Biochemical Pharmacology, Vol. 26, pp. 2297–2305. Pergamon Press, 1977. Printed in Great Britain.

EFFECTS OF DISOPHENOL ON THE ISOLATED, PERFUSED RAT HEART*

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(*Received* 26 *October* 1976; *accepted* 18 *February* 1977)

Abstract—Perfusion of hearts with disophenol caused significant alterations in spontaneous heart rate, coronary flow, isometric systolic tension, metabolite levels and electrical activity. Phosphorylase *a* levels were normal at 10 and 100 ng/ml but decreased significantly at 1000 ng/ml. Disophenol markedly depressed spontaneous heart rate at 1000 ng/ml and similar decreases were noted in coronary flow and isometric systolic tension. Diastolic tension, however, increased greater than threefold after 60 min of perfusion at a level of 1000 ng/ml. At this concentration, serious disturbances in electrical activity were also evident. Disophenol depleted tissue glycogen, adenosine-5′-triphosphate, total adenine nucleotides and creatine phosphate levels while it elevated lactate levels. No disophenol-induced lesions, gross or microscopic, were found.

Disophenol (2,6-diiodo-4-nitrophenol, DNP) is a drug used in veterinary medicine recommended for use in the treatment of dogs infested with hook worms (*Ancylostoma caninum*, *A. braziliense* and *Uncinaria stenocephala*). While the pharmacology of this agent in dogs is considered similar to that of 2,4-dinitrophenol in that both drugs increase respiration, body temperature, and heart rate in this species, and both drugs accelerate the onset of rigor mortis [1, 2], the effects of disophenol on cardiac metabolism have not been previously reported. Our interest in disophenol was further stimulated by the work of several investigators who have demonstrated the ability of 2,4-dinitrophenol and related compounds to markedly alter ion transport and metabolic activity in various tissues, including the heart, obtained from different species [3–8]. The isolated, perfused rat heart was selected for our studies with disophenol because of its potential as an *in vitro* model with which to assess the cardiotoxic properties of drugs [9, 10].

METHODS AND MATERIALS

Animals, surgical preparation and perfusion methods. Wistar male albino rats (220–250 g) given Purina Lab Chow and water *ad libitum* were sacrificed by decapitation and perfused by the Langendorff technique with Krebs–Ringer bicarbonate buffer (K–R buffer) as previously described [10].

Freezing and preparation of tissue for histochemical evaluation. Hearts selected for biochemical analysis were frozen in position on the perfusion apparatus by clamping the tissue with Wollenberger tongs [11] which had been precooled in liquid nitrogen while hearts that were to be subjected to histochemical evaluation were quickly placed in 10 per cent buffered formalin solution at the conclusion of the experiment. Frozen and fixed tissue was stored and prepared as previously described [10].

Analytical methods. The analytical methods used in these studies to determine tissue metabolite and enzyme levels have been presented in detail in a previous paper [10].

Electrocardiographic recording techniques. Wick type electrodes were placed close to the surface of the heart so K–R buffer flowing over the surface bridged the small gap between the electrode and the myocardium, thereby providing a pathway for conduction of electrical impulses. The electrodes were positioned to record from the right atrium and the apex of the right ventricle. A 22 gauge needle inserted into the flow of perfusion fluid just above the glass cannula was connected to complete the circuit to ground. The Hewlett–Packard bioelectric amplifier (Model 8811–A) was set with a lower cut off frequency of 0.5 Hz and an upper cut off frequency of 300 Hz. Recordings were made at a sensitivity of 2 mv/cm with the electrode input selector switch in the Lead I position. The Hewlett–Packard recording oscillograph (Model 7782-A) was operated at a paper speed of 50 mm/second.

The durations of the PR, QT*a* (a measurement taken from the Q to the apex of the T wave) and QT intervals were calculated from the tracing using established guidelines [12–15], and corrected for variability in heart rate [16].

Statistical methods. The standard error of the mean for each experimental group was computed, and the data were examined by either the paired variate or independent *t* test [17]. Significance was established at the 5 per cent level ($P \leq 0.05$).

Drugs. The disophenol (DNP) used in these studies was a gift from the American Cyanamid Company, Princeton, New Jersey.

RESULTS

Mechanical measurements. Disophenol had little effect on spontaneous heart rate except at the highest concentration, 1000 ng/ml, which reduced it to approximately 17 per cent of control (Table 1) after 60 min of perfusion with the disophenol-containing

* This work was supported by a research contract (No. 223-74-7174) with the United States Food and Drug Administration.

Table 1. Effect of disophenol on heart rate, coronary flow, isometric systolic tension and diastolic tension in the isolated perfused rat heart*

Group	Drug and concentration	Perfusion time† (min)	N‡	Heart rate (beats/min)	Coronary flow (ml/min)	Isometric systolic tension (g)	Diastolic tension§ (g)
1	None	0	11	286 ± 6.2	10.1 ± 0.7	16.1 ± 0.9	4.9 ± 0.10
		15		272 ± 7.5	9.6 ± 0.7	16.8 ± 0.9	4.6 ± 0.13
		30		267 ± 4.9‖	9.7 ± 0.7	15.7 ± 1.0	4.6 ± 0.14‖
		45		259 ± 7.3‖	9.5 ± 0.8	14.6 ± 0.9‖	4.6 ± 0.18
		60		256 ± 8.5	8.8 ± 0.8	13.2 ± 1.3	4.9 ± 0.35
2	Disophenol (10 ng/ml)	0	7	283 ± 12.9	7.3 ± 0.2	15.0 ± 0.4	5.0 ± 0.04
		15		244 ± 7.8‖	7.1 ± 0.5	17.1 ± 0.7	4.3 ± 0.11
		30		253 ± 6.1	7.4 ± 0.4	16.9 ± 0.7	4.1 ± 0.08‖
		45		257 ± 6.1	7.2 ± 0.5	15.7 ± 0.7	4.1 ± 0.08
		60		257 ± 8.9	7.5 ± 0.6	14.2 ± 0.6	4.2 ± 0.17‖
3	Disophenol (100 ng/ml)	0	7	296 ± 12.1	8.1 ± 1.1	13.2 ± 1.0	5.2 ± 0.31
		15		266 ± 17.8‖	8.3 ± 1.3	12.6 ± 1.4	5.5 ± 0.48
		30		257 ± 14.4‖	8.3 ± 1.1	12.1 ± 1.4	5.6 ± 0.46
		45		257 ± 12.9‖	8.6 ± 1.3	10.5 ± 1.3	5.8 ± 0.46
		60		249 ± 12.6	8.0 ± 1.4	8.9 ± 1.1	6.1 ± 0.45
4	Disophenol (1000 ng/ml)	0	7	291 ± 8.6	8.5 ± 0.7	15.9 ± 0.6	5.0 ± 0.03
		15		107 ± 41.3‖	7.7 ± 1.6	2.8 ± 1.6	16.1 ± 2.35
		30		77 ± 38.1‖	6.3 ± 1.6	1.8 ± 1.4	15.5 ± 1.94
		45		58 ± 19.3‖	3.8 ± 0.4‖	1.5 ± 0.7‖	16.8 ± 0.69
		60		49 ± 21.9‖	3.0 ± 0.5‖	0.6 ± 0.2‖	16.5 ± 0.67

