INCREASE IN HEAT PRODUCTION OF MUSCULAR CONTRACTION CAUSED BY 2,4-DINITROPHENOL

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An increase in the heat production of a single contraction of the isolated rat diaphragm under the influence of 2,4-dinitrophenol (DNP) was demonstrated by a highly sensitive thermometric method ($1 \cdot 10^{-5}$ °C/mm). The action of low concentrations of DNP caused no visible changes in the resting muscle but changes were clearly visible during contraction. After exposure to DNP and during contraction of the muscle of equal force, its heat production was increased by almost 50%. This fact confirms the hypothesis that uncoupling of oxidation and phosphorylation in the respiratory chain of the muscle cell plays the leading role in the mechanism of increased heat production of muscular contractions in animals after adaptation to cold. KEY WORDS: heat production; rat diaphragm; dinitrophenol; muscular contraction.

Uncoupling of oxidation and phosphorylation in the respiratory chain caused by 2,4-dinitrophenol (DNP) and the thermoregulatory uncoupling accompanying adaptation to cold are regarded as analogous in their biochemical mechanism [4, 6]. In both cases it is considered that the degree of coupling between oxidation and phosphorylation in the oxidative phosphorylation system of the cell is reduced. As a result, cell respiration is activated but phosphorylation, i.e., ATP formation from ADP, is reduced, and this is expressed as a decrease in the P/O_2 ratio or, what amounts to the same thing, an increase in energy expenditure and a decrease in heat production while the working function of the cell remains unchanged.

Biochemical and electron-microscopic evidence of uncoupling of oxidative phosphorylation has been obtained in experiments on isolated mitochondria from muscles of homeothermic animals, previously acclimatized to cold. Electron-microscopic studies have shown that the typical picture of uncoupling of oxidation and phosphorylation, expressed by considerable swelling of the mitochondria [8], was disturbed in mitochondria of the same animals after incubation in the presence of submaximal doses (40 μM) of DNP. Experiments such as these led many writers [4-6, 9-12] to assert that maintenance of a normal body temperature in cold-adapted

TABLE 1. Heat Production (Q, in cal) and Tension (P, in g) of Single Muscular Contraction of Rat Diaphragm during Electrical Stimulation with a Strength of 1 V before and after Treatment with DNP (M ± m)

Expt. No.	Weight of muscle, mg	Without DNP		With DNP	
		Q	P	Q	P
1	350	0,695±0,150	11,5±1,2	1,370±0,080	11,0±0,5
2	340	0,679±0,063	11,5±0,4	1,000±0,030	9,5±1,5
3	300	1,100±0,118	11,5±0,7	1,634±0,055	10,8±1,5
4	220	1,707±0,125	10,8±1,0	2,080±0,100	11,0±0,4
5	220	1,244±0,010	11,0±1,5	1,640±0,116	10,0±1,5
6	330	1,516±0,234	10,8±2,1	2,000±0,180	10,8±1,0
7	330	1,325±0,100	10,5±1,0	1,820±0,140	10,0±0,7
8	270	0,785±0,165	11,0±0,6	1,385±0,046	10,5±0,4
ΣM	—	1,131±0,136	—	1,616±0,124 ($P<0,05$)	—

Legend. ΣM denotes total value of Q.

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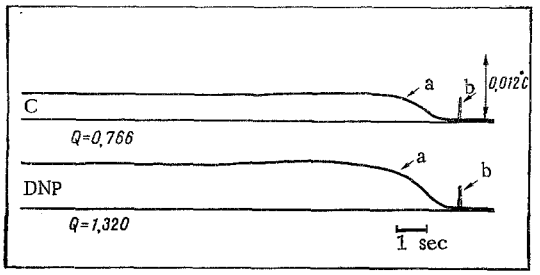

Fig. 1. Heat production per single muscular contraction of rat diaphragm before (C) and after treatment with DNP. a) Heat production; b) mechanical contraction. Q) Total heat production of contraction (in cal).

