acetate-infused dogs. r is correlation coefficient, and the solid line is the least-squares linear fit through the data. bpm, beats per minute.

coronary circulations (Tables I and II). There was also no significant increase in total body oxygen consumption (from 116±8 to 124±7 ml/min) or arterial blood pH (from 7.38±0.01 to 7.40±0.02).

Infusion of Tris–HCl solution (pH 10) in five dogs (21.5±0.4 kg) increased arterial blood pH from 7.35 ±0.01 to 7.41±0.02 and 7.44±0.02, at 10 and 20 min of infusion, respectively. Corresponding cardiac output values, 3.70±0.56, 4.06±0.59, and 3.85±0.42 liters/min, were not statistically different, nor did other systemic hemodynamic parameters change significantly during Tris–HCl infusion.

Metabolic effects of acetate and pyruvate infusions on arterial and skeletal muscle concentrations of tricarboxylic acid cycle and glycolytic metabolites. Ace-

TABLE I

Effects of Acetate and Pyruvate Infusions on Systemic Hemodynamics

Infusion rate	Cardiac output	Heart rate	Left ventricular: End-diastolic pressure	Left ventricular: *dP/dt*	Left ventricular: *dP/dt/P*	Mean aortic pressure	Total peripheral vascular resistance	Mean pulmonary pressure
mmol/kg/min	*liters/min*	*beats/min*	*mm Hg*	*mm Hg/s × 0.001*	*per s*	*mm Hg*	*dyn-s-cm^{-5} × 0.001*	*mm Hg*
Acetate infusion (n = 7, 21.4±0.9 kg)								
Control	3.16±0.07	112±8	7.9±0.5	3.89±0.30	49.8±3.2	145±6	3.66±0.18	13.1±0.5
0.0375	3.49±0.08*	151±5*	6.8±0.7	4.92±0.44*	59.8±6.0*	144±7	3.20±0.13*	16.5±1.4*
0.075	4.10±0.47*	161±9*	6.6±0.5	5.46±0.28*	63.0±6.2*	146±5	2.98±0.31*	16.7±1.6*
0.15	4.91±0.46*	163±7*	7.7±0.6	5.56±0.37*	63.4±4.8*	139±5	2.45±0.22*	17.3±1.1*
Pyruvate infusion (n = 6, 21.0±1.0 kg)								
Control	3.36±0.32	133±10	7.6±1.0	4.20±0.52	54.7±4.2	155±3	3.84±0.34	12.9±0.7
0.0375	3.16±0.33	132±8	7.5±1.2	4.43±0.65	54.0±5.0	161±3	4.27±0.42	12.7±0.9
0.075	3.17±0.42	143±7	7.0±1.0	4.33±0.43	53.1±4.2	159±5	4.20±0.47	12.6±0.9
0.15	3.10±0.38	145±7	7.7±1.4	4.11±0.49	51.6±5.1	153±5	4.21±0.45	12.9±1.1

* The number of experiments and body weights of the animals are given in the parentheses after each subheading. Values are averages of three measurements made between 5 and 20 min of each infusion. The experimental results between the two groups were compared by two-way analysis of variance with trend analysis (28). Asterisks (*) indicate values that are significantly different from the preinfusion control at $P < 0.05$, as determined by Dunnett's test (29).

TABLE II

Effects of Acetate and Pyruvate Infusions on Coronary Hemodynamics and Myocardial Energetics

Infusion rate	Coronary blood flow	Coronary sinus oxygen saturation	Myocardial oxygen extraction	Diastolic coronary vascular resistance	Myocardial oxygen consumption	Left ventricular work	Mechanical efficiency
mmol/kg/min	*ml/100 g/min*	%	%	*dyn-s-cm^{-5} × 0.001*	*ml/100 g/min*	*kg·m/min*	%
Acetate infusion							
Control	90±6	29±2	75±2	52.8±5.6	13±1	6.7±0.3	25±2
0.0375	169±13*	67±5*	40±3*	22.5±2.5*	14±1	7.5±0.6	28±3
0.075	227±22*	72±4*	38±5*	19.1±3.8*	17±2*	8.8±1.1*	28±3
0.15	258±13*	76±4*	36±5*	16.4±2.3*	18±1*	9.2±1.0*	26±4
Pyruvate infusion							
Control	107±12	29±6	73±3	52.3±7.9	15±2	8.1±0.8	27±4
0.0375	100±12	33±2	72±2	59.6±11.0	14±1	7.9±0.9	29±2
0.075	112±14	32±3	73±2	47.1±7.5	15±1	7.6±1.1	25±2
0.15	107±14	31±2	74±2	50.6±8.4	13±1	6.9±0.9	26±3