* Hearts obtained from untreated normal male animals (220–250 g).
† Duration of perfusion time after initial 15 min equilibration period.
‡ Number of hearts in each group.
§ An initial diastolic tension of 5 g was imposed upon the heart at the start of the 15 min equilibration period.
‖ Significant compared to 0 perfusion time within each group by paired variate t-test ($P \leq 0.05$).

medium. While a spontaneous change in heart rate (10 per cent) occurred in the control group, this magnitude of change is consistent with previous findings [10] and the drug-induced effect is clearly of greater magnitude (83 per cent).

Disophenol, 1000 ng/ml, reduced coronary flow (Table 1) to approx. 35 per cent of control at the end of 60 min perfusion, whereas it was without effect at lesser concentrations.

Isometric systolic tension decreased to 67 per cent of control in hearts perfused for 60 min with 100 ng/ml; tension was reduced to 4 per cent of pre-drug initial values in hearts perfused for 60 min with medium containing 1000 ng/ml of disophenol. Spontaneous changes in isometric systolic tension which occurred in the control group are similar to previously reported values for this preparation [10].

Disophenol produced contracture of the cardiac muscle at concentrations of 100 and 1000 ng/ml and at the latter dose, diastolic tension after 60 min perfusion was approximately 300 per cent of its initial value (Table 1). While not shown in Table 1, the above mentioned increase in diastolic tension occurred within 3–5 min after the start of perfusion with disophenol-containing medium. No change occurred in the control group and a slight, but signifi-

Table 2. Effect of disophenol on the electrical activity of the isolated perfused rat heart*

Group	Drug and concentration	Perfusion time† (min)	N‡	PR interval (milliseconds)	PR / heart rate	QT_a interval (milliseconds)	QT_a / heart rate	QT interval (milliseconds)	QT / heart rate
1	None	0	11	41 ± 0.8	2.45 ± 0.03	20 ± 1.1	1.18 ± 0.06	72 ± 2.1	4.28 ± 0.15
		15		41 ± 0.6	2.49 ± 0.04	20 ± 0.9	1.24 ± 0.06	71 ± 1.2	4.33 ± 0.09
		30		41 ± 0.7	2.53 ± 0.04	21 ± 0.8	1.26 ± 0.05	72 ± 1.4	4.42 ± 0.09
		45		42 ± 1.0	2.63 ± 0.07	20 ± 0.9	1.26 ± 0.06	73 ± 1.7	4.55 ± 0.15
		60		41 ± 0.6	2.57 ± 0.05	19 ± 0.7	1.21 ± 0.04	73 ± 1.5	4.58 ± 0.14
2	Disophenol (10 ng/ml)	0	7	40 ± 0.9	2.39 ± 0.07	21 ± 0.6	1.23 ± 0.04	69 ± 1.6	4.13 ± 0.12
		15		41 ± 1.5	2.66 ± 0.14§	22 ± 1.2	1.43 ± 0.08§	73 ± 2.2§	4.69 ± 0.16§
		30		42 ± 2.0	2.65 ± 0.14	23 ± 1.4	1.44 ± 0.09§	75 ± 3.3	4.71 ± 0.21§
		45		43 ± 2.0	2.70 ± 0.15§	24 ± 1.1§	1.49 ± 0.07§	74 ± 2.7§	4.68 ± 0.19§
		60		43 ± 2.0	2.72 ± 0.15§	24 ± 1.1§	1.52 ± 0.08§	73 ± 2.8	4.63 ± 0.19§
3	Disophenol (100 ng/ml)	0	7	39 ± 0.6	2.30 ± 0.04	21 ± 0.9	1.21 ± 0.02	71 ± 1.1	4.14 ± 0.04
		15		41 ± 0.9	2.52 ± 0.07§	24 ± 1.0§	1.46 ± 0.06§	76 ± 1.8§	4.71 ± 0.13§
		30		41 ± 1.6	2.60 ± 0.12§	23 ± 0.7§	1.43 ± 0.05§	78 ± 2.5§	4.90 ± 0.24§
		45		40 ± 1.2	2.50 ± 0.04§	23 ± 1.2	1.48 ± 0.08§	80 ± 2.9§	5.05 ± 0.27§
		60		42 ± 2.0	2.65 ± 0.12§	24 ± 1.6§	1.52 ± 0.10§	81 ± 3.4§	5.11 ± 0.27§
4	Disophenol (1000 ng/ml)	0	7	40 ± 0	2.35 ± 0.03	21 ± 0.7	1.22 ± 0.05	70 ± 0	4.11 ± 0.06
		15		40 ± 0‖	2.92 ± 0.34§	20 ± 0‖	1.46 ± 0.17‖	70 ± 0‖	5.12 ± 0.60‖
		30		45 ± 5.0‖	3.11 ± 0.34‖	20 ± 0‖	1.38 ± 0‖	85 ± 5.0‖	5.87 ± 0.34‖
		45		-	-	-	-	-	-
		60		40¶	4.22¶	34¶	3.58¶	80¶	8.43¶

* Hearts obtained from untreated normal male animals (220–250 g).
† Duration of perfusion time after initial 15 min equilibration period.
‡ Number of hearts in each group.
§ Significant compared to 0 perfusion time within each group by paired variate t-test ($P \leq 0.05$).
‖ N = 2.
¶ N = 1.

cant relaxation was present in those hearts perfused with 10 ng/ml of disophenol.

