peak on day 3 of SD and resembling somewhat the excretion changes of 5-HIAA during SD[3]. The increase persists in the 2nd control period.

Elevation of free TP levels is assumed to be related to a change in the binding capacity to albumin influenced by exogenous substances, e.g. different drugs[6,7,9,10], and endogenous substrates. Thus, for example, the TP-albumin binding is known to interfere with the binding of free fatty acids (FFA) to this carrier[11]. The FFA level increases under stress, including SD[12]. Therefore this factor may be involved in the changes in TP blood level and 5-HT metabolism observed both in this and in previous investigations. Our observations indicate that elevated excretion of 5-HIAA during SD may be inter alia induced by enhanced synthesis of 5-HT, probably caused by the increased free TP level. Changes in 5-HT metabolism are assumed to be at play in the therapeutical effect of SD in endogenous depression.

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Some Properties of External NADH Oxidation by Human Placental Mitochondria[1]

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Summary. Isolated human term placenta mitochondria catalyse oxidation of external NADH in the presence of cytochrome c. This reaction is insensitive to the respiratory chain inhibitors such as rotenone and antimycin A, and is not coupled to phosphorylation. Comparison of the effect of Mg^{++} ion on NADH plus cytochrome c oxidation by human term placental, human skeletal muscle and rat skeletal mitochondria showed that Mg^{++} ion exerts an inhibitory effect in the case of human mitochondria and a stimulatory effect in the case of rat skeletal muscle mitochondria.

It has been shown that cytochrome c greatly stimulates the exogenous NADH oxidation by rat liver mitochondria[2]. This reaction is insensitive to the respiratory chain inhibitors such as rotenone, amytal and antimycin A, and is not coupled to phosphorylation[2]. HEDMAN et al.[3] reported that also rat and human skeletal muscle mitochondria oxidize external NADH with a high rate in the presence of cytochrome c. However, in contrast to liver mitochondria, the oxidation by rat skeletal muscle mitochondria was shown to be inhibited by respiratory chain inhibitors[3]. Recently we observed that some steroids exert an inhibitory effect on the cytochrome c induced NADH oxidation, this inhibition being partial in the case of human muscle and almost complete in the case of rat muscle mitochondria[4]. This indicates the existence of differences between mitochondria isolated from various tissues as far as some properties of NADH oxidation in the presence of cytochrome c are concerned. In the present work, some properties of NADH oxidation by human placental mitochondria are presented.

Materials and methods. NADH, NADPH, ADP, heparin, rotenone and antimycin A were obtained from Sigma Chemical Co.; cytochrome c was from Koch-Light; CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone) from Calbiochem; Mannitol from UCB (Belgium), sucrose from BDH; all other compounds were of the highest purity available commercially from POCh Gliwice (Poland). 0.25 *M* sucrose solution (used while preparing human placental mitochondria) and the solution of 0.21 *M* mannitol + 0.07 *M* sucrose (used while preparing human and rat skeletal muscle mitochondria) were deionized by passing through a mixed-bed ion exchange resin (Amberlit-MB-/BDH).

Human term placentas were obtained fresh from the maternity unit of a local hospital. Human muscle was obtained from patient undergoing orthopaedic surgery. The patient having no known history of metabolic diseases were anesthetized with brevinarcorn and $N_2O + O_2$ (6:3). Rat skeletal muscle was obtained from the hind legs of mal Wistar rats immediately after decapitation.

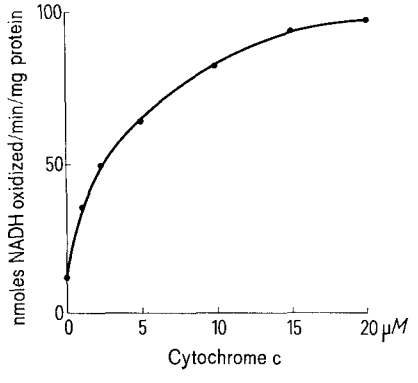

Fig. 1. The dependence of NADH oxidation by human placental mitochondria on cytochrome c concentration. NADH oxidation was measured by following the decrease of absorbancy at 340 nm using Unicam SP-800 recording spectrophotometer in the medium described under materials and methods. The medium contained additionaly 0.2 m*M* NADH and cytochrome c at concentrations indicated on the Figure. Reaction was started by addition of 0.5 mg mitochondrial protein suspended in 0.1 ml of 0.25 *M* sucrose + 10 m*M* *tris*-HCl (pH 7.4).

