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Research Article

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Comparison of Cardiac Output Responses to 2,4-Dinitrophenol-Induced Hypermetabolism and Muscular Work

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ABSTRACT Both electrically induced exercise and infusion of 2,4-dinitrophenol (DNP) increased oxygen consumption and tissue metabolism in chloralose-anesthetized dogs. Cardiac output increased with oxygen consumption at the same rate in both experimental conditions. The increase in cardiac output induced by exercise was, as expected, accompanied by increases in both lactate-to-pyruvate ratio and "excess lactate" in arterial blood. However, these parameters did not increase after DNP infusion until the rate of oxygen consumption had increased four- to fivefold, perhaps due to facilitation of mitochondrial electron transport by DNP. Anaerobic tissue metabolism therefore probably did not contribute significantly to increased cardiac output during the mild-to-moderate tissue hypermetabolism induced by DNP. The increased cardiac output may have been the result of metabolic changes common to both exercise and DNP infusion; muscular activity alone may not have been the primary determinant of the cardiac output response during exercise.

INTRODUCTION

The principal factor initiating increased cardiac output during exercise, the "work stimulus," still is not fully understood (1). Both mechanical and metabolic factors have been identified with the "work stimulus." The former have been related to the stimulation of mechanical sensory receptors inside the muscles or tendons, and the latter to metabolic changes that occur in exercising muscles.

In this study, dogs were infused with 2,4-dinitrophenol (DNP)[1] to produce tissue hypermetabolic changes fundamentally like those that occur in exercise but not associated with muscular movement. The cardiac output responses to DNP infusion and exercise were compared to assess the relative contributions of mechanical and metabolic factors in the regulation of cardiac output. The rate of oxygen consumption was taken as the index of tissue metabolism.

In addition, concentrations of lactate and pyruvate in arterial blood were measured. A change in the lactate-to-pyruvate ratio indicates the presence or absence of tissue hypoxia (2), and its correlation with cardiac output provides an estimate of the role of anaerobic tissue metabolism in the cardiac output response to tissue hypermetabolism.

METHODS

Studies were performed on 22 healthy dogs of both sexes weighing between 14 and 33 kg. Anesthesia was induced with vaporized methoxyflurane, (Penthrane, Abbott Laboratories, North Chicago, Ill.) followed by intravenous chloralose (60 mg/kg). The trachea was cannulated with a T tube connected to a Benedict-Roth metabolic spirometer to record the rate of oxygen consumption. A femoral artery and the right ventricle were cannulated and connected to pressure transducers and a Sanborn 7700 recorder (Hewlett-Packard Co., Waltham Div., Waltham, Mass.) to measure blood pressures and heart rate. Another catheter was inserted into a carotid artery and connected to a Gilford Colson 103 densitometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) to determine cardiac output (3) with indocyanine green (Cardio-Green, kindly supplied by Hynson,

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[1] *Abbreviations used in this paper:* DNP, 2,4-dinitrophenol; *L/P*, lactate to pyruvate; PC, phosphorylcreatine; XL, excess lactate.

TABLE I

Circulatory and Metabolic Data on Eight Dogs before and after 2, 4-Dinitrophenol Infusions