Electrical measurements. The effects of disophenol perfusion on the electrical activity of the heart are shown in Table 2. In the control group (number 1), no alteration in depolarization, conduction or repolarization was evident during the 60 min perfusion period with drug-free Krebs–Ringer bicarbonate medium. Disophenol-induced effects, however, were seen at 10 ng/ml (group 2). At this concentration, there was prolongation of the QT_a and QT intervals as well as an increase in the corrected PR, QT_a and QT values. Similar effects occurred when the dose was increased to 100 ng/ml (group 3).

Severe conduction disturbances, decreased spontaneous rate and cardiac arrest occurred in a number of hearts perfused with 1000 ng/ml (group 4) for 60 min. Since these effects usually took place within 5 min or less after the switch to disophenol-containing medium (Fig. 2), It was often impossible to obtain meaningful measurements of electrical activity by 15 min and at subsequent time intervals, hence the data for group 4 appears incomplete even though 7 hearts were perfused. The marked alterations produced by disophenol (1000 ng/ml) on the electrical activity of hearts in group 4 (Table 2) are seen in Figs. 1 and 2. While there was no evidence of any abnormality in the predrug control tracing (Fig. 1), ectopic ventricular beats were present in the 15 min tracing. A sinus rhythm was seen at 30 min followed by ventricular tachycardia at 42 min, a series of very small ectopic ventricular beats at 45 min, and an occasional ectopic ventricular beat at 60 min (not shown).

In tracings from a second heart (Fig. 2), also perfused with 1000 ng/ml, the control pattern appears normal with a sinus rhythm, but a few non-conducted ventricular beats were detected. After 6 min of perfusion with disophenol, there was a sinus rhythm with bradycardia and ventricular ectopic beats. At 14 min, a very slow sinus rhythm was evident with coupled ectopic ventricular beats, while at 15 min, ventricular standstill occurred with evidence of a few ectopic ventricular beats. Ectopic ventricular beats, with sinus beats following at a constant interval, were seen at 18 min. Some of these sinus beats appeared to be conducted while others were blocked. Ventricular tachycardia occurred at 30 min with some aberrations. Ectopic ventricular beats were seen at 45 min, and at 60 min, there was a sinus rhythm with ectopic ventricular beats.

Biochemical measurements. In hearts perfused with 10 ng/ml of disophenol, metabolite levels did not differ significantly from control values with the exception of pyruvate which was elevated (Table 3). The 100 ng/ml dose reduced glycogen levels to about 65 per cent of control while 1000 ng/ml lowered it to 12 per cent. Glucose-6-phosphate (G6P) levels increased in the 1000 ng/ml group, whereas lower doses of disophenol showed no increase. Fructose-1,6-diphosphate (F-1, 6-DP) levels were elevated in the 100 and 1000 ng/ml groups, but D-glyceraldehyde-3-phosphate (GAP) levels were below normal. Total triose phosphate levels were reduced in the 100 ng/ml group. The concentration of L-(−)-glycerol-1-phosphate in hearts perfused with 1000 ng/ml of disophenol was significantly higher than that in control hearts. At this dose, there was also a two-fold increase in the tissue content of lactate in the same heart (Table 3).

Adenosine-5′-triphosphate (ATP) was significantly lower in hearts perfused with 1000 ng/ml of disophenol for 60 min than in control hearts, while the tissue level of adenosine-5′-monophosphate (AMP)

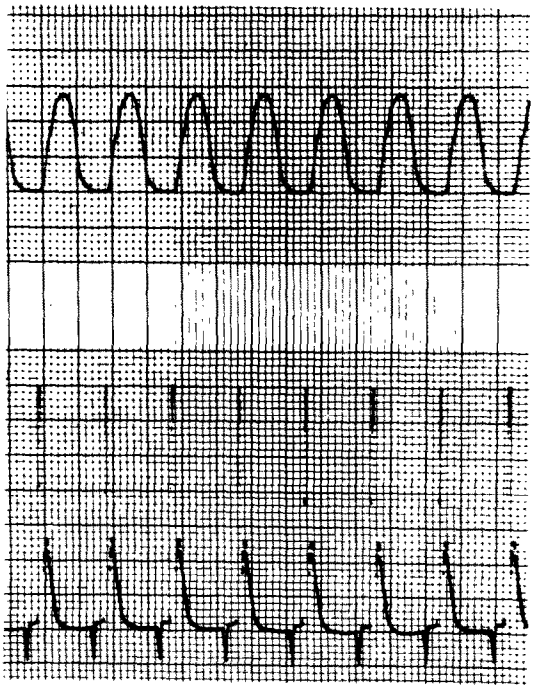


Fig. 1(*A*)

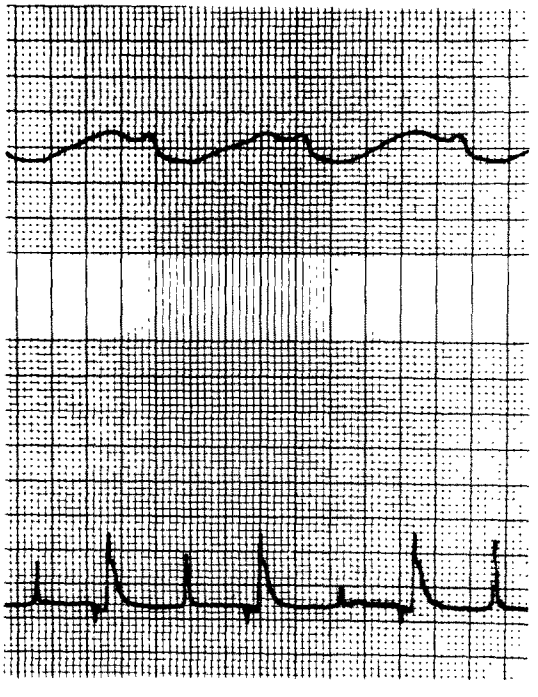


Fig. 1(*B*)

was elevated. The total adenine nucleotide content was also significantly lower in 1000 ng/ml hearts than in controls. The amount of creatine phosphate present in the 1000 ng/ml group was less than that in controls, whereas no effect occurred with 10 ng/ml or 100 ng/ml of disophenol.