[1] This work has been supported by a grant from Ministry of Higher Education Science and Technology within the project No. 01.02.
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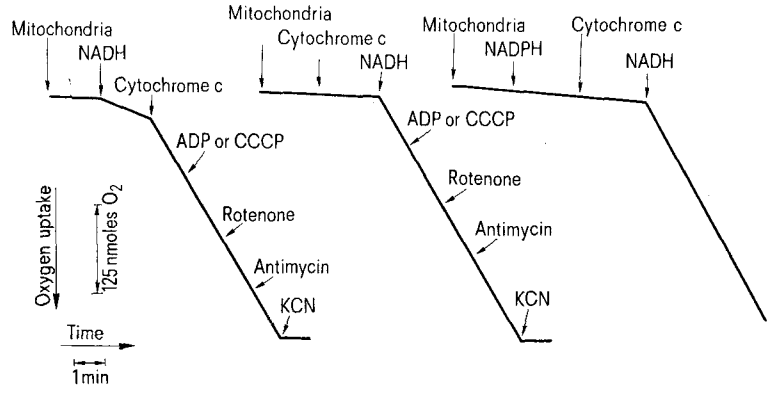

Fig. 2. Polarographic recordings of oxygen uptake by human placental mitochondria. The experiments were carried out using a Clark electrode. Human placental mitochondria (3 mg mitochondrial protein suspended in 0.1 ml of 0.25 *M* sucrose + 10 m*M* *tris*-HCl pH 7.4) were added to 2.4 ml medium described under materials and methods. Further additions were: NADH or NADPH (0.5 μmoles), cytochrome c (37.5 nmoles), ADP (2.5 μmoles), rotenone (2 μg), antimycin A (4 μg) KCN (1.25 μmoles), mitochondria.

Human placental[5] and human and rat skeletal muscle[4] mitochondria were prepared as described previously. The protein content of the mitochondria was determined by ultraviolet absorption as described previously[6]. NADH oxidation was measured by polarographic technique using a Clark electrode or by following the decrease of absorbancy at 340 nm using Unicam SP-800 recording spectrophotometer. In both cases, the reaction was carried out in the medium containing 40 m*M* KCl, 20 m*M* *tris*-HCl (pH 7.4) and 6 m*M* potassium phosphate buffer (pH 7.4). Other additions were as indicated in the corresponding figures. Final volume of the reaction mixture was 2.5 ml. Assay temperature was25 °C.

Results and discussion. Figure 1 presents the effect of adding increasing concentrations of cytochrome c on NADH oxidation by human placental mitochondria. It may be seen that the stimulation by cytochrome c of NADH oxidation depended on cytochrome c concentration: 15–20 μ*M* concentration of cytochrome c was needed to achieve maximal stimulation.

The polarographic traces in Figure 2 indicate that exogenous NADH was oxidized only slightly by human placental mitochondria. Addition of cytochrome c greatly stimulated oxygen uptake, but neither respiratory control by ADP or stimulation by CCCP, nor the sensitivity to rotenone and antimycin A could have been shown. KCN in these conditions completely inhibited the reaction. In this respect mitochondria from human placenta resemble mitochondria obtained from rat liver[2]. NADPH in the conditions described in Figure 2 was not oxidized by human placental mitochondria, either in the presence or in the absence of added cytochrome c. Microsomes from human placenta possess very low ability to oxidize NADH plus cytochrome c (not shown); therefore interference in oxygen uptake by contaminating microsomes may be excluded.