Wt	Experimental conditions	V_{O_2}	$\dot{Q}$	HR	SV	$\overline{P_a}$	TPVR	$S_{\bar{v}O_2}$	P_{aCO_2}	pH	$\Delta\dot{Q}/\Delta\dot{V}_{O_2}$	[L]	[P]	*L/P*
kg		*ml/min*	*ml/min*	*beats/min*	*ml*	*mm Hg*	*mm Hg per liter/min*	%	*mm Hg*			*mmoles per liter*	*mmoles per liter*	
19.7	A	90.6	2,135	100	21.4	143	67.0	79.6	40	7.30	—	2.787	0.271	10.30
	B	202.1	2,959	112	26.4	142	48.0	63.0	41	7.29	7.39	1.645	0.180	9.13
	C	347.7	3,629	129	28.1	136	37.5	46.3	41.5	7.28	5.81	1.086	0.110	9.87
	D	624.4	4,953	140	35.4	117	23.6	32.1	41	7.31	5.28	1.206	0.116	10.34
	E	982.4	7,218	212	34.1	125	17.3	—	40	7.30	5.70	2.999	0.199	15.07
19.5	A	89.8	2,078	94	22.1	142	68.3	69.8	42.5	7.32	—	1.062	0.175	6.07
	B	215.3	3,310	125	26.5	142	42.9	57.6	43	7.32	9.81	0.780	0.133	5.87
	C	393.0	4,253	144	29.5	155	36.4	41.0	41.5	7.31	7.17	1.269	0.142	8.94
	D	634.3	5,279	147	35.9	152	28.8	24.9	39	7.32	5.88	1.840	0.145	12.69
18.5	A	81.2	2,932	116	25.3	167	57.0	87.4	47	7.30	—	1.930	0.208	9.30
	B	207.6	3,984	124	32.1	164	41.2	66.6	47	7.29	8.33	1.055	0.136	7.76
	C	361.2	4,193	130	32.3	157	37.4	51.0	48	7.28	4.51	1.057	0.115	9.19
	D	690.8	5,965	146	40.9	166	27.8	29.6	54	7.24	4.98	1.683	0.124	13.57
19.1	A	68.8	2,213	88	25.2	119	53.8	84.4	40.5	7.30	—	1.846	0.175	10.55
	B	165.1	2,990	98	30.5	126	42.1	66.7	40.5	7.30	8.07	1.302	0.143	9.11
	C	266.8	3,752	115	32.6	129	34.4	57.2	41.5	7.30	7.77	1.054	0.108	9.76
	D	400.1	4,242	133	31.9	133	31.4	38.7	34	7.34	6.12	1.906	0.152	12.54
19.8	A	86.7	3,009	108	27.9	121	40.2	90.8	35	7.32	—	1.515	0.166	9.13
	B	225.0	3,911	104	37.6	122	31.2	72.5	36	7.31	6.52	1.395	0.140	9.96
	C	364.9	4,008	98	40.9	125	31.2	52.8	35.5	7.32	3.59	0.973	0.119	8.18
	D	568.8	4,636	108	42.9	128	27.6	30.5	34	7.32	3.38	1.169	0.118	9.91
19.1	A	125.8	3,806	108	35.3	156	41.0	83.9	41	7.32	—	1.880	0.211	8.91
	B	270.9	3,970	108	36.8	149	37.5	66.3	41	7.32	1.13	1.654	0.193	8.57
	C	476.2	5,115	132	38.8	148	28.9	52.8	32	7.39	3.74	0.752	0.096	7.83
	D	798.4	6,034	155	38.9	158	26.2	34.9	22	7.44	3.31	1.213	0.113	10.73
20.5	A	97.2	4,098	132	31.0	142	34.7	83.2	37	7.30	—	2.535	0.232	10.93
	B	228.7	4,813	142	33.9	145	30.1	70.2	37	7.30	5.44	1.433	0.156	9.19
	C	413.5	5,345	152	35.2	150	28.1	52.3	37	7.30	3.94	1.019	0.123	8.28
	D	712.9	6,761	132	51,2	155	22.9	31.7	33	7.31	4.33	1.601	0.140	11.44
20.4	A	132.0	3,874	149	26.0	154	39.8	92.7	42	7.33	—	2.918	0.231	12.63
	B	317.8	4,945	156	31.7	160	32.4	74.3	42	7.32	5.76	2.743	0.218	12.58
	C	521.1	6,265	150	41.8	158	25.2	63.6	41	7.34	6.15	2.296	0.183	12.55
	D	1,056.0	9,840	260	37.9	140	14.2	50.8	42	7.32	6.46	2.061	0.158	13.04

A, control state; B, C, D, and E, after the first, second, third, and fourth infusions of DNP (2 mg/kg); Wt, body weight; HR, heart rate; [L], Lactate concentration in arterial blood water; $\overline{P_a}$, mean arterial blood pressure; [P], pyruvate concentration in arterial blood water; P_{aCO_2}, arterial blood carbon dioxide tension; SV, stroke volume; $S_{\bar{v}O_2}$, right ventricular blood oxygen saturation; TPVR, total peripheral vascular resistance.

Westcott & Dunning, Inc., Baltimore, Md.). The dye curves, registered on a linear Hewlett-Packard 1701 BM strip chart recorder (Hewlett-Packard Co., Palo Alto, Calif.), were corrected for recirculation of dye, and the area measured planimetrically. Mean arterial blood pressure was divided by cardiac output to calculate total peripheral vascular resistance.

Cardiac output was also measured by the Fick principle, and these data were used exclusively for statistical comparisons (see Results). Outputs were obtained by drawing simultaneous 4-ml samples of right ventricular and arterial blood over a period of 2 min and determining the rate of oxygen consumption during the same interval. However, during exercise, the blood was sampled at double speed. The blood samples were analyzed promptly for oxygen content using a Beckman GC-2A gas chromatograph (Beckman Instruments, Fullerton, Calif.) by the method of Ramsey (4); in our laboratory, this method yields results consistent with those of the method described by Van Slyke and Neill (5).

The oxygen capacity of blood was determined by a cyanmethemoglobin method (6). Oxygen saturation was calculated by dividing blood oxygen content by oxygen capacity. Blood pH and gas tensions were measured on a Radiometer PHM 71 Acid Base Analyzer (The London Company, Westlake, Ohio).