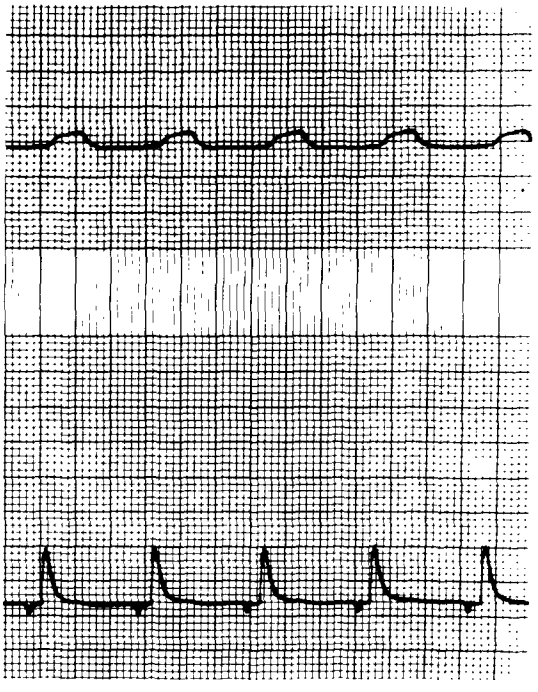


Fig. 1(*C*)

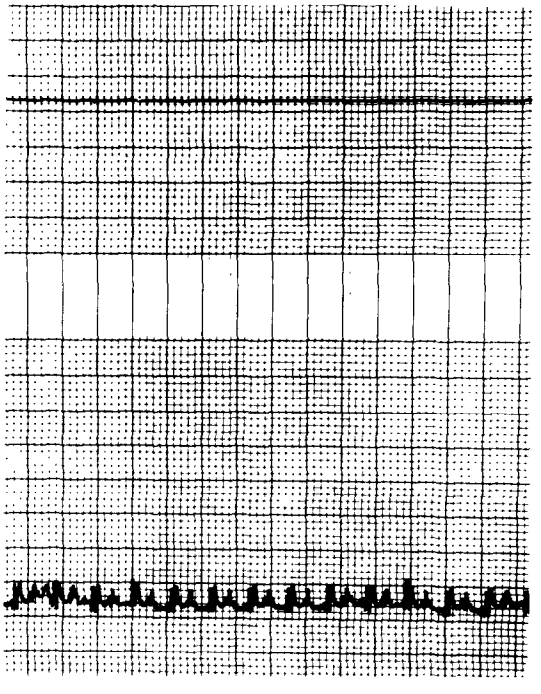


Fig. 1(*D*)

Lactate levels were stable in effluent samples obtained from control hearts throughout the 60 min perfusion period (group 1, Table 4) and no significant departures from normal were found in samples from those perfused with 10 ng/ml of disophenol. In hearts perfused with 100 ng/ml (group 3), there was an elevation in the amount of lactate measured in the coronary effluent at 45 min and it was further increased by 60 min. There was a significant decrease in lactate present in 60 min samples from 1000 mg/ml-perfused hearts.

Disophenol had no effect on cardiac phosphorylase *a* following 60 min of perfusion at 10 and 100 ng/ml (groups 2 and 3, Table 5) and phosphorylase *a* activity was significantly below control levels following a 60 min perfusion with medium containing 1000 ng/ml of disophenol.

Histochemical evaluation. Disophenol-containing K–R buffer (10, 100, 1000 ng/ml) produced no significant alterations in the histology of rat hearts exposed to these solutions for a period of 60 min.

The degree of fuchsinorrhagia [18], a measure of myocardial ischemia, found in disophenol-perfused hearts was not appreciably different from that in control hearts.

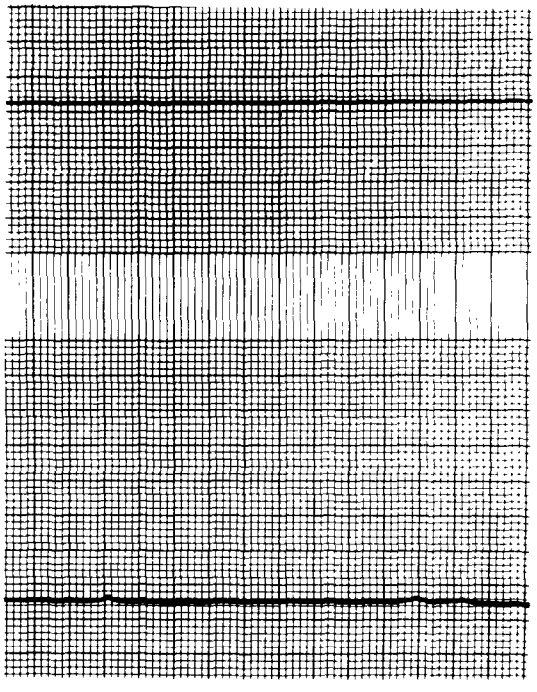


Fig. 1(*E*)

Fig. 1. This series of recordings was made from a heart perfused with Krebs–Ringer bicarbonate buffer containing disophenol (1000 ng/ml). Isometric systolic tension (1 g/mm) is shown in the upper part of each tracing, while electrical activity (2 mV/cm) is shown below. Paper speed was 50 mm/s. The first, or *O* time, recording (*A*) was made at the end of the 15 min equilibration period with drug-free buffer after which the heart was switched to medium containing disophenol. Subsequent recordings were made at the following time intervals) *B*—15 min, *C*—30 min, *D*—42 min, *E*—45 min. Abnormalities are described in the Results section.

