SHORT COMMUNICATIONS

BBA 63274

NAD- and NADP-specific dehydrogenases in cord-factor-treated mice

BLOCH[1] described the extraction from virulent tubercle bacilli of a toxic lipid fraction which was later named 'cord factor'. This compound was purified and identified as trehalose-6,6'-di-mycolate[2,3]. There are reports of a decrease in the rate of oxidation of malate, of a 50% decrease in NAD content and of a two- to three-fold increase in NAD glycohydrolase activity of lung, liver and spleen of tuberculous mice[4,5]. Similar findings have been reported with cord factor and it has been suggested that the biochemical changes elicited by tubercle bacilli can be duplicated by cord factor[6]. Studies with cord-factor-treated mice demonstrated a marked decrease in fatty acid synthesis in liver and adipose tissue[7]. Inhibition of fatty acid synthesis and decrease in the activities of pyridine nucleotide-linked dehydrogenases in the 10 000 × *g* supernatant fraction of liver and adipose tissue were observed in tuberculous guinea pigs[7]. It was of interest to ascertain whether the derangement in enzyme activities observed in tuberculosis could be duplicated in mice treated with cord factor. The toxic manifestations of cord factor might throw light on the direct role of this toxin in the pathological biochemistry of tuberculosis. The present investigation was therefore undertaken with these objectives in mind.

Activities of pyridine nucleotide-linked dehydrogenases such as combined hexose monophosphate pathway dehydrogenases [phosphogluconate dehydrogenase (decarboxylating) (6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating), EC 1.1.1.44) and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49)], malate dehydrogenase (decarboxylating) (L-malate: NADP oxidoreductase (decarboxylating), EC 1.1.1.40), isocitrate dehydrogenase (*threo*-D_s-isocitrate:NADP oxidoreductase (decarboxylating), EC 1.1.1.42) and malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37) have been assayed in the liver and adipose tissue of cord-factor-treated mice.

Mice were injected intraperitoneally with 0.2 mg cord factor in olive oil. Control and cord-factor-treated mice were sacrificed after 48 h. Animals of both the groups were fasted for 18 h prior to sacrifice. Mitochondria free fractions (10 000 × *g* supernatant) of liver and epididymal fat pads were prepared and dehydrogenases assayed by recording changes in absorbance at 340 mμ in a Beckman model DU spectrophotometer. Assay of hexose monophosphate pathway dehydrogenases was performed by the method of GLOCK AND MCLEAN[8]. Isocitrate dehydrogenase, malate dehydrogenase and malate dehydrogenase (decarboxylating) were assayed according to the procedure of OCHOA[9–11]. The extinction coefficient for NADH and NADPH at 340 mμ was taken as $6.22 \cdot 10^6$ cm²/mole (ref. 12). Protein was estimated by the method of LOWRY *et al.*[13]. Table I records the enzyme activities in 10 000 × *g* supernatant of liver and adipose tissue of control and cord-factor-treated mice. All activities are significantly decreased with the exception of isocitrate dehydrogenase in adipose tissue.

The results indicate appreciable differences in the activities of NAD- and NADP-specific dehydrogenases in control and cord-factor-treated mice. Earlier studies on

TABLE I

ACTIVITIES OF NAD- AND NADP-SPECIFIC DEHYDROGENASES IN THE LIVER AND ADIPOSE TISSUE OF NORMAL (CONTROL) AND CORD-FACTOR-TREATED MICE

Enzyme activities were assayed in 10 000 × *g* supernatant. Values are expressed as μmoles of pyridine nucleotides oxidized or reduced per min per mg protein. Each value is mean ± S.E. of the results of 4 separate experiments. Results have been statistically evaluated and $P \leqslant 0.05$ has been considered significant. HMP, hexose monophosphate pathway.

Enzymes	*Control*	*Cord-factor-treated*	*P*
Liver			
HMP dehydrogenase	0.053 ± 0.0098	0.013 ± 0.0010	<0.010
Isocitrate dehydrogenase (NADP)	0.292 ± 0.0162	0.113 ± 0.0122	<0.001
Malate dehydrogenase (decarboxylating)	0.134 ± 0.0235	0.020 ± 0.0037	<0.005
Malate dehydrogenase	10.190 ± 0.9030	2.950 ± 0.3430	<0.001
Lactate dehydrogenase	1.742 ± 0.3240	1.965 ± 0.2370	>0.05
Adipose tissue			
HMP dehydrogenase	0.298 ± 0.330	0.163 ± 0.0210	<0.020
Isocitrate dehydrogenase (NADP)	0.070 ± 0.0176	0.054 ± 0.0096	>0.400
Malate dehydrogenase (decarboxylating)	0.075 ± 0.0050	0.022 ± 0.0072	<0.001