* The number of experiments, body weights of the animals, and the notation of asterisks (*) are the same as in Table I.

tate and pyruvate infusions produced comparable, progressive increases in arterial blood and skeletal muscle concentrations of citrate and malate. During a 60-min acetate infusion ($n = 7$), arterial blood citrate increased from 127±11 to 208±16 μM, and malate from 7±1 to 10±1 μM. These changes are significant at $P < 0.05$. Similarly, during pyruvate infusion ($n = 6$), arterial citrate concentration changed significantly from 140 ±19 to 192±26 μM, and malate from 8±2 to 12±2 μM. In skeletal muscle, the citrate concentration increased from 76±11 to 214±45, and from 96±8 to 193±25 nmol/g wet wt, after the highest doses of acetate and pyruvate, respectively. Similarly, the malate concentration increased from 87±24 to 151±47, and from 101±11 to 190±31 nmol/g wet wt after infusions of acetate and pyruvate, respectively. These changes are statistically significant at $P < 0.05$. Tissue water content did not change significantly after either acetate (from 75.2±1.3 to 75.1±0.8%) or pyruvate (from 75.4±1.0 to 75.7±0.7%) infusion.

Arterial lactate concentration increased from 2.22 ±0.42 to 3.47±0.72 mM, and pyruvate concentration decreased from 0.23±0.03 to 0.14±0.02 mM, during acetate infusion. Simultaneously, coronary sinus blood lactate concentration increased from 1.12±0.17 to 2.67 ±0.60 mM. In contrast, both arterial lactate (from 2.79±0.36 to 4.50±0.85 mM) and pyruvate (from 0.29 ±0.03 to 3.90±0.71 mM) concentrations increased significantly during pyruvate infusion. The arterial blood lactate:pyruvate ratio increased (from 8.33±0.31 to 23.72±3.03) during acetate infusion but decreased (from 9.16±0.80 to 1.19±0.49) during pyruvate infusion.

Plasma osmolality increased during infusions of acetate and pyruvate. The osmolalities achieved during the highest doses of these two substances were 324±3 and 325±4 mosmol/kg H_2O, respectively.

Effects of acetate and pyruvate on adenine nucleotide contents of skeletal muscle and the heart. Table III shows that skeletal muscle content of AMP increased and that the ATP:AMP ratio decreased during the last acetate infusion. Contents of ATP, ADP, and creatine phosphate, however, did not change significantly. None of the adenine nucleotides measured changed significantly in pyruvate-infused dogs.

Similarly, the myocardial ATP:AMP ratio obtained during acetate infusion (23.2±3.5) was significantly lower than that during pyruvate infusion (48.3±8.0). The respective ATP and AMP concentrations of the heart were 17.1±1.0 and 0.84±0.11 μmol/g dry wt in the acetate group, and 22.8±0.9 and 0.50±0.05 μmol/g dry wt in the pyruvate group.

Effects of acetate and pyruvate infusions on plasma and myocardial adenosine, inosine, and hypoxanthine concentrations. Infusion of acetate produced a significant increase in the hypoxanthine concentration but not in adenosine and inosine concentrations of coronary sinus plasma (Table IV). Arterial hypoxanthine concentration did not change significantly (from 0.27±0.05 to 0.48±0.12 μM). In contrast, infusion of pyruvate caused no significant changes in the hypoxanthine con-

TABLE III

Skeletal Muscle Contents of Adenine Nucleotides and Creatine Phosphate during Acetate and Pyruvate Infusions

Substance infused	ATP	Creatine phosphate	ADP	AMP	ATP:AMP ratio
			μmol/g dry wt		
Control (n = 7)	19.6±2.0	50.0±4.8	4.0±0.4	0.40±0.04	51.8±3.1
Acetate (n = 7)	17.7±1.3	47.0±5.6	5.6±1.2	0.72±0.12*	27.6±7.7*
Control (n = 6)	19.7±1.8	44.9±6.6	3.7±0.4	0.45±0.04	50.0±7.9
Pyruvate (n = 6)	17.7±2.0	44.7±6.1	3.7±0.4	0.37±0.08	50.2±6.8

* The number of experiments is given by n. Asterisks (*) indicate values that are significantly different from the preinfusion control at $P < 0.05$, as determined by Dunnett's test (29). The mean ATP:AMP ratio was calculated from values of individual ratios.

centration of coronary sinus plasma. Adenosine and inosine concentrations also did not change. On the other hand, the adenosine content of the myocardium was significantly higher at the end of infusion of acetate (2.85±0.68 nmol/g wet wt) as compared to pyruvate (0.60±0.11 nmol/g wet wt). Inosine and hypoxanthine contents of the myocardium did not differ between the two infusions.