Externally added NADH is not readily oxidized by mammalian mitochondria[7]. This is due to the impermeability of inner mitochondrial membrane to NADH. In mammalian mitochondria, NADH is oxidized at high rate only when either the mitochondrial membrane is damaged or cytochrome c is added[7]. As indicated above, human placental mitochondria possess similar properties. In the presence of cytochrome c, the oxidation of NADH occurs through a non-phosphorylating external pathway, and is mediated by rotenone-insensitive NADH-cytochrome c reductase[8]. For the NADH-cytochrome c reductase activity in outer mitochondrial membrane, both NADH-cytochrome b_5 reductase and cytochrome b_5 are responsible[8]. During oxidation of NADH by intact mitochondria in the presence of cytochrome c, cytochrome c mediates the electron flow from cytochrome b_5 to cytochrome oxidase of inner mitochondrial membrane[8]. Thus the activity of NADH-cytochrome b_5 reductase might be determined by measuring oxygen uptake by intact mitochondria oxidizing external NADH in the presence of cytochrome c. Sottocasa[9] showed that divalent cation stimulate NADH oxidation by outer mitochondrial membrane preparation from rat liver. In the intermembrane space, there is a relatively high con-

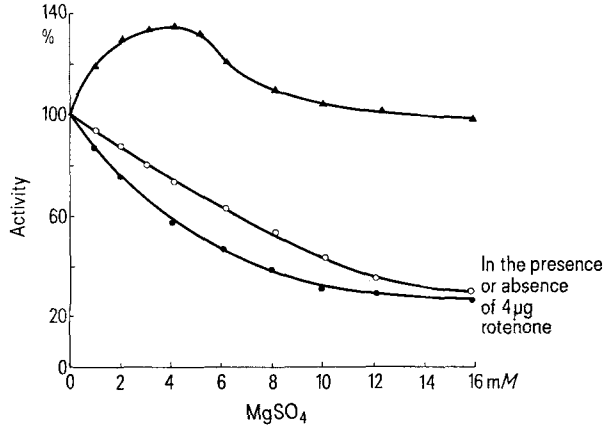

Fig. 3. Effect of $MgSO_4$ on NADH plus cytochrome c oxidation by human placental (●), human skeletal muscle (○) and rat skeletal muscle (▲) mitochondria. Experimental conditions as described in the section on methods and in Figure 1. Cytochrome c concentration was 15 μ*M*; $MgSO_4$ was added to the incubation medium before mitochondria. The figures on the ordinate represent relative rates. The control rates were: 100 nmoles NADH oxidized per min per mg protein for both human skeletal muscle mitochondria and human placental mitochondria, and 350 for rat skeletal muscle mitochondria.

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centration of Mg^{++} ion[10], which might influence the enzyme systems localized in outer mitochondrial membrane. Therefore we have examined the effect of Mg^{++} ion on the external NADH oxidation in the presence of cytochrome c by human placental, human and rat skeletal muscle mitochondria.

