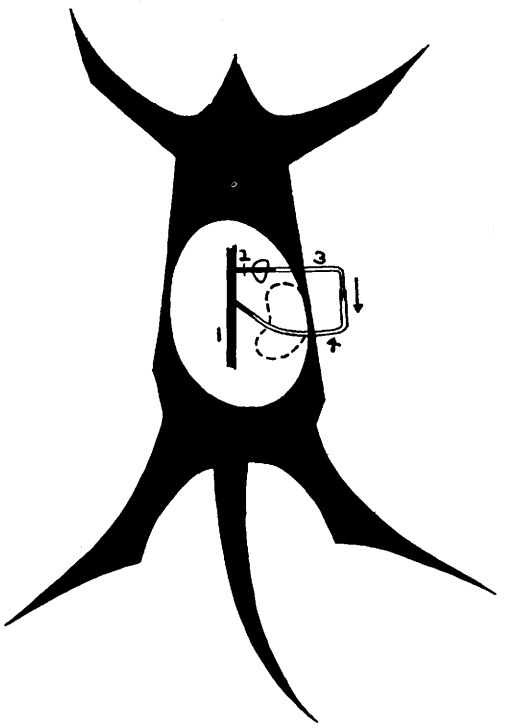

FIG. 1. Schematic illustration of "adrenal-renal shunt". Shown are inferior vena cava (1), ligated adrenal vein (2), cannulated adrenal lumbar vein (3), cannulated renal vein (4) and outline of kidney. Arrow indicates direction of blood flow.

turbance may interfere with adrenal hemeodynamics or influence the secretory activity of the adrenal. The "adreno-renal shunt" also has the advantage that measurements of flow rate can be easily obtained during the shunt period. It is not necessary to utilize the renal vein to return blood to the animal. Other abdominal veins may also serve this purpose.

Summary. A method is described for collecting intermittent samples of adrenal venous blood. This method is applicable to rats, dogs, cats and other laboratory animals. It has the advantage of involving no manipulation, tension or interference with adrenal hemodynamics during periods of collection or when the adrenal effluent is being returned to the animal through a cannula placed in an ipsilateral abdominal vein.

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Temperature-dependence of Dinitrocresol Stimulation of Aerobic and Anaerobic Lactate Production in Ascites Tumor Cells. (24368)

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Stimulation by 4,6-dinitro-o-cresol (DNC) of glucose use and lactate production in Ehrlich carcinoma and Crocker sarcoma 180 ascites tumor cells has been described(1). Further studies of 2 features of this stimulation are here reported. These are: (1) stimulation by DNC occurs under anaerobic as well as aerobic conditions and, (2) stimulated aerobic rate exceeds stimulated anaerobic rate at certain concentrations of DNC. The earlier experiments were carried out at 25°. In the present studies, similar experiments were carried out at 15° and 37° as well as 25°; it has been found, especially with the Ehrlich carcinoma, that degree of stimulation by DNC is markedly temperature-dependent and that it has a different value aerobically than it has anaerobically. Thus, the ratios of stimulated aerobic rates to anaerobic rates also vary with temperature.

Methods. Ehrlich carcinoma ascites tumor cells were maintained, harvested, and washed free of red and white blood cells as previously described(1) except that a solution of 0.15 M

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TABLE I. Influence of Temperature on 4,6-dinitro-o-cresol Stimulation of Lactate Production and Oxygen Consumption in the Ehrlich Carcinoma and the Crocker Sarcoma 180 Ascites Tumors. The experiments were carried out as described in the text. The duration of incubation was 20 min. at 37°, 40 min. at 25°, and 90 min. at 15°. The values for lactate production and oxygen consumption are expressed as micromoles per hr per 250 mg wet wt of cells.