Hemodynamic effects of acetate infusion in conscious dogs. Seven conscious dogs (9.8±0.7 kg) received intravenous infusions of acetate (0.075 mmol/kg per min) for 20 min. As in anesthetized dogs, acetate infusion increased cardiac output, left ventricular *dP/dt* and *dP/dt/P*, but had no effect on mean aortic blood pressure (Fig. 4). Pulmonary arterial blood pressure increased significantly from 11.8±1.1 to 14.0±1.5 mm Hg at 20 min of acetate infusion; total body oxygen consumption rose from 59±3 to 80±9 ml/min ($P < 0.05$).

Fig. 5 shows that infusion of acetate resulted in increases in blood flow to right and left ventricles, the gastrointestinal tract, pancreas, kidneys, intercostal muscle, and diaphragm. However, blood flow to liver, brain, lungs, femoral muscles, and skin did not change significantly.

Effects of propranolol on hemodynamic responses to acetate infusion in conscious dogs. Propranolol pretreatment of six conscious dogs (10.3±1.0 kg) increased the dose of isoproterenol required to accelerate heart rate by 25 beats/min from 1.6±0.2 μg to 16.5±2.4 μg ($P < 0.01$). Fig. 4 shows that propranolol pretreatment did not abolish the increase in cardiac output produced by acetate infusion. Fig. 4 also shows that although left ventricular *dP/dt* and *dP/dt/P* were reduced by propranolol during the preinfusion control period, myocardial contractility still increased significantly during acetate infusion. Expressing values as percents of the control, left ventricular *dP/dt* and *dP/dt/P* increased to 124±5 and 122±5%, respectively, in propranolol-pretreated dogs; values did not differ from those in untreated dogs (125±3 and 116±3%, respectively). In addition, propranolol pretreatment did not alter the organ blood flow responses to acetate infusion (Fig. 5).

Effects of fluoroacetate on hemodynamic responses to acetate infusion in conscious dogs. Acetate (0.075 mmol/kg per min) was administered to six conscious dogs (12.1±0.8 kg) that had been pretreated with fluoroacetate (2 mg/kg). These dogs did not exhibit an increase in total body oxygen consumption during acetate infusion (from 80±9 to 87±14 ml/min). Furthermore, arterial plasma acetate concentration increased by 3.11 ±0.30 mmol/liter, which was significantly higher than that in untreated dogs (1.03±0.24 mmol/liter, n = 7).

TABLE IV

Concentrations of Adenosine, Inosine, and Hypoxanthine in Coronary Sinus Plasma during Acetate and Pyruvate Infusions

Infusion rate	Adenosine	Inosine	Hypoxanthine
mmol/kg/min		*μM*	
Acetate infusion (n = 7)			
Control	0.10±0.03	0.13±0.06	0.37±0.12
0.0375	0.17±0.06	0.11±0.02	0.54±0.12
0.075	0.23±0.10	0.11±0.04	0.67±0.17*
0.15	0.19±0.08	0.26±0.07	1.26±0.22*
Pyruvate infusion (n = 6)			
Control	0.06±0.04	0.07±0.05	0.23±0.08
0.0375	0.06±0.05	0.03±0.02	0.20±0.08
0.075	0.09±0.03	0.03±0.03	0.20±0.10
0.15	0.05±0.02	0.06±0.02	0.26±0.06

* The number of experiments is given by n. Asterisks (*) indicate values that are significantly different from the preinfusion control at $P < 0.05$, as determined by Dunnett's test (29).