To determine lactate and pyruvate concentrations, arterial blood was allowed to flow freely into a tube of ice-cooled trichloroacetic acid. The filtrate was analyzed for lactate by the enzymatic method of Friedland and Dietrich (7), and for pyruvate by the method of Friedemann and Haugen, as modified by Huckabee (8). Blood water content was determined by the reduction in weight after drying. Concentrations of "excess lactate" (XL) were calculated from changes in pyruvate and lactate concentrations by the following equation (2):

$$XL = (L_n - L_o) - (P_n - P_o)(L_o/P_o),$$

where L_o and L_n and P_o and P_n are lactate and pyruvate concentrations in arterial blood water in control and experimental conditions.

The experimental animals were divided into two groups. In one group of eight dogs, tissue hypermetabolism was induced by three or four successive infusions of a 200 mg/100 ml DNP aqueous neutral solution, spaced at 15-min intervals. The dose of DNP for each infusion was 2 mg/kg given into the femoral artery catheter over a 2-min period. In another group of 14 dogs, muscular work was induced by direct stimulation of limb muscles with electrical pulses of 3/s frequency, 5 ms duration, and 80 V intensity delivered from a Grass SD 5 stimulator (Grass Instrument Co., Quincy, Mass.) over a period of 10 min. Each dog was stimulated once.

Circulatory and metabolic measurements were made at frequent intervals before and after both kinds of experimental intervention. Preliminary experiments using the indicator dilution method showed that cardiac output rose to a steady state within 3 min after onset of exercise and within 5 min after each infusion of DNP. Therefore it was decided to obtain Fick cardiac outputs between 6 and 9 min after the start of exercise and DNP infusion.

In each group of experiments, the significance of differences between control and experimental values was determined using t tests for paired comparisons. Correlations and regression coefficients were computed for both groups of animals, and the slopes of the regression lines for these two groups were compared (9).

RESULTS

Experimental results obtained in animals that received DNP infusions are shown in Table I. Table II provides a statistical summary of both exercise and DNP infusion experiments. With the exception of stroke volume, there were not significant differences between the control values for the two groups of animals, as determined by unpaired t tests.

Oxygen consumption and cardiac output. The rate of oxygen consumption increased 2–10-fold after DNP infusion and exercise, and was accompanied by an increase in cardiac output. Fig. 1 illustrates the stepwise increase of both oxygen consumption and cardiac output after successive doses of DNP. Fig. 2 shows that cardiac output increased with oxygen consumption at the same rate in both groups of dogs. The regression coefficient and its standard error were 5.460 and 0.462 for the DNP-treated dogs, and 5.235 and 0.655 for the dogs subjected to electrically induced exercise. There was no difference between the regression coefficients of these two groups ($t = 0.27$, $P > 0.5$).

The slope of the regression line in Fig. 2, which is the ratio of increment in cardiac output to increment in oxygen consumption ($\Delta\dot{Q}/\Delta\dot{V}o_2$), is called the "exercise factor" when employed in studies of exercise (10). It has been shown repeatedly (11–16) that cardiac output increases linearly with oxygen consumption during exercise, to rates of oxygen consumption up to 10–15 times the normal resting value. The calculated $\Delta\dot{Q}/\Delta\dot{V}o_2$ is constant over this range of oxygen consumption. This ratio was calculated for individual animals with both exercise and DNP infusion; their means in these two groups did not differ significantly (Table II).

Heart rate and stroke volume. Fig. 3 shows that the increases in cardiac output were associated with increases in both heart rate and stroke volume in both groups of animals.

Arterial and right ventricular pressures and total peripheral vascular resistance. Mean arterial blood pressure did not change significantly in dogs after DNP infusion (Tables I and II), whereas it fell immediately 20–25% below control after initiation of electrically induced exercise. As exercise continued, it rose and stabilized at about 15% below the control (Table II). Right ventricular end-diastolic pressure did not change significantly after DNP infusion. Right ventricular pressure tracings for dogs undergoing exercise were distorted and are not reported. The calculated total peripheral vascular resistance was reduced both with exercise and DNP infusion, and its changes were inversely related to changes in cardiac output.

Blood oxygen saturation and blood gases. Arterial blood was fully saturated with oxygen in dogs at rest,

TABLE II

Statistical Summary of

Wt	Experimental conditions	$\dot{V}_{O_2}$	$\dot{Q}$	HR	SV	$\overline{P_a}$	TPVR
kg		*ml/kg/min*	*ml/kg/min*	*beats/min*	*ml/kg*	*mm Hg*	*mm Hg/liter/min*
							2, 4-Dinitrophenol
19.6±0.2	A	4.92±0.37	153.8±14.2	111.9±7.2	1.37±0.08	143.0±5.9	50.2±4.6
	B	11.72±0.77	196.8±12.3	121.1±7.0	1.66±0.08	143.8±5.2	38.2±2.3
	C	19.32±1.14	232.7±15.0	131.3±6.4	1.78±0.09	144.8±4.6	32.4±1.7
	D	34.95±3.24	303.6±29.4	152.6±16.1	2.01±0.10	143.6±6.0	25.3±1.9
							Exercise
20.8±1.6	Control	5.85±0.28	120.8±10.6	121.4±5.9	1.01±0.07	132.8±3.2	61.6±7.0
	Exercise	28.54±3.21	246.5±16.0	153.6±6.9	1.56±0.09	113.4±3.5	24.7±2.3