cord-factor-treated mice had revealed a decrease in fatty acid synthesis in liver and adipose tissue, fatty infiltration of liver and derangement in mitochondrial function[7]. A significant decrease in the combined hexose monophosphate pathway dehydrogenases is noted in the present study. These observations are consistant with the earlier findings of depressed lipogenesis, mitochondrial derangement and reduced activity of liver and adipose shunt dehydrogenases in tuberculosis[7]. The activity of malate dehydrogenase (decarboxylating) of both liver and adipose tissue in the present study is decreased as a result of treatment with cord factor. Comparable results were obtained in experimental tuberculosis[7]. A 70% decrease in the activity of malate dehydrogenase is observed in toxin-treated mice. ARTMAN, BEKIERKUNST AND GOLDENBERG[6] have reported a markedly lower rate of O_2 uptake by liver homogenates and an inhibition in oxidation of substrates whose dehydrogenases are linked to NAD in cord-factor-treated mice. A decrease in NAD content of liver, spleen and lung and an increase in NAD glycohydrolase activity of these tissues have also been reported by these workers. The decrease in activity of malate dehydrogenase observed in the present study may be due to a reduced availability of NADH since hepatic NAD^+ levels have been shown to be depressed by administration of cord factor[6]. Earlier observations with cord factor in mice indicated an increase in gluconeogenesis in liver[7]. The decreased activity of malate dehydrogenase and malate dehydrogenase (decarboxylating) enzyme system observed in the present investigation could be due to decreased availability of oxaloacetate, a substrate utilized in both gluconeogenesis and lipogenesis. Activity of isocitrate dehydrogenase decreases in liver supernatant on cord factor treatment. A similar decrease in both liver and adipose tissue was observed in tuberculous infection[7]. The present investigation reveals that the activities of pyridine nucleotide-specific dehydrogenases decrease on cord factor treatment. Previous studies demonstrated a decrease in fatty acid synthesis in tuberculous guinea pigs as well as cord-factor-treated mice[7] indicating that the biochemical alterations in experimental tuberculosis represent toxic manifestations of the cord

factor. The metabolic derangement observed in tuberculosis could be due to the presence of this toxic component in the tubercle bacilli. The present investigation has revealed that cord factor simulates experimental tuberculosis in producing a depression of NAD- and NADP-specific dehydrogenases.

We thank Prof. A. S. PAINTAL, Director of this Institute for his interest in this work. This investigation was supported in part by funds from the Council of Scientific and Industrial Research, New Delhi and Grant No. E 3427, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

Department of Biochemistry,
V. Patel Chest Institute,
University of Delhi,
Delhi (India)

H. S. R. MURTHY
K. SAMPATH KUMAR*
T. A. VENKITASUBRAMANIAN

1 H. BLOCH, *J. Exptl. Med.*, 91 (1950) 197.
2 H. NOLL, H. BLOCH, J. ASSELINEAU AND E. LEDERER, *Biochim. Biophys. Acta*, 20 (1956) 299.
3 H. BLOCH, J. DEFAYE, D. LEDERER AND H. NOLL, *Biochim. Biophys. Acta*, 23 (1957) 312.
4 A. BEKIERKUNST AND M. ARTMAN, *Am. Rev. Respirat. Diseases*, 86 (1962) 832.
5 A. BEKIERKUNST, M. ARTMAN AND N. SILMAN, *Am. Rev. Respirat. Diseases*, 89 (1964) 575.
6 M. ARTMAN, A. BEKIERKUNST AND I. GOLDENBERG, *Arch. Biochem. Biophys.*, 105 (1964) 80.
7 H. SREERAMAMURTHY, *Fatty Acid Metabolism in Experimental Tuberculosis*, Dorctoral thesis, University of Delhi, 1966.
8 G. F. GLOCK AND P. MCLEAN, *Biochem. J.*, 55 (1933) 40.
9 S. OCHOA, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press, New York, 1955, p. 739.
10 S. OCHOA, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press, New York, 1955, p. 735.
11 S. OCHOA, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press, New York, 1955, p. 699.
12 A. KORNBERG AND B. L. HORECKER, *Biochem. Prepn.*, 3 (1953) 24.
13 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.

Received June 30th, 1967

* Deceased January 1, 1967.