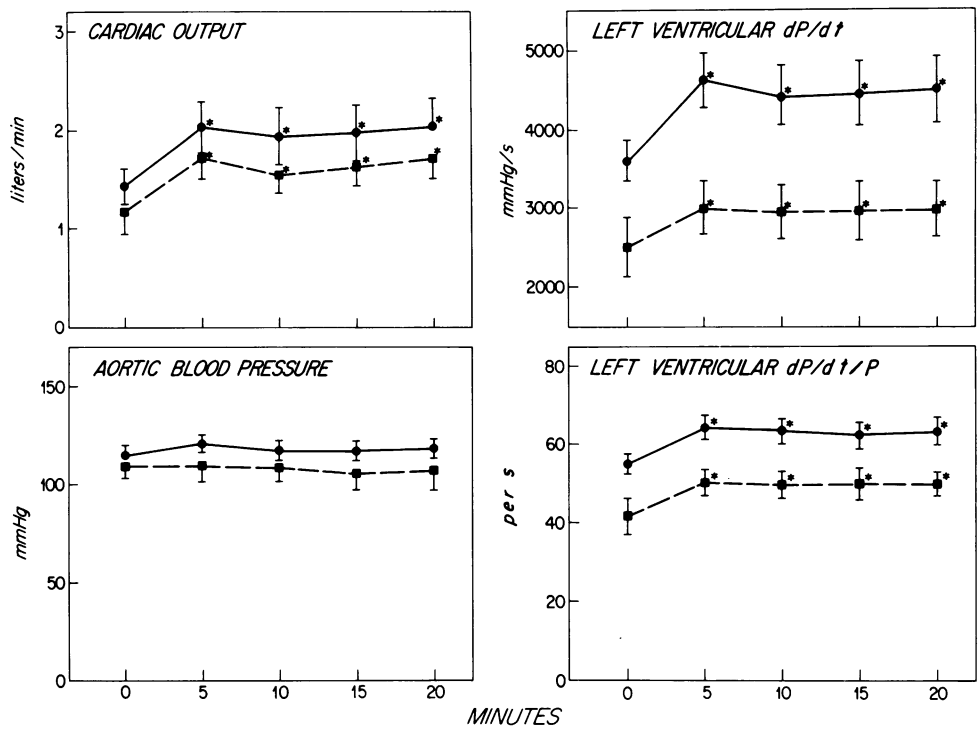

FIGURE 4 Changes in cardiac output, mean aortic blood pressure, left ventricular *dP/dt* and *dP/dt/P* during acetate (0.075 mmol/kg per min) infusion in seven normal untreated (circles) and six propranolol-pretreated (squares) conscious dogs. Asterisks (*) indicate values that are statistically different from the zero-time value (control) at $P < 0.05$, as determined by Dunnett's test (29).