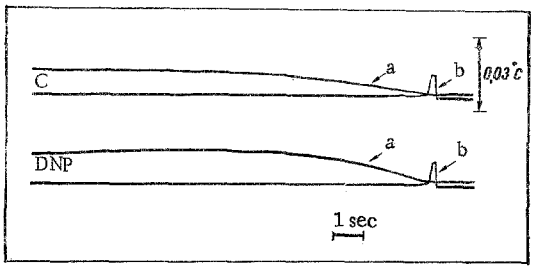

Fig. 2. Heat production per single muscular contraction of frog sartorius muscle before and after exposure to DNP. Legend as in Fig. 1.

animals depends on so-called chemical nonshivering thermogenesis, due to specific changes of metabolism in the cells. Muscular contraction, as the main source of thermoregulatory heat production, from this point of view, is completely ignored on the basis of the fact that during prolonged exposure to cold the cold muscle tremor gradually diminishes.

However, according to Ivanov's views [1], after acclimatization to cold the efficiency of mechanical work of muscular contraction falls, i.e., more heat is liberated per unit of muscular work than normally.

In the present investigation an attempt was made to study the energetics of the elementary muscular act – a single isometric contraction of the rat diaphragm before and after treatment with low concentrations of DNP.

EXPERIMENTAL METHOD

The diaphragm was excised from intact Wistar albino rats weighing 220 g (8 animals), cut into two parts along the central tendon, and the right half of the muscle was fixed to a thermopile in a special transparent plastic frame fitted with a miniature strain gauge. Under the control of the strain gauge the diaphragm was stretched to a "standard" level, after which the frame with the muscle was placed in a thermometric system to record the temperature effect of a single muscular contraction [3]. The sensitivity of the thermometric system was $1 \cdot 10^{-5}$ °C/mm; the error of the heat measurements did not exceed 3%. The experiments were carried out at 37°C.

The experiment was as follows. The diaphragm fixed to the frame was incubated in Krebs–Henseleit buffer solution for 60 min, after which the frame was lifted above the solution into the part of the chamber containing air and directly stimulated with single electric pulses applied to it through thin silver electrodes from an ESL-2 stimulator. The muscle was stimulated every 5 min in the course of 30 min (voltage 1 V, pulse duration 50 msec). The preparation was then again lowered into the incubation solution, to which DNP ($0.6 \cdot 10^{-5}$ mM) was added 10 min later. The frame with the muscle was lifted into the upper part of the chamber 3 min after addition of the DNP and the diaphragm was again stimulated electrically in the same order as in the control part of the experiment.

The temperature effect and the tension developed by the muscle during each contraction were recorded on photographic film. The total heat production of a single muscular contraction of the diaphragm was calculated from the area bounded by the temperature curve. Altogether 63 temperature records were analyzed.

EXPERIMENTAL RESULTS

Values of heat production of a single muscular contraction of eight preparations of isolated rat diaphragm before and after treatment of the muscle with DNP solution of low concentration are given in Table 1. Clearly the intact isolated diaphragm (weighing about 300 mg) gives out on average 1.131 cal during a single contraction, during which it develops a tension of about 11 g. Brief treatment of the muscle with a weak solution of DNP led to a significant increase in the heat production per contraction, the increase being about 50% to a mean value of 1.616 cal. In other words, for every gram of tension developed by the muscle the intact diaphragm gives out about 0.1 cal of heat; DNP increases this amount significantly to about 0.15 cal. Under these circumstances the resting muscle shows no evident changes in heat production.

The uncoupling action of DNP on the energetics of muscular contraction is illustrated in Fig. 1, which shows a record of single contractions of one muscle before and after exposure to DNP.

Several experiments were carried out on the frog sartorius muscle at 20°C. One record of the temperature curves of single isometric contractions of the sartorius muscle before and after treatment of the muscle with DNP is illustrated in Fig. 2. Contraction of the muscle of a poikilothermic animal also was found to be subject to the uncoupling action of DNP. In both cases the increase in heat production of muscular contraction (while the tension developed by the muscle remained relatively unchanged after brief exposure to DNP) points to a reduction in the energetic efficiency of muscular contraction.

An increase in the heat production of contraction of the isolated muscle under the influence of DNP was thus demonstrated. The action of low concentrations of DNP caused no visible changes in the resting muscle, but was clearly manifested during contraction. After treatment with DNP and during the same strength of contraction heat production increased considerably – by almost 50%. This fact confirms the previous hypothesis [1-3, 7] of the role of uncoupling of oxidation and phosphorylation in the increased heat production of muscular contractions in animals after adaptation to cold.

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