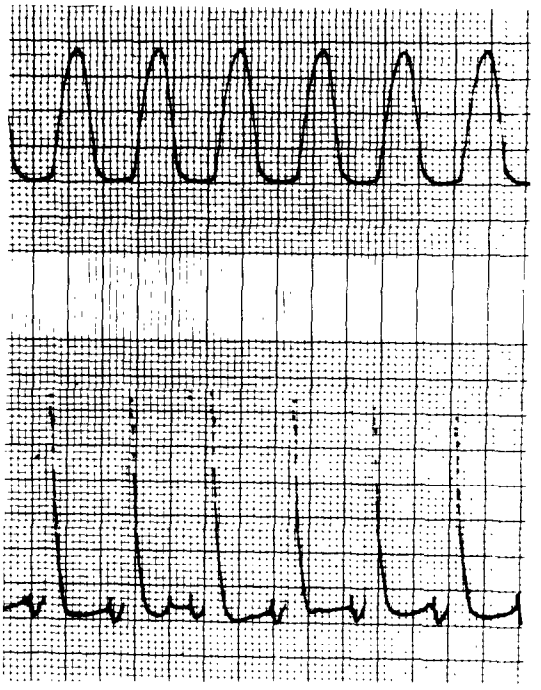


Fig. 2(*A*)

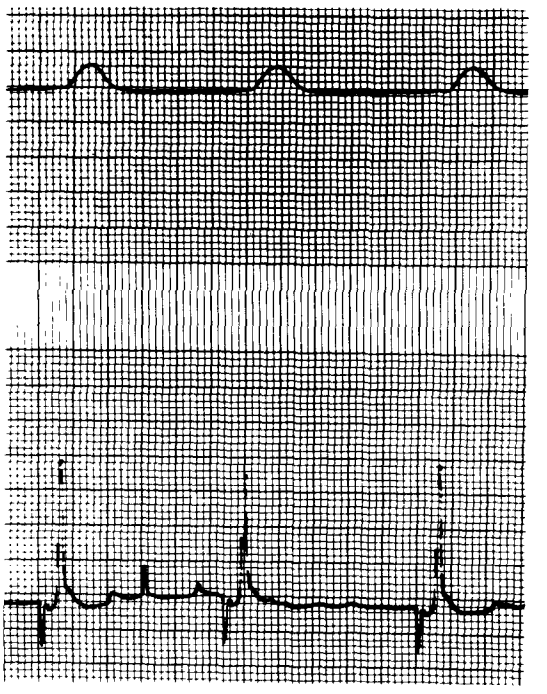


Fig. 2(*B*)

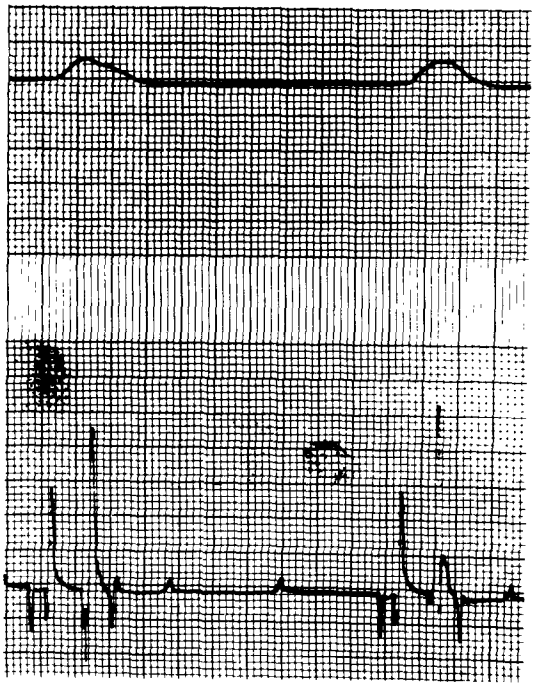
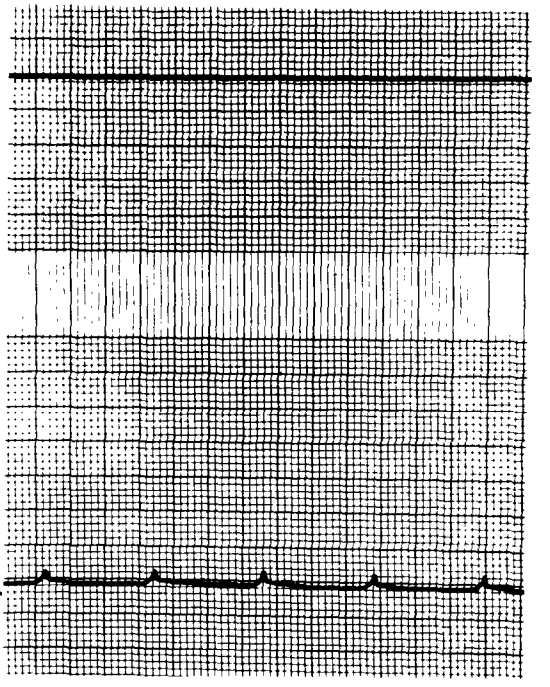


Fig. 2(*C*)



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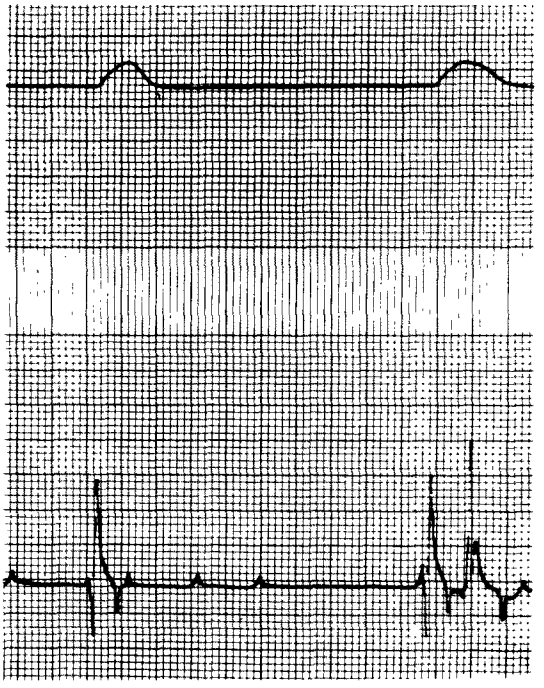

Fig. 2(*E*)

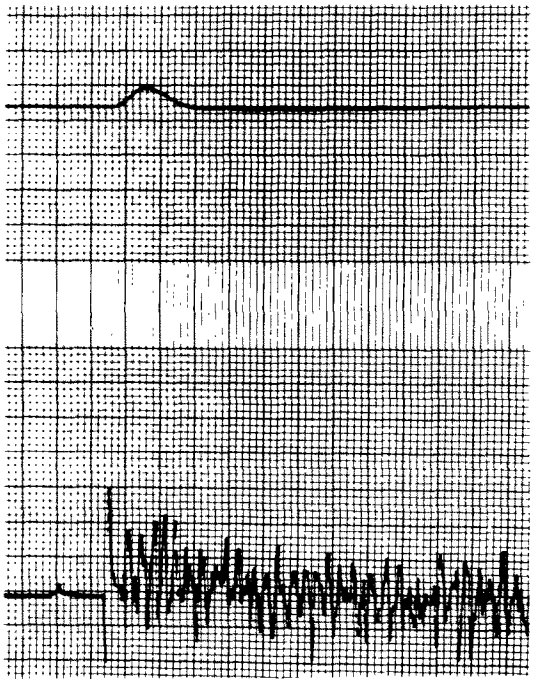

Fig. 2(*F*)