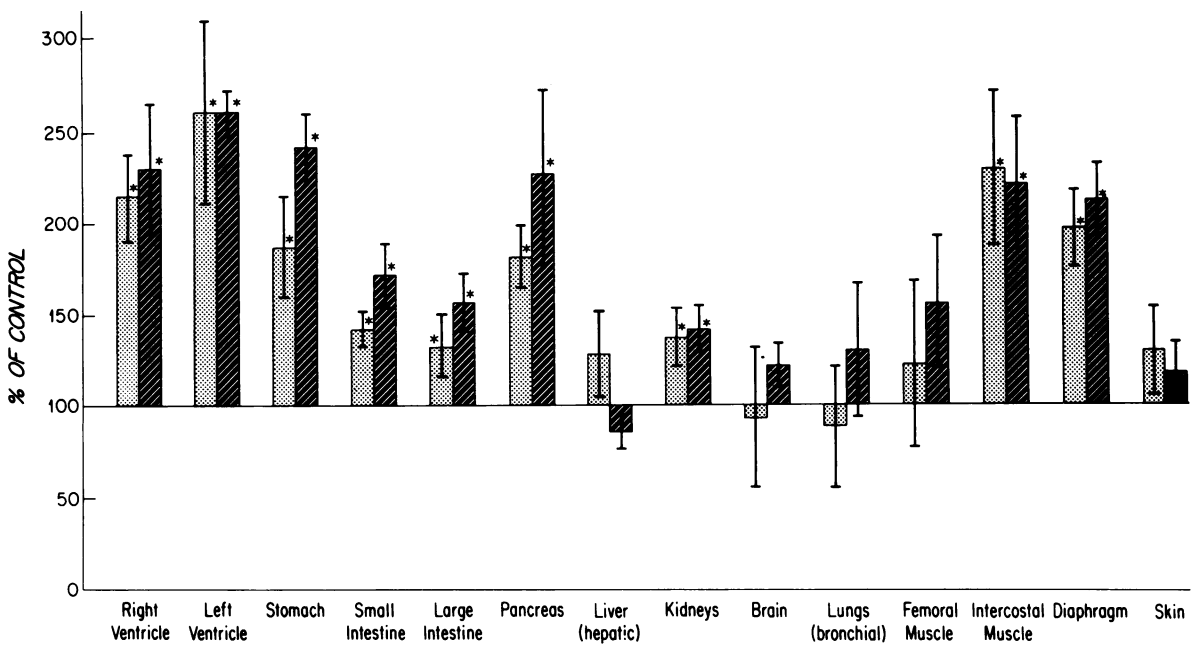

FIGURE 5 Changes in organ blood flow during acetate infusion in normal untreated (shaded columns, $n = 7$) and propranolol-pretreated (hatched columns, $n = 6$) conscious dogs. Asterisks (*) indicate values that are statistically different from the preinfusion control at $P < 0.05$, as determined by paired t test.

In the fluoroacetate-pretreated dogs, cardiac output values were 1.74±0.27, 1.79±0.28, 1.83±0.29, 1.83 ±0.31, and 1.84±0.30 liters/min at 0, 5, 10, 15, and 20 min of acetate infusion, respectively; these values were not statistically different. Similarly, left ventricular *dP/dt* and *dP/dt/P* did not change significantly from the control (101±2 and 100±4%, respectively). Mean aortic blood pressure, however, fell from 122±9 to 109±11 mm Hg at 5 min of infusion, and then increased slightly to 112±13 mm Hg at 20 min.

DISCUSSION

This study shows that cardiac output, myocardial contractility, and coronary blood flow increase during infusion of acetate but not of pyruvate. The circulatory stimulation of acetate is probably related to its activation to acetyl CoA by acetyl CoA synthetase (acetate thiokinase), a reaction which requires ATP and yields AMP and inorganic pyrophosphate (12). The metabolic effects of acetate are associated with increases in skeletal muscle and myocardial contents of AMP and with decreases in their ATP:AMP ratios. The rise in AMP concentration is also related, at least in part, to the increase in total body oxygen consumption observed during acetate infusion because it leads to the formation of ADP (30). In contrast, the conversion of pyruvate to acetyl CoA does not require ATP, and has no effects on oxygen consumption.

Previous work has shown that perfusion of rat heart with acetate leads to an increase in the content of AMP and to a disequilibrium of the myokinase reaction (31): 2 ADP ↔ AMP + ATP. This disequilibrium may be facilitated by the direct formation of AMP that occurs in the acetyl CoA synthetase (acetate thiokinase) reaction (12, 32): Acetate + ATP + CoA → acetyl CoA + AMP + pyrophosphate. The increased content of AMP can be expected to result in an increase in the rate of conversion of AMP to adenosine by 5′-nucleotidase. The adenosine formed is subsequently deaminated to inosine which is cleaved to hypoxanthine (31). Furthermore, perfusion of rat heart with acetate increases the cytoplasmic NADH:NAD ratio (32). This change in cytoplasmic redox potential during acetate oxidation probably is responsible for the increase in the arterial blood lactate:pyruvate ratio observed by us.

Tricarboxylic acid cycle intermediates in the blood and skeletal muscle, and plasma osmolality increased by the same extent during acetate and pyruvate infusions, which indicates that these changes are not responsible for the circulatory stimulation produced by acetate. The increase in arterial blood pH also was not responsible for the hemodynamic changes produced by acetate; similar changes in arterial pH by Tris–HCl infusion produced no significant hemodynamic alterations. Moreover, the present study shows that Tris–pyruvate infusion produced no significant hemodynamic effects; therefore, the changes produced by Tris–acetate infusion cannot be attributed to Tris alone.

In addition to increasing cardiac performance, acetate infusion increased blood flow to various organs, particularly the myocardium and the gastrointestinal tract. Left ventricular work and myocardial oxygen consumption increased, which might explain, in part, the increase in coronary blood flow and the changes in myocardial high energy phosphate levels. The increase in coronary sinus oxygen saturation and the decrease in myocardial oxygen extraction, however, suggest that an active coronary vasodilation occurs which is not related to the increased cardiac work. Nevertheless, myocardial mechanical efficiency did not change. Although coronary sinus blood lactate concentration increased during acetate infusion, coronary sinus blood pH did not decrease; this excludes lactic acidosis as a factor which contributes to the increase in coronary blood flow. Our purine nucleoside and hypoxanthine measurements indicate that the increase in myocardial blood flow probably is caused by the release of adenosine from the myocardium. These results support Berne and Rubio's contention (33) that adenosine production is not obligatorily associated with tissue hypoxia.