Figure 3 presents the effect of increasing concentrations of $MgSO_4$ on the oxidation of NADH in the presence of added cytochrome c in human and rat skeletal muscle and human placental mitochondria. The results are expressed as a percent of the control oxidation rate in the absence of $MgSO_4$. It may be seen that $MgSO_4$ inhibited NADH oxidation in the presence of cytochrome c in the case of both human skeletal muscle and placental mitochondria. The inhibition depended on $MgSO_4$ concentration reaching about 50% and 75% at 9 m*M* and 16 m*M* $MgSO_4$ respectively in the case of human skeletal muscle mitochondria; the same degree of inhibibition in the mitochondria from human placenta was observed at 5 m*M* and 13 m*M* $MgSO_4$, respectively. In the case of rat skeletal muscle mitochondria, $MgSO_4$ exerted a stimulatory effect on cytochrome c induced NADH oxidation. Maximal stimulation was obtained at the concentration range 2–5 m*M* $MgSO_4$. $MgSO_4$ concentration higher than 8 m*M* stimulated only slightly NADH oxidation in the presence of cytochrome c. At 16 m*M* no stimulatory effect was observed. $MgCl_2$ exerted similar effect as $MgSO_4$ (not shown), indicating that Mg^{++} ion is responsible for the inhibition in the case of human skeletal muscle mitochondria and placental mitochondria, and for the stimulation in the case of rat skeletal muscle mitochondria. In human placental and skeletal muscle mitochondria, $MgSO_4$ inhibited NADH oxidation to the same extent both in the presence and absence of rotenone. In the case of rat skeletal muscle, mitochondria NADH plus cytochrome c oxidation was studied in the absence of rotenone only because this inhibitor exerted inhibitory effect on NADH plus cytochromec oxidation[3]. Data presented in Figure 3 indicate that with regard to the action of Mg^{++} ion on NADH oxidation in the presence of cytochrome c, rat skeletal muscle mitochondria resemble rat liver mitochondria[9], whereas in human mitochondria Mg^{++} ion exerted an inhibitory effect on this process.

The question arises whether the inhibitory effect of $MgSO_4$ is caused by other contaminating ions. We checked the $MgSO_4$ solution in atomic absorption spectrophotometer and showed that Zn^{++} ion is present, although at a concentration which was without effect on NADH oxidation. At present it is difficult to say what is the mechanism of Mg^{++} action on NADH oxidation in the presence of cytochrome c. It seems that two possibilites may be taken into consideration: a) that Mg^{++} is either inhibiting or stimulating NADH-cytochrome b_5 reductase localized in the outer mitochondrial membrane, b) that Mg^{++} is limitting the permeability of outer membrane to cytochrome c, inhibiting in this manner electron flow from cytochrom b_5 to cytochrome oxidase of the inner mitochondrial membrane. It is not excluded that both mechanisms are responsible for the observed Mg^{++} ion effect on NADH plus cytochrome c oxidation.

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The Extracellular Protease from *Pseudomonas fluorescens*

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Summary. An extracellular protease has been purified from cultures of *Pseudomonas fluorescens*. It is a metalloenzyme with a molecular weight of 37,000 ± 3,700, able to digest casein, hemoglobin and gelatine.

The extracellular proteolytic enzymes produced by some *Pseudomonas* strains are important in the process of meat spoilage at low temperatures[2]. Extracellular proteases have been isolated from the psychrophile *Ps. fragi*[3], and from *Ps. maltophilia*[4] and *Ps. aeruginosa*[5]. We report in this communication the purification and some properties of the extracellular protease produced by *Ps. fluorescens*, a fluorescent facultatively psychrophilic *Pseudomonas*, which differs from *Ps. aeruginosa* by its ability to grow at 4 °C and from *Ps. putida* by its ability to hydrolize gelatine[6].

Methods. Ps. fluorescens, strain R-12, was isolated from water of the Paraná River at Rosario[7] and grown at 25 °C in Nutrient Broth No 1 (Oxoid), 13 g/l, with the addition of $CaCl_2$ (0.3 g/l), in a New Brunswick water bath girotory shaker. When the cultures reached the stationary phase ($A_{680\,nm}$ of 1.4), the cells were separated by centrifugation at 15,000 *g* for 20 min at 4 °C. To the clear coloured supernatant was added solid ammonium sulfate to 50% saturation at 0 °C. The precipitate was separated by centrifugation at 37,000 *g* for 20 min at 4 °C and discarded. The supernatant was brought to 60% saturation with solid ammonium sulfate. The precipitate was dissolved in 1 ml of 20 m*M* *Tris*-HCl buffer (pH 7.6) containing 2 m*M* $CaCl_2$ and filtered through a Sephadex G-200 column (52 × 1.2 cm) equilibrated with the same buffer. The active fractions were pooled and dialyzed against 20 m*M* glycine-NaOH buffer (pH 9.7) for 21 h

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