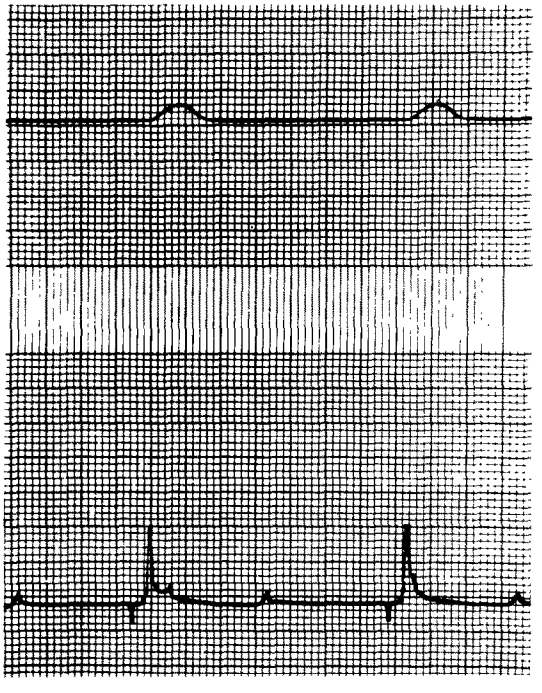

Fig. 2(*G*)

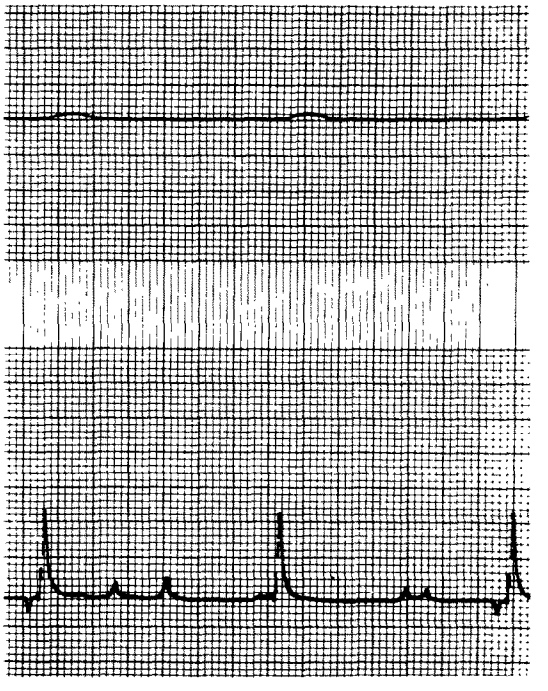

Fig. 2(*H*)

Fig. 2. This series of recordings was made from a heart perfused with Krebs–Ringer bicarbonate buffer containing disophenol (1000 ng/ml). Isometric systolic tension (1 gm/mm) is shown in the upper part of each tracing, while electrical activity (2 mV/cm) is shown below. Paper speed was 50 mm/s. The first, or *O* time, recording (*A*) was made at the end of the 15 min equilibration period with drug-free buffer after which the heart was switched to medium containing disophenol. Subsequent recordings were made at the following time intervals: *B*—6 min, *C*—14 min, *D*—15 min, *E*—18 min, *F*—30 min, *G*—45 min, *H*—60 min. Abnormalities are described in the Results section.

Table 3. Effect of disophenol on metabolite levels in the isolated perfused rat heart*

Metabolite	Concentration of Disophenol in Perfusion Medium							
	0		10 ng/ml		100 ng/ml		1000 ng/ml	
	N†	μM/gram ± SEM‡	N†	μM/gram ± SEM‡	N†	μM/gram ± SEM‡	N†	μM/gram ± SEM‡
Glycogen	9	12.06 ± 0.99	5	12.03 ± 1.04	5	7 91 ± 0.64§	5	1.48 ± 0.21§
D-Glucose-1-phosphate	9	0 ± 0	5	0 ± 0	5	0 ± 0	5	0 ± 0
D-Glucose-6-phosphate	9	0.0497 ± 0.0033	5	0.0432 ± 0.0049	5	0.0422 ± 0.0032	5	0.0724 ± 0.0072§
D-Fructose-6-phosphate	9	0 ± 0	5	0 ± 0	5	0 ± 0	5	0 ± 0
D-Fructose-1,6-diphosphate	9	0.0114 ± 0.0013	5	0.0140 ± 0	5	0.0244 ± 0.0058§	5	0.0284 ± 0.0075§
Dihydroxyacetone phosphate	9	0.0354 ± 0.0066	5	0.0330 ± 0.0054	5	0.0330 ± 0.0136	5	0.0490 ± 0.0189
D-Glyceraldehyde-3-phosphate	9	0.0362 ± 0.0057	5	0.0362 ± 0.0074	5	0.0194 ± 0.0077§	5	0.0248 ± 0.0095§
Total triose phosphate	9	0.0878 ± 0.0066	5	0.0712 ± 0.0118	5	0.0524 ± 0.0143§	5	0.0738 ± 0.0148
L-(−)-Glycerol-1-phosphate	9	0.1662 ± 0.0485	5	0.0564 ± 0.0167	5	0.1112 ± 0.0188	5	0.3522 ± 0.0418§
Pyruvate	9	0.0177 ± 0.0094	5	0.0556 ± 0.0146§	5	0.0480 ± 0.0094	5	0.0338 ± 0.0112
L-(+)-Lactate	9	0.4294 ± 0.0643	5	0.2430 ± 0.0569	5	0.5148 ± 0.1068	5	0.9336 ± 0.1546§
Adenosine-5'-triphosphate	9	2.0650 ± 0.1429	5	2.3898 ± 0 1507	5	1.9532 ± 0.1359	5	0.6814 ± 0.1223§
Adenosine-5'-diphosphate	9	0 2131 ± 0.0331	5	0.1326 ± 0.0264	5	0.1434 ± 0.0141	5	0.1114 ± 0.0282
Adenosine-5'-monophosphate	9	0.0782 ± 0.0100	5	0.1068 ± 0.0295	5	0.0770 ± 0.0191	5	0.2580 ± 0.0296§
Total adenine nucleotides	9	2.3563 ± 0.1657	5	2.6292 ± 0.1284	5	2.1736 ± 0.1245	5	1.0508 ± 0.1050§
Creatine phosphate	9	1.8450 ± 0.0892	5	1.6768 ± 0.1585	5	1.8704 ± 0.2014	5	1.1708 ± 0.1106§