The different responses of various organs to the infusion of acetate suggest that the type and degree of the vascular responses might be determined, at least in part, by the differences in the levels of 5′-nucleotidase in various organs. Different organs also exhibit different sensitivities to neurohumoral factors that regulate the blood flow. The amount of adenosine produced in a tissue is determined not only by the level of AMP, but also by the relative activities of AMP deaminase and 5′-nucleotidase which compete for AMP. In the rat, 5′-nucleotidase activity of heart is two–four times greater than that of skeletal muscle and liver (7). A particularly high level of 5′-nucleotidase occurs in the smooth muscle of the small intestine (34). Adenosine causes vasodilation in the mesenteric artery (35), and it is possible that adenosine is responsible for the increased blood flow to the gut, which we observed in our experiments during acetate infusion (Fig. 5). Adenosine has also been postulated to mediate blood flow in skeletal muscle (36) and brain (37). Femoral muscle and cerebral blood flows, however, did not increase significantly in our study.

Minute pulmonary ventilation and respiratory rate increase during acetate infusion in dogs (38). This might account for the increases in blood flows to intercostal muscle and diaphragm. Renal blood flow also increases during acetate infusion; this is attributable either to the increase in cardiac output or to renal vasodilation, as was shown previously in isolated perfused kidneys (5).

Pretreatment with propranolol indicates that the effects of acetate infusion on cardiac output, myocardial

contractility, and organ blood flows are not mediated via the β-adrenergic receptors. The experiments with fluoroacetate pretreatment, on the other hand, show that the hemodynamic effects of acetate were no longer present when acetate utilization was inhibited. Fluoroacetate is activated to fluoroacetyl CoA which is then converted to fluorocitrate, a competitive inhibitor of aconitase. The result is an accumulation of citrate (39). Inhibition of acetate activation after pretreatment with fluoroacetate was apparent by the high plasma acetate concentration and the absence of a rise in oxygen consumption during acetate infusion. These results suggest that the hemodynamic effects of acetate are caused not by acetate itself but by metabolic changes associated with acetate metabolism.

Neither the heart rate nor myocardial contractility is affected by administration of acetate into a coronary artery in dogs (40). Moreover, acetate does not exert an inotropic effect in the isolated perfused rat heart (32, 41). These results suggest that the stimulus that increases cardiac output and myocardial contractility during intravenous infusion of acetate in intact dogs probably originates in peripheral tissues. A neural afferent pathway is involved in the hemodynamic and ventilatory responses to increases in oxygen consumption produced by 2,4-dinitrophenol (11). Whether such a mechanism is also involved in the changes that occur during acetate infusion warrants further study.

In recent times, acetate has become the most widely used anion in hemodialysis (42). Arterial acetate concentration in patients may reach 3–5 mM, equal to or greater than the highest concentrations achieved in this study. Hemodialysis is usually accompanied by a decline in the mean arterial blood pressure, and occasionally, severe hypotension ensues (43). Effects of acetate on cardiac performance and regional blood flows during dialysis have not been ascertained.

Acetate accumulates as an intermediate during the metabolism of ethanol. The plasma concentration of acetate after ethanol administration may reach levels as high as those achieved in the present experiments during acetate infusion (44). Ethanol exerts a negative inotropic action in isolated perfused hearts (45), but its hemodynamic effects in intact animals are controversial. Myocardial function and coronary blood flow have been said to increase (46, 47) and to decrease (48, 49) after ethanol administration. It has been postulated that the positive inotropic effect of ethanol in vivo is caused by the sympathomimetic action of acetaldehyde (50), the immediate product of ethanol oxidation. Our results suggest that acetate may play an important role in mediating the hemodynamic effects of ethanol.

In summary, this study demonstrates that acetate infusion increases cardiac output, myocardial contractility, and blood flow to the myocardium, gastrointestinal tract, kidneys, and respiratory muscles. These changes, which are not seen after pyruvate infusion, are probably associated with AMP formation that accompanies acetate activation. It is likely that the increase in myocardial blood flow is caused by the vasodilatory action of adenosine formed in response to the increase in AMP. Acetate may also play a role in the hemodynamic changes produced by ethanol. Our results suggest the need for an investigation of possible circulatory effects of acetate-containing dialysates in clinical use.

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