A, control state; B, C, D, and E, after the first, second, third, and fourth infusions of DNP (2 mg/kg); Wt, body weight; HR, heart rate; [L], Lactate concentration in arterial blood water; $\overline{P_a}$, mean arterial blood pressure; [P], pyruvate concentration in arterial blood water; P_aCO_2, arterial blood carbon dioxide tension; SV, stroke volume; $S\bar{v}O_2$, right ventricular blood oxygen saturation; TPVR, total peripheral vascular resistance.

after DNP infusion, and during exercise. On the other hand, right ventricular blood oxygen saturation fell as oxygen consumption increased in both groups of animals (Fig. 4). Neither DNP infusion nor exercise produced significant changes in arterial pH or P_{CO_2} (Table II).

Arterial blood lactate and pyruvate concentrations. Arterial blood concentrations of lactate and pyruvate fell progressively reaching their lowest values of 40–50% of the control after the second infusion. With additional doses of DNP, both lactate and pyruvate increased. In three experiments, lactate concentrations even rose above the control values after the third infusion (Table I). On the other hand, exercise was consistently accompanied by increased concentrations of lactate and pyruvate; at no

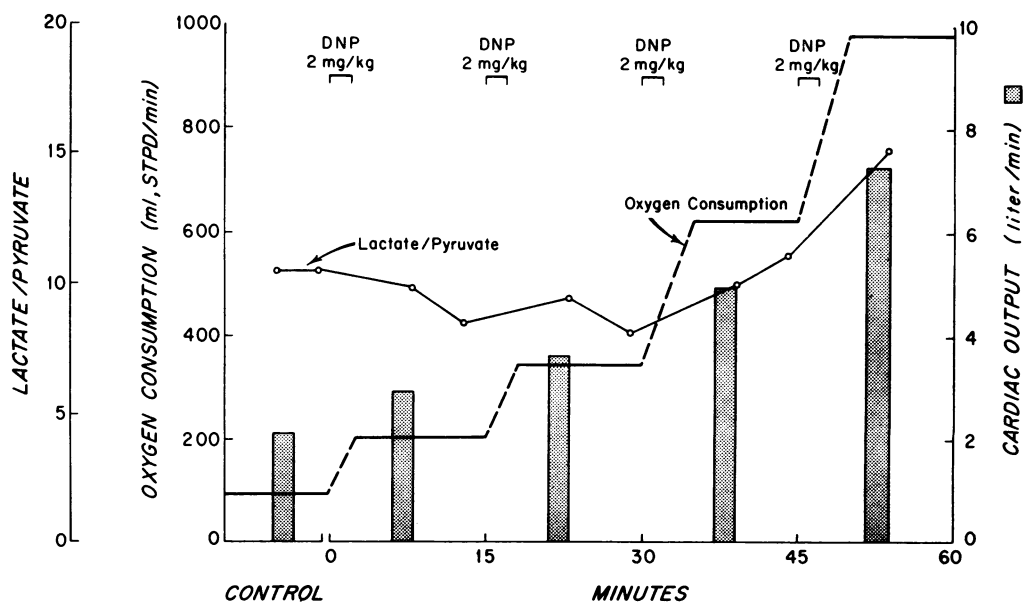

FIGURE 1 A typical experiment showing the changes in oxygen consumption, cardiac output and *L/P* ratio in a dog after four successive infusions of 2,4-DNP. Straight lines depicting oxygen consumption were calculated from the slopes of spirometer tracings taken at those time intervals.

$S_{\bar{v}O_2}$	P_{aCO_2}	pH	$\Delta\dot{Q}/\Delta\dot{V}O_2$	[L]	[P]	*L/P*	XL
%	*mm Hg*			*mmoles per liter*	*mmoles per liter*		*mmoles per liter*
Infusion (n = 8)							
84.0±2.5	40.6±1.3	7.311±0.004	—	2.059±0.226	0.209±0.012	9.73±0.67	—
67.2±1.9	40.9±1.2	7.306±0.004	6.56±0.93	1.500±0.205	0.162±0.011	9.02±0.67	−0.130±0.045
52.1±2.4	39.8±1.7	7.315±0.013	5.34±0.57	1.188±0.165	0.125±0.009	9.33±0.53	−0.036±0.072
34.2±2.8	37.4±3.3	7.325±0.019	4.97±0.43	1.584±0.123	0.133±0.005	11.78±0.48	0.280±0.114
(n = 14)							
72.4±6.7	39.3±1.6	7.331±0.042	—	1.755±0.629	0.186±0.014	9.30±1.53	—
41.4±3.2	41.4±4.0	7.301±0.025	5.73±0.30	4.504±0.770	0.223±0.018	19.12±1.60	2.165±0.620

time did either of them fall below control values (Table II).