		Ehrlich carcinoma				Crocker sarcoma 180			
		Lactate production				Lactate production			
Temp., °C	Molar conc. DNC $\times 10^{-6}$	Aerobic	An-aerobic	Ratio: aerobic/anaerobic	O_2 use	Aerobic	An-aerobic	Ratio: aerobic/anaerobic	O_2 use
37	none	24.6	41.4	.59	6.7	33.9	68.4	.50	17.1
	8	41.7	46.8	.95	12.3	60.0	68.4	.88	24.9
	16	52.5	49.5	1.06	15.9	71.1	65.7	1.08	29.1
	32	62.4	49.5	1.32	13.5	82.2	62.7	1.30	23.1
	64	80.5	49.5	1.63	10.2	68.4	60.0	1.14	15.6
	128	81.3	46.2	1.76	7.5	57.3	54.0	1.06	14.4
	256	66.3	46.8	1.41	7.5	47.8	46.8	1.01	15.3
	512	57.9	45.6	1.28	6.0	40.2	39.6	1.00	17.4
25	none	10.3	20.8	.50	3.6	10.6	25.5	.42	3.6
	8	22.8	25.8	.88	6.1	26.4	25.5	1.03	8.6
	16	27.4	26.4	1.03	8.7	32.0	23.7	1.35	11.4
	32	36.2	31.0	1.11	5.4	27.8	22.6	1.22	4.1
	64	40.4	33.2	1.22	4.5	22.2	20.8	1.07	3.0
	128	40.8	30.9	1.32	3.5	18.6	16.7	1.12	2.1
	256	33.3	29.1	1.15	2.7	13.3	12.4	1.07	1.7
	512	27.8	23.1	1.20	2.7	10.8	10.2	1.06	2.1
15	none	2.2	3.7	.59	.73	3.7	7.5	.50	1.3
	8	4.3	5.5	.78	1.7	10.3	9.8	1.05	3.0
	16	5.2	6.3	.82	2.1	10.8	9.0	1.21	2.4
	32	8.2	9.0	.91	1.9	8.8	8.5	1.04	1.5
	64	10.3	10.0	1.04	1.6	8.2	7.3	1.11	1.1
	128	11.2	10.6	1.06	1.4	5.4	5.4	1.00	.9
	256	9.5	9.8	.97	1.2	3.8	3.6	1.04	.9
	512	8.8	8.4	1.05	1.0	2.7	2.1	1.29	.8

NaCl containing 0.025 M glycylglycine, pH 7.4, was used in washing the cells and for making final suspension. Experiments were carried out in Warburg flasks as before(1), except that the main compartment contained 0.6 cc of glycylglycine, 0.02 M, pH 7.4, 0.1 cc $KHCO_3$, 0.02 M, 1 cc of cell suspension, and enough of above washing solution to bring the final total volume to 3 cc. Glucose, 0.5 cc, and the substituted phenol, 0.3 cc, each in the amount required to give the desired final concentrations, were placed in a side arm. Sarcoma 180 ascites tumor cells and Freund sarcoma ascites tumor cells were harvested and handled similarly.

Results. Values for aerobic and anaerobic lactate production and for oxygen consumption by the Ehrlich carcinoma and the Sarcoma 180 ascites tumor cells at 3 different temperatures and in the presence of various concentrations of DNC are shown in Table I.

Ehrlich carcinoma. At all 3 temperatures, lactate production by the Ehrlich carcinoma cells was stimulated by DNC both aerobically and anaerobically. Characteristically, degree of stimulation increased with graded concentrations of DNC, achieved a maximal value, and decreased at highest concentrations used. These data and the ratios derived from them suggest the following salient features of DNC stimulation: 1. Degree of stimulation was, at all temperatures, greater under aerobic than under anaerobic conditions. It should be noted, however, that these cells exhibit a Pasteur effect and, in the absence of added DNC, aerobic lactate production is less than anaerobic by 40 to 50%. 2. Maximal value reached aerobically with increasing DNC was greater than maximal anaerobic value at 37° and 25° but not at 15°. 3. Maximal stimulation of lactate production occurred aerobically at 128×10^{-6} M DNC in all instances, whereas anaerobically the concentration of DNC required for maximal stimulation appeared to change with temperature. At 15°, however, maximal stimulation an-

aerobically also occurred at 128×10^{-6} M DNC. This value may be contrasted to the value of 16×10^{-6} M, the concentration of DNC producing maximal stimulation of oxygen consumption at all 3 temperatures.

Crocker sarcoma 180. A significantly different pattern of DNC action was obtained with the Crocker sarcoma 180 ascites tumor. Although stimulation of lactate production was obtained under aerobic conditions at all temperatures, its degree did not increase in a regular manner as temperature was reduced from 37°. Moreover, stimulation of anaerobic lactate production occurred only at 15° and was, in that instance, not large. It is perhaps significant that concentration of DNC (16 or 32×10^{-6} M) which produced maximal stimulation of lactate production in this tumor was the same or nearly the same as that (16×10^{-6} M) which produced maximal stimulation of oxygen consumption.

Freund sarcoma. Cells of the Freund sarcoma, a chemically-induced tumor, were studied at 37°. In their response to graded concentrations of DNC they resembled that Crocker sarcoma 180 rather than the Ehrlich carcinoma.

