THE EFFECT OF PYROGEN FROM *E. COLI* ON THE ACTIVITY OF SUCCINIC ACID DEHYDROGENASE IN LIVER MITOCHONDRIA

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To investigate more accurately the effect of pyrogen from *E. coli* on liver metabolism, its influence on mitochondrial fractions and on the activity of succinic acid dehydrogenase in this fraction has been studied. It appears that the pyrogen acts by directly activating the enzyme system chosen. Antagonism by malonate confirms the enzymatic and specific character of the phenomenon. No effect of pyrogen was found on the activity of lactic acid dehydrogenase. The results further the conception of a peripheral action of pyrogen.

In our previous studies[1–3] it was possible, amongst other things, to demonstrate increase of oxygen consumption in the sections of liver by the pyrogen from *Escherichia coli*. This action takes place both after the administration of pyrogen incubated with serum, directly into the section of liver *in vitro*, and also in rabbits killed at the peak of post-pyrogenic fever. This suggests the possibility that the increase of temperature here is of a peripheral character and the increase of metabolism is a primary phenomenon.

To investigate more accurately the effect of our pyrogen on liver metabolism we have studied its influence on mitochondrial fractions and on the activity of succinic acid dehydrogenase in this fraction.

Material and Methods

Rabbits of determined sensitivity to pyrogen were used. After killing the animals and bleeding, the tissue for examination was immediately taken and placed on ice in a small vessel.

Pyrogen from *E. coli* was obtained by the method of Palmer and Gerlough[4] as modified by Westphal, Luderitz, Eichenberger, and Keiderling[5]. The pyrogen was added to Warburg vessels and incubated for 1 hour at 37° with rabbit serum diluted with an equal amount of fluid of the following composition: NaCl, 0·154M 48 ml., KCl, 0·154M 1 ml., $CaCl_2$ 0·11M 1 ml., phosphate buffer 1/15M pH 7·2 5 ml., glucose 0·1 g.

After homogenising in 0·25M sucrose the liver homogenate was fractionated by centrifugation[6,7]. Nuclei and large residues were separated by centrifugation at 700 *g* and mitochondria were obtained at 4,500 *g*. After resuspension and rinsing they were again separated at 12,500 *g*. All the procedures were carried out below 4°.

The activity of succinic acid dehydrogenase was determined in the Warburg apparatus employing Slater and Bonner's[8] manometric method in which oxygen consumption is determined during the oxidation of sodium succinate in the presence of potassium cyanide as inhibitor of cytochrome and methylene blue as acceptor of hydrogen. For this purpose

there was added to the Warburg vessels: 0·8 ml. phosphate buffer, 0·2M; 0·2 ml. pyrogen in the amount of 2 ng. or 0·002 ng.; 0·2 ml. sodium succinate, 0·4M; 0·3 ml. methylene blue, 0·01M; 0·3 ml. KCN, 0·1M; 0·5 ml. suspension of mitochondria from 50 mg. of tissue and into the middle part 0·2 ml. of 10 per cent NaOH.

Succinic acid dehydrogenase was inhibited by adding 0·2 ml. of sodium malonate, 1·6M.

The activity of lactic acid dehydrogenase was estimated by means of Green and Brosteaux's[9] method which consists of the measurement of oxygen consumption during the oxidation of sodium lactate in the presence of methylene blue. The following were added into the Warburg vessels: 0·2 ml. sodium lactate, 2M; 0·2 ml. of pyrogen, 2 ng.; 0·1 ml. methylene blue, 0·0017M; 1·8 ml. mitochondria suspension from 180 mg. of tissue in phosphate buffer 7·2, and into the middle part 0·2 ml. of 10 per cent NaOH.

RESULTS

Succinic acid dehydrogenase is one of the most abundant enzymes concerned with oxygen consumption. Thus, it was the first enzyme which attracted our attention after we observed the increase of respiration

TABLE I

THE EFFECT OF PYROGEN ON THE ACTIVITY OF SUCCINIC ACID DEHYDROGENASE

Substrate	Number of experiments	O_2 consumption in microlitres by 1 mg. of dry mass of mitochondria during 2 hours	P
Mitochondria	35	88·2 ± 2·96	—
Mitochondria with incubated serum	31	114·8 ± 3·06	$P_1 < 0·001$
Mitochondria with 2 ng. incubated pyrogen	26	167·2 ± 3·20	$P_1 < 0·001$ $P_2 < 0·001$
Mitochondria with 0·002 ng. incubated pyrogen	24	230·4 ± 11·29	$P_1 < 0·001$ $P_2 < 0·001$
Mitochondria from a rabbit killed at the peak temperature	37	120·4 ± 3·22	$P_1 < 0·001$
Mitochondria with 0·002 ng. incubated pyrogen and sodium malonate	20	14·7 ± 1·95	$P_4 < 0·000001$

of liver sections by pyrogens. Numerous authors[10–12] have demonstrated the main site of succinic acid dehydrogenase to be the mitochondria, which we, in turn, have used as an enzyme source. Pyrogen incubated in the serum was added to the vessel in amounts of 2 ng. and 0·002 ng. for 2·5 ml. of liquid. The latter quantity corresponds approximately to the amount of pyrogen which would reach 100 mg. of liver with a pyrogen injection of 0·02 ng./kg. In one group of the experiments the activity was examined in mitochondria obtained from rabbits killed at the peak of fever after intravenous injection of pyrogen in the dose of 0·2 ng./kg.

To confirm the specificity of the observed mechanism in one group of experiments we made measurements in the environment containing 0·2 ml. of 1·6M sodium malonate in addition to the proper substrate.

Double control was possible by estimating the activity of mitrochondria in normal environment and in the environment enriched by serum. The results obtained are presented in Table I.

Another enzyme which we considered in our study was lactic acid dehydrogenase. Because of its less abundant appearance as a source of mitochrondria we have used 180 mg. of liver for each vessel. To pyrogen incubated with serum we have added the concentration of 2 ng./2·5 ml. of liquid. The results obtained are presented in Table II.

TABLE II

THE EFFECT OF PYROGEN ON THE ACTIVITY OF LACTIC ACID DEHYDROGENASE

Substrate	Number of experiments	O_2 consumption in microlitres by 1 mg. of dry mass of mitochondria during 2 hours	P
Mitochondria	27	16·35 ± 0·65	—
Mitochondria with incubated serum	18	12·10 ± 0·68	$P_1 < 0·001$
Mitochondria with 2 ng. incubated pyrogen	20	13·90 ± 0·65	$P_1 = 0·02$ $P_2 = 0·07$

DISCUSSION

The system of succinic acid dehydrogenase chosen by us is one of the more important oxidising systems and the basic link of the Krebs cycle. From our studies it appears that pyrogen from *E. coli* acts by directly activating this system. This activity is reflected by the effect of minute amounts of pyrogen which confirms its observed action on the metabolism of liver cells. Antagonism of the described phenomenon by malonate confirms its enzymatic and specific character. No effect of pyrogen was found on the activity of lactic acid dehydrogenase. Our results are a further step in favour of the conception of a peripheral action of pyrogen which does not negate and does not exclude the existence of a central action suggested by numerous authors. The problem whether the system of succinic acid dehydrogenase is the only one which becomes activated under the influence of pyrogen remains an open question.

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