Lactate to pyruvate ratio and "excess lactate." Lactate to pyruvate (L/P) ratio was reduced slightly and no XL was formed after the first two infusions of DNP; but after the third infusion, L/P ratio rose slightly and XL appeared (Fig. 1, Table I). On the other hand, exercise was always accompanied by increases in both

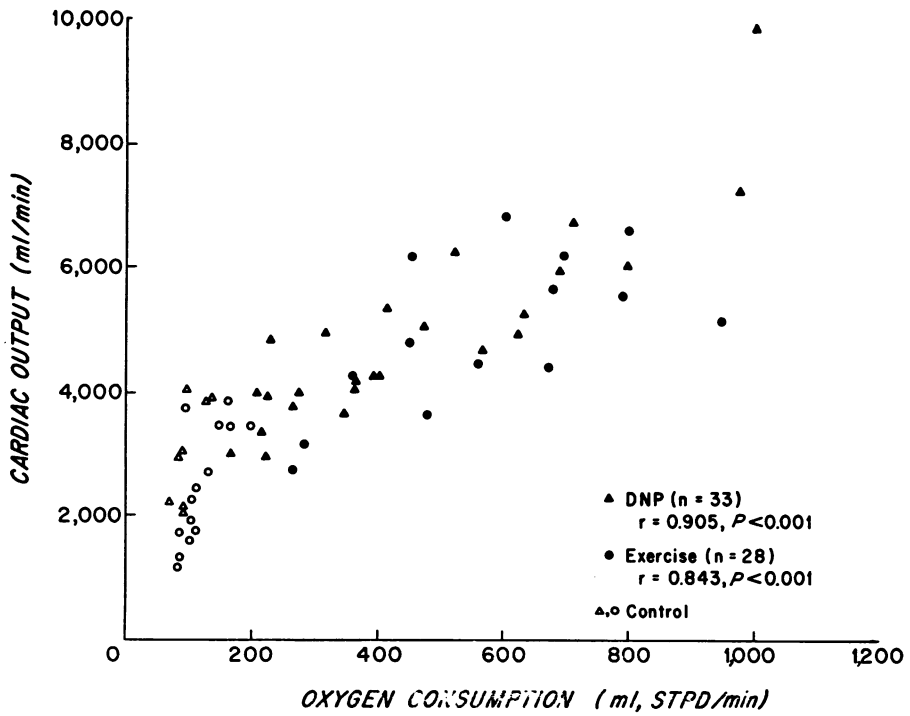

FIGURE 2 A graphic representation of the relationship between cardiac output and oxygen consumption in dogs both during exercise and after DNP infusions.