Effects of other substituted phenols. Various other substituted phenols were tested for their influence on lactate production by the Ehrlich carcinoma ascites tumor cells. These were o-nitrophenol, p-nitrophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4-dibromophenol, 2,6-dibromophenol, 2,4,5-trichlorophenol, 2,4,6-tribromophenol, and 2,4,6-triiodophenol. At 37°, all stimulated aerobic lactate production except o-nitrophenol, which had no effect at any concentration tested, and none stimulated anaerobic lactate production. Only 2,4,5-trichlorophenol was tested at 25°. It did not stimulate anaerobic lactate production.

Discussion. In the Ehrlich carcinoma ascites tumor, the difference between concentration of DNC which maximally stimulates lactate production and that which maximally stimulates oxygen consumption is large (8-fold) and it seems justified to conclude that the two phenomena are unrelated. The latter phenomenon and its probable mechanisms have been extensively reviewed(2,3,4). The mechanism by which lactate production may be stimulated by DNC has so far eluded detection.

Two aspects of the stimulation of lactate production by DNC require explanation: 1, Locus and mode of action of DNC which results in stimulation and, 2, identity of the rate-limiting steps of metabolism which account for absolute and relative values of lactate production when maximally stimulated by DNC. Concerning the latter, the following may be considered. Maximum values of aerobic and anaerobic lactate production when stimulated by DNC are the same at 15° and different at 25° and 37°. This relationship suggests that there is, at 15°, a common rate-limiting step and at higher temperatures a different rate-limiting step for the 2 experimental conditions. If this assumption is correct a possible explanation for the results at 15° may be that glucose penetration into the cell, which is not rate-limiting in absence of DNC, becomes rate-limiting when glucose metabolism is stimulated. In support of this suggestion it may be noted that the large Q_{10} values (approximately 4-5) for lactate production between temperatures of 15° and 25° are similar to those reported by Crane, Field, and Cori(5) for sugar penetration into these cells. Furthermore, penetration of sugar has the same rate under aerobic and anaerobic conditions and is not directly influenced by DNC(5).

In continuing the search for loci of action of DNC which can result in stimulation of lactate production and in differing degrees of stimulation under aerobic and anaerobic conditions, the following points may be considered. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and oxidized pyridine nucleotide are required in conversion of glucose to lactate and their individual availabilities are rate-controlling factors in this process(6,7). Since at all temperatures maximal stimulated rate occurs at a concentration of DNC which has presumably suppressed aerobic phosphorylation to minimal levels (8), ATP available as a phosphate donor in the hexokinase and phosphohexokinase reactions and ADP available as a phosphate acceptor in later reactions are likely glycolytic in

origin and very nearly the same in amount in both aerobic and anaerobic experiments. Thus the proposition is appealing that the influence of DNC on some aerobic reaction other than aerobic phosphorylation, possibly reduced pyridine nucleotide oxidation, underlies the difference between aerobic and anaerobic values for maximal stimulation at 37° and 25°. In fact, interference with reduced triphosphopyridine nucleotide (TPNH) oxidation as a mechanism of DNC action for both aerobic and anaerobic stimulation seems a reasonable speculation on which to base further work. Some evidence pointing in this direction has been obtained with the eggs of *Arbacia punctulata* in which it has been found(9) that DNC alters the pathway of glucose utilization from one requiring a continuing supply of oxidized triphosphopyridine nucleotide (phosphogluconate pathway) to one requiring a supply of oxidized diphosphopyridine nucleotide (glycolytic pathway). In addition, substituted phenols can inhibit flavin nucleotide oxidations in general(10) and TPNH-cytochrome-c reductase in particular(11).

Summary. Conversion of glucose to lactate by ascites tumor cells derived from the Ehrlich carcinoma, Crocker sarcoma 180, and Freund sarcoma is stimulated by 4,6-dinitro-o-cresol under both aerobic and anaerobic conditions. Degree of stimulation increases as temperature is lowered from 37° to 15°, although the absolute rate of lactate production diminishes. Aerobic lactate production is stimulated to a greater degree than anaerobic and the former exceeds the latter under certain conditions. The implications and possible mechanisms of these phenomena are discussed.

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Mucopolysaccharide, Protein and Desoxyribosenucleic Acid Concentration of Granulation Tissue Induced by Polyvinyl Sponges.* (24369)

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Granulation tissue free of inflammatory infiltrate has been observed to enter polyvinyl sponges inserted under skin of various animals (1,2). This tissue provides proliferating, metabolically active connective tissue suitable for experimental study. Protein and collagen content of tissue which enters these sponges and some of the enzymatic activities of this tissue have been studied(2,3). We explored the metabolism of mucopolysaccharides in this tissue. The present report concerns mu-

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