* Hearts obtained from untreated normal male animals (220–250 g).
† Number of hearts in each group.
‡ Expressed per gram of tissue (wet weight).
§ Significant compared to control (0 drug level) by an independent *t*-test ($P \leq 0.05$).

Table 4. Effect of disophenol on lactate levels in coronary effluent*

Group	Drug and concentration	Perfusion time† (min)	N‡	Lactate level§ (μmole/g/min)
1	None	0	3	0.584 ± 0.239
		15		0.500 ± 0 217
		30		0.644 ± 0.296
		45		0.619 ± 0.229
		60		0.617 ± 0 170
2	Disophenol (10 ng/ml)	0	7	0.332 ± 0.033
		15		0.410 ± 0.074
		30		0.325 ± 0.082
		45		0.295 ± 0.081
		60		0.411 ± 0.113
3	Disophenol (100 ng/ml)	0	7	0.515 ± 0.133
		15		0.594 ± 0.156
		30		0.498 ± 0.103
		45		0.634 ± 0.123‖
		60		0.708 ± 0.127‖
4	Disophenol (1000 ng/ml)	0	7	0.442 ± 0.061
		15		0.481 ± 0.069
		30		0.463 ± 0.103
		45		0.338 ± 0.039
		60		0.283 ± 0.030‖

* Hearts obtained from untreated normal male animals (220–250 g).
† Duration of perfusion time after initial 15 min equilibration period.
‡ Number of hearts in each group.
§ Calculated on the basis of tissue wet weight.
‖ Significant compared to 0 perfusion time within each group by paired variate *t*-test ($P \leq 0.05$).

Table 5. Effect of disophenol on cardiac phosphorylase *a* activity*

Group	Drug	Drug concentration (ng/ml)	N†	% Phosphorylase *a*‡
1	None	—	9	13.7 ± 0.8
2	Disophenol	10	5	14.5 ± 0.2
3	Disophenol	100	5	15.0 ± 0.7
4	Disophenol	1000	5	8.0 ± 0.5§

* Hearts obtained from normal male animals (220–250 g) were perfused with control Krebs–Ringer bicarbonate solution for 15 min before being perfused with perfusion medium containing disophenol for 60 min.
† Number of hearts in each group.
‡ % phosphorylase *a* = Cori Units of phosphorylase *a*/Total cori units of phosphorylase ($a + b$) × 100.
§ Significant compared to control hearts (group 1, perfused with control medium for a total of one hour and 15 min) by an independent *t*-test ($P \leq 0.05$).

DISCUSSION

When Chang *et al.* [19] perfused isolated guinea pig hearts with 2,4-dinitrophenol at concentrations ranging from 8×10^{-7} to 4×10^{-5} M, they observed an increase in the force of contraction without any apparent toxic effect on the heart. At 8×10^{-6} M, 2,4-dinitrophenol caused a large positive inotropic response in their preparation which lasted approximately 4–5 min and was followed by a smaller sustained response which persisted for the balance of the 30 min perfusion. They attributed this positive inotropic action to a 2,4-dinitrophenol-induced release of endogenous catecholamines within the heart. In our experiments with the isolated, perfused rat heart, disophenol (1000 ng/ml, 2.56×10^{-6} M) depressed isometric systolic tension, spontaneous heart rate and coronary flow (Table 1). Perfusion of our preparation with 10 ng/ml disophenol-containing medium (2.56×10^{-8} M) caused a small elevation of isometric systolic tension which was significant at the 15 and 30 min time intervals, however, it returned to control levels before 60 min when the hearts were frozen for subsequent biochemical analysis.

Force of contraction was markedly reduced while mechanical alternation was increased in an electrically driven cat papillary muscle preparation bathed in a 10 μg/ml solution of 2,4-dinitrophenol-containing medium [20]. Jose and Stitt [21] found that 2,4-dinitrophenol (0.2 mg/kg/min) caused a progressive slow decline in contractile force, which was terminated by spontaneous cardiac arrest, and at 1×10^{-4} M, it had a similar effect in isolated rat embryo hearts [22]. The beating of cultured rat heart cells was also inhibited and tissue levels of ATP in these cells were reduced by 5×10^{-4} M 2,4-dinitrophenol [23]. Our results with disophenol, are quite similar to the studies listed above utilizing 2,4-dinitrophenol. The positive inotropic effects of 2,4-dinitrophenol in the heart reported by Chang *et al.* [19] may be due, in part, to the fact that these investigators used a perfusion medium containing a lower concentration of Ca^{2+} (1.8 mM) than our Krebs–Ringer bicarbonate solution (2.5 mM Ca^{2+}). It is quite possible, however, that greater control isometric systolic tension associated with 2.5 mM Ca^{2+} partially or completely masked the effect of any endogenously released catecholamines in our preparation. Bianchi* has observed that certain drugs which release endogenous catecholamines from adrenergic nerve terminals are less effective in producing a positive inotropic response in isolated hearts perfused with medium containing a high Ca^{2+} concentration than in those perfused with medium containing a lower Ca^{2+} concentration.