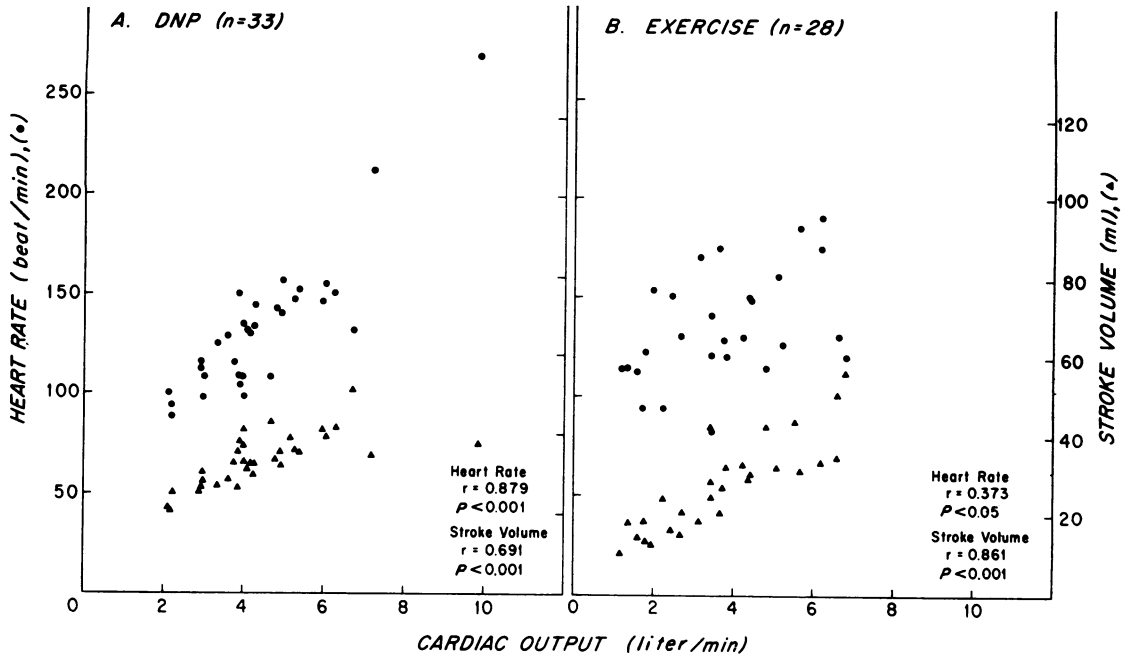

FIGURE 3 Relationships of heart rate and stroke volume to cardiac output in dogs after DNP infusions (A), and during exercise (B).

L/P ratio and XL. The means of these two parameters during exercise were significantly higher than those observed after DNP infusion ($P < 0.01$).

DISCUSSION

In the present experiments, oxygen consumption was increased in dogs by both DNP infusion and electrically induced exercise. DNP uncouples oxidation and phosphorylation in the mitochondrial cytochromes (17), resulting in a net increase in ADP and a decrease in ATP (18, 19). ATP hydrolysis also occurs in muscles during contraction (20, 21). The increased availability of tissue ADP in turn increases tissue metabolism and oxygen consumption (22, 23). Thus, the hypermetabolism induced by DNP is a result of mechanisms basically similar to those occurring during exercise.

The validity of the assumption that electrically induced work simulates spontaneous exercise has been previously established (24, 25). Fig. 2 shows that cardiac output increased with oxygen consumption during electrically induced exercise. The regression line is similar to those obtained in unanesthetized dogs running on a treadmill (11, 12).

Both heart rate and stroke volume were augmented during electrically induced exercise, but systemic arterial blood pressure was reduced (Fig. 3, Table II). A similar change in arterial blood pressure was observed earlier by Euler and Liljestrand (26) using the same method of muscle stimulation in chloralose-anesthetized animals, but it did not occur when muscular work was induced by ventral root stimulation (27). Therefore, it appears likely that the hypotensive response was peculiar to the site of electrical stimulation employed in our experiments.

DNP-induced hypermetabolism was accompanied by an increase in cardiac output comparable to that which occurred during exercise (Fig. 2). Furthermore, as occurred during exercise, the increase in cardiac output after DNP infusion was brought about by increases in both heart rate and stroke volume (Fig. 3). DNP infusion has been reported to increase cardiac output in intact dogs (28, 29). However, these reports did not provide sufficient data to allow quantitative comparison with our experimental results. Before we can attribute this increased cardiac output to the metabolic effects of DNP on peripheral tissues, the effects of DNP on the heart, arterial chemoreceptors, and central nervous system must be considered. Fawaz and Tutunji (30) found that DNP did not change left ventricular output in a heart-lung preparation within 1 h, even at concentrations more than 10 times those achieved in the present experiments; left ventricular output fell after more prolonged DNP exposure. DNP is capable of stimulating carotid chemoreceptors (31, 32). The immediate and transient hyperventilation and hypertension which occur when DNP is injected into the carotid artery are abolished by the denervation of carotid chemoreceptors. This indicates that these changes are not caused by direct effects of

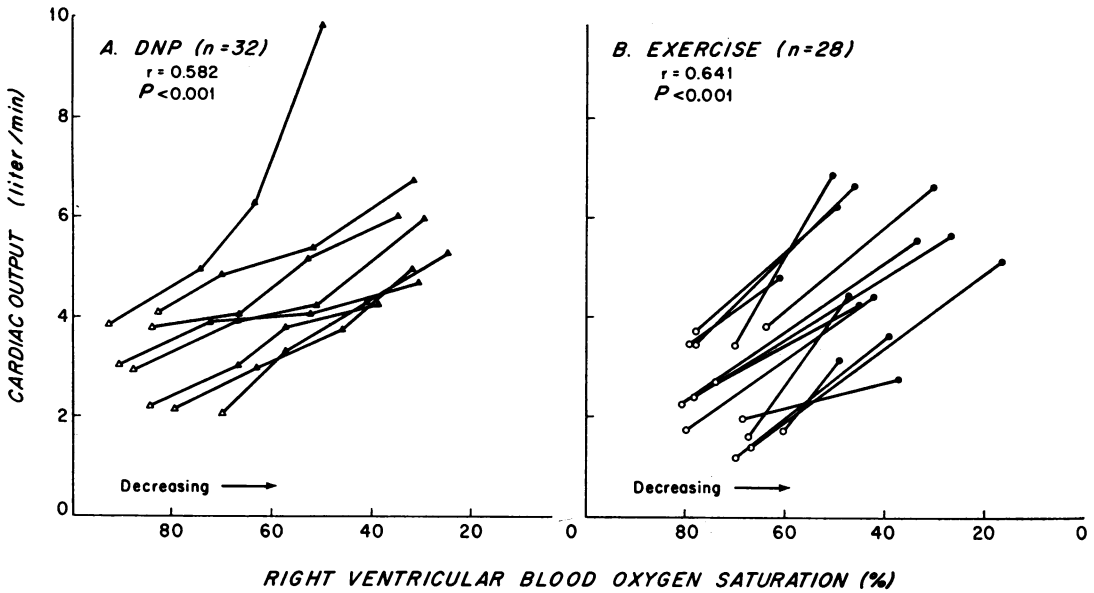

FIGURE 4 Relationship of cardiac output to right ventricular blood oxygen saturation in dogs after DNP infusions (A), and during exercise (B).

DNP on the central nervous system. Relatively large concentrations of DNP are required to stimulate carotid chemoreceptors (31, 32). Similarly high concentrations probably were not attained in the present experiments because the observed ventilatory and circulatory responses were gradual in onset and in 5 min reached a steady state well adjusted to the tissue metabolic rate with no change in arterial blood P_{CO_2}. Carbon dioxide tension fell only during severe hypermetabolism. Nor in all likelihood was the central nervous system important in the regulation of the cardiac output response to DNP infusion because, as shown by Banet and Guyton (29), cardiac output increased normally after DNP infusion in decapitated dogs as long as arterial blood pressure was maintained. Furthermore, using a cross-perfusion technique in which the head of one dog (recipient) was perfused by a second dog (donor), Levine and Huckabee (33) found that DNP, when given to the head of the recipient dog, did not produce any change in the recipient dog's ventilation, whereas the donor dog's ventilatory rate increased markedly. On the basis of these results from other investigators, it seems likely that the hemodynamic and respiratory changes observed in intact dogs after DNP infusion were related to the hypermetabolic state created by DNP in peripheral tissues.

Both isocapnic (hyperpnea) and hypocapnic hyperventilation occurred in dogs after DNP infusion. Richardson, Kontos, Raper, and Patterson (34) found that hyperpnea had no effects on cardiac output, whereas hypocapnic hyperventilation produced a transient increase in cardiac output which disappeared in 4 min. These observations make it unlikely that the sustained increase in cardiac output observed in the present experiments after DNP infusion was caused by either a decreased arterial P_{CO_2} or an increased respiratory movement of the chest.

It should be noted that, except for the increases in respiratory movement, there was no visible muscular hyperactivity in dogs that received DNP infusions, even when the rate of oxygen consumption increased eight to nine times above control values. Since cardiac output increased to the same extent in response to tissue hypermetabolism, whether associated with muscular movement or not, it appears that mechanical movement of the working muscles and the stimulation of mechanoreceptors did not play an important role in the regulation of cardiac output. Instead, the increased cardiac output was closely related to the metabolic changes common to both exercise and DNP infusion experiments, suggesting that the "work stimulus" is a metabolic one. This is certainly true during DNP infusion, in which muscular movement is absent. However, it cannot be totally excluded that exercise may be associated with effects upon either cardiac output or oxygen consumption, both of which are related to mechanoreceptor stimulation. Furthermore, because we achieved only a doubling of cardiac output with DNP-induced hypermetabolism, these experiments do not exclude the possibility that the stimulation of mechanoreceptors may

contribute to the increased cardiac output during severe exercise, which can increase cardiac output as much as 500% above its resting value.

The conclusion that the "work stimulus" is primarily metabolic in origin is supported by the recently published electrophysiological studies of Pérez-González and Coote (35). They studied the activity of afferent nerve fibers originating from muscle spindles and tendons during tetanic muscular contraction, and found that afferent nerve activities did not correlate with either the intensity of muscular contraction or the anticipated pressor responses. They, too, concluded that mechanoreceptors do not play a role in the circulatory response to exercise.

The concept that the "work stimulus" is metabolically linked was first postulated by Alam and Smirk (36), who found that the pressor response to exercise in man was greatly exaggerated when the circulation of the working muscles was occluded, and that the blood pressure remained elevated, even after cessation of muscular activity, as long as the circulation through the previously working muscles was arrested. Similar results were reported more recently by Asmussen and Nielsen (37).

The exact metabolic nature of the "work stimulus" has never been ascertained. Since the breakdown of ATP to ADP is common and fundamental to both muscular exercise and the uncoupling effect of DNP, the hydrolysis of ATP probably is linked to the "work stimulus."