The increase in diastolic tension produced by disophenol perfusion at 100 and 1000 ng/ml (Table 1) was probably caused by drug-induced changes in ion movements across cardiac muscle cell membranes. While our experiments did not include measurement of Na^+, K^+ and Ca^{2+} levels in the heart, McDonald and MacLeod [3] reported that 2,4-dinitrophenol (0.1 mM) produced a large dose-dependent loss of K^+ from guinea pig ventricular muscle and this loss of K^+ was not related to a Na^+ gain. The effect of 2,4-dinitrophenol on K^+ efflux was attributed to a direct effect on the cell membrane. The reduction in the duration of the action potential which accompanied it was believed due to an increase in K^+ efflux and a reduction in the inward current due to Na^+ and Ca^{2+} that was previously assumed to be almost entirely dependent on ATP produced through glycolysis. The authors [3] concluded that electrogenic Na^+ pumping may play a role in maintaining the resting potential in K^+-depleted, 2,4-dinitrophenol-treated cardiac muscle. The disophenol-induced elevation in diastolic tension that occurred in the isolated, perfused rat heart (Table 1) may be related to a combination of excessive K^+ loss and a build up of free Ca^{2+} within the tissue as ATP levels diminished. Additional studies, however, would be required to confirm this possibility.

The data in Table 2, as well as Figs. 1 and 2, illustrate the ability of disophenol to produce severe disturbances in the electrical activity of the isolated, perfused heart. Kaiser [2] reported that, in dogs, 2,4-dinitrophenol and disophenol produced diphasic T waves, T wave reversal, S–T segment elevation or depression and a reduction in the amplitude of the R wave. 2,4-Dinitrophenol also caused a high incidence of ectopic beats in dogs [21]. It was also reported that while 25 or 125 mg/kg of 2,4-dinitrophenol in dogs produced bizarre ECG tracings devoid of characteristic wave patterns, disophenol had no such effect [2]. Our studies, however, indicate that disophenol can cause bizarre recordings in rat hearts perfused with 1000 ng/ml of the drug, a concentration a hundred times smaller than the plasma level of disophenol reported to cause death in dogs [2]. Disophenol-induced electrical disturbances were not unexpected in light of the severe ionic changes produced by 2,4-dinitrophenol, a similar compound. Our finding that the isolated perfused rat heart preparation is considerably more sensitive than intact animals to the cardiotoxic properties of disophenol strongly suggests that protein binding of disophenol may be a protective factor *in vivo*. If this assumption is correct, hearts would actually be exposed to a lower level of free (active) drug than *in vivo* plasma level studies would seem to indicate. Since Kaiser [2] did not distinguish between free and bound forms, one can only assume that his method measured total (free plus bound) plasma disophenol. His data indicates that some binding of disophenol probably takes place, since little disophenol was excreted in the urine by dogs during the first 24 hr following ingestion of the drug.

The enzymes and metabolites measured in these studies (Tables 3–5) were selected because they related to important energy producing pathways in the heart, and 2,4-dinitrophenol, a drug very similar to disophenol, has been shown to markedly alter the function of one or more of these pathways [3–8, 24–28].

The most striking disophenol-induced changes in metabolic activity (Table 3) were seen at the 1000 ng/ml level. At this concentration, glycogen content was reduced to 12 per cent of the control value. The elevated level of D-glucose-6-phosphate suggests possible phosphoglucomutase stimulation, while phosphofructokinase stimulation may account for the accumulation of D-fructose-1,6-diphosphate.

* C. P. Bianchi, personal communication.

Several drugs, including 2,4-dinitrophenol and related agents, uncouple oxidative phosphorylation presumably by hydrolyzing a high energy intermediate interposed between the electron transport chain and ATP [24]. 2,4-Dinitrophenol also markedly stimulates glycolysis and reduces ATP concentrations [4, 25]. While Lardy and Wellman [26] have demonstrated the ability of 2,4-dinitrophenol to stimulate mitochondrial ATPase, Klahr *et al.* [27] proposed that 2,4-dinitrophenol-induced hydrolysis of an high energy intermediate involved in ion transport serves to stimulate glycolysis and divert ATP to this pathway from others, including ATP otherwise destined to support electrical and mechanical activity. Disophenol probably reduced myocardial ATP in our preparation by one or more of these mechanisms. Creatine phosphate levels were also substantially reduced when hearts were perfused with 1000 ng/ml of disophenol.

Depression of oxidative-type reactions by 2,4-dinitrophenol-like drugs leads to build up of NADH which favors stimulation of L-(−)-glycerol-1-phosphate and lactate production as shown in Table 3. The decrease in effluent lactate production seen at 60 min in Table 4 (group 4) may be a function of lower than normal glycogen breakdown, and maximal lactate production probably occurred within minutes after the drug entered the heart. Additional time course studies with more frequent sampling intervals would be necessary to firmly establish this point.

In our experiments, disophenol did not elevate phosphorylase *a* activity in the perfused rat heart, but caused a significant decrease in the conversion of phosphorylase *b* to *a* (137 ± 0.8% to 8.0 ± 0.5%) at the highest level studied (1000 ng/ml) (Table 5). Our results are similar to those of Simoes *et al.* [5] who reported that 1,2,4-dinitrophenol, a compound similar in structure to disophenol, anomalously was a strong inhibitor of glycogen phosphorolysis, yet a glycogenolytic agent in skeletal muscle from rabbits. They demonstrated that this drug inhibited phosphorylase *b* by competition with its coenzyme AMP, but not phosphorylase *a* in their system. Their data also showed that when lactic acid levels were high in rabbit skeletal muscle following treatment with 1,2,4-dinitrophenol, glycogen phosphorolysis was inhibited in muscle. In our experiments, disophenol, at 100 ng/ml (Table 3), significantly elevated tissue lactic acid levels in the heart, while reducing the conversion of phosphorylase *b* to *a* (Table 5), but tissue glycogen levels were also reduced to 12 per cent of the control value (Table 3) by 60 min. In a subsequent paper, Focesi *et al.* [28] reported the ability of 2,4-dinitrophenol to stimulate phosphorylase *b* kinase activity and subsequent conversion of phosphorylase *b* to *a* in hearts from rats pretreated with 2,4-dinitrophenol. More recently, Vercesi and Focesi [6] demonstrated a 3-fold elevation of phosphorylase *a* and a 4-fold increase in tissue lactic acid levels in isolated rat hearts perfused with 10^{-5} M 2,4-dinitrophenol. It should be pointed out, however, that their hearts were frozen after a 10 min perfusion with 2,4-dinitrophenol-containing medium, whereas, in our experiments, hearts were perfused with disophenol for 60 min. Differences between the actions of disophenol and 2,4-dinitrophenol, sampling time sequences, freezing techniques, depletion of available glycogen stores, and assay methods are all factors which may account for the differences in results observed by various investigative groups.

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