In intact tissues, as soon as ATP is hydrolyzed to ADP, ATP is resynthesized via oxidative phosphorylation, anaerobic glycolysis, or breakdown of phosphorylcreatine (PC) to creatine and inorganic phosphate. There usually is little or no change in tissue ATP concentrations. Fawaz, Hawa, and Tutunji (38) found that DNP, when infused into a canine heart-lung preparation, significantly lowered the PC content with no appreciable change in the ATP content. Similarly, Hultman, Bergström, and McLennan Anderson (39) found in man that PC in working muscles decreased markedly, while the change in tissue ATP concentration was relatively small. They also showed that the PC concentration in the muscles during exercise was inversely related to the work load but directly related to the pulse rate. We may speculate that the changes in high energy phosphate and the intimately associated metabolic changes may cause the increase in cardiac output that occurs during exercise and after DNP infusion. However, direct evidence that would either substantiate this hypothesis or implicate any of the substances involved in these metabolic processes as the "work stimulus" is still not available.

Another feature common to tissue hypermetabolism induced by both DNP infusion and exercise is the reduction in tissue oxygen tension as oxygen consumption increases. In our experiments, no direct measurements of tissue oxygen tension were made, but the right ventricular blood oxygen saturation, which represents the summation of tissue oxygen saturation in all parts of the body, was determined. Fig. 4 shows that cardiac output increased as right ventricular blood oxygen saturation fell in dogs both during exercise and after DNP infusion. Although there is no direct evidence that lowering of tissue oxygen tension itself can increase cardiac output, such a possibility is not excluded and warrants further studies.

The reduction in total peripheral vascular resistance probably was caused in part by the reduced tissue oxygen tension or the metabolic changes produced by DNP. This change, however, did not decrease systemic arterial blood pressure or increase right ventricular end-diastolic pressure (Tables I and II). Vasodilation in exercising muscles probably is not responsible for the increased cardiac output, because cardiac output remains elevated even when the circulation through exercising limbs is separated from the general circulation by occlusion of blood flow to the limbs (37). Furthermore, Banet and Guyton (29) showed that when DNP was infused into dogs in which the central nervous system had been destroyed, total peripheral vascular resistance declined as much as it did in intact dogs, but cardiac output increased only slightly.

Relative tissue hypoxia may occur during tissue hypermetabolism. Tissue hypoxia impairs cellular oxidation and phosphorylation, resulting in an increase in mitochondrial NADH/NAD ratio. Based on the following relationship:

$$[\text{Pyruvate}] + [\text{NADH}] \xleftrightarrow{\text{Lactic dehydrogenase}} [\text{Lactate}] + [\text{NAD}],$$

$$\frac{[\text{Lactate}]}{[\text{Pyruvate}]} = \text{Equilibrium constant} \times \frac{[\text{NADH}]}{[\text{NAD}]}.$$

Huckabee (2, 40) suggested the use of L/P ratio and XL as measures of tissue anaerobic metabolism. Both L/P ratio and XL increased during exercise (Table II). However, when tissue hypermetabolism was induced by DNP, the increase in cardiac output was accompanied initially by a decreased L/P ratio and a negative XL. The L/P ratio and XL did not increase until oxygen consumption had exceeded four to five times its control value (Tables I and II). Therefore, anaerobic metabolism probably was not linked to the "work stimulus" that caused the increased cardiac output during the mild

to moderate tissue hypermetabolism, but the present study does not preclude the possibility that anaerobic metabolism might contribute to increased cardiac output when it occurs during severe tissue hypermetabolism. In fact, since the circulatory occlusion of exercising muscles exaggerated the hemodynamic responses during steady-state exercise (36, 37), it appears likely that anoxia could potentiate either the production of the "work stimulus" or the responsiveness of receptor organs to the "work stimulus."

Presumably glycolysis is accelerated after DNP infusion because phosphofructokinase activity is enhanced by the decrease in ATP and the increases in AMP, ADP, and inorganic phosphate (41). However, despite this increased rate of glycolysis, blood lactate and pyruvate concentrations and the L/P ratio actually fell after the two lowest doses of DNP in these experiments. This may have resulted from concurrent facilitation of electron transport by DNP (19) of a magnitude that exceeded its effects on glycolysis. This facilitation of electron transport is, however, counteracted by the falling tissue oxygen tension. A critical oxygen tension may exist below which the NADH/NAD or *L/P* ratios rise, in spite of maximum facilitation of electron transport. Oxygen consumption probably increases to roughly the same extent in all parts of the body after DNP infusion, although it would be expected to increase chiefly in the working muscles during exercise. In the latter situation, muscle oxygen tension might decrease to the critical level, causing anaerobic metabolism with a relatively small increase in total body oxygen consumption. A comparable DNP-induced increase in oxygen consumption might result in only a slight reduction in tissue oxygen tension in all parts of the body. The *L/P* ratio did not increase until oxygen consumption had increased more than fivefold.

It should be noted that cardiac output increased in proportion to the increase in oxygen consumption no matter where oxygen was consumed. It appears that the "work stimulus" for the increase in cardiac output could have been produced not only in working muscles but also in other parts of the body.

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