content. The relative increase during the ageing period is due mainly to a decrease in other acids (especially citric and malic).

Juice vesicles have been found to contain all the enzymes of TCA and of some auxiliary cycles from an early date[10]; juice at maturity contains very large amounts of citric and malic acids; in comparison malonic acid is only a negligible fraction, though it tends to accumulate with age.

The trend of malonic acid accumulation from incipient senescence onwards is therefore common to all these tissues, notwithstanding their widely different capabilities. Our data confirm previous findings of CLEMENTS[11] related to peel of Washington Navel oranges during maturation and delayed picking. Due to less sensitive methods, however, he was unable to detect malonic acid in juice. Malonic acid is a recognized competitive inhibitor of succinic dehydrogenase, blocking the classical TCA cycle. The concentrations we found in flavedo tissues are similar to those causing malonate inhibition of 0_2 uptake in vitro (1 to $5 \times 10^{-2} M$)[12]. Also pH values of peel tissues are pH 5–5.5, i.e. similar to those needed for the reaction in vitro[12]. On the other hand, malonic acid is probably located in vacuoles away from mitochondria. Malonic acid through its activated derivative malonyl-CoA is active in many metabolic paths (as lipid and phenolic biosyntheses, etc.) and its accumulation could also be connected with slowing down of biosynthetic activities at incipient senescence.

Although the reasons for malonic acid accumulation have not yet been explained, such accumulation seems to provide a reliable and adequate indicator of fruit tissue senescence in oranges.

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Changes in Blood Tryptophan Level During Sleep Deprivation

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Summary. Prolonged sleep deprivation elicits a significant elevation of the plasma level of free tryptophan which appears to be involved in increased excretion of 5-HIAA during this state through enhanced 5-HT synthesis.

In an earlier paper we have demonstrated that sleep deprivation (SD) is associated with changes in the excretion of the end-product of serotonin metabolism (5-HT), i.e. 5-hydroxyindole acetic acid (5-HIAA)[3].

Temporary elevation of 5-HIAA excretion on days 2 and 3 of SD was explained by an increased 5-HT release from tissues, on one hand, and by a stress manifestation of SD, on the other. Latest investigations showed that changes in blood tryptophan (TP) levels[4], particularly in the free tryptophan[5–7], may be a reliable index of the changes of 5-HT synthesis. We therefore investigated the changes of blood TP during SD.

Method. 6 healthy volunteers (20–23 years) were observed for total, free and bound TP during 120-hour SD and during 2 control periods under experimental conditions described in the preceding paper[3]. The plasma TP was measured with the spectrofluorometric method according to ECLESTONE[8], the free TP was filtered before processing through a 2 100 CF 50 membrane filter. Heparinized blood was withdrawn at 06.00 h and at 18.00 h and the average of the 2 values was used for the final evaluation.

Results and discussion. The Table shows that both the free and bound TP increase during SD. However, this increase is significant only for the free fraction, reaching its

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		1st control period	Sleep deprivation (h)				2nd control period	
			24–48	48–72	72–96	96–120	48–72	72–96
Bound tryptophan	µg/ml	10.58	12.117	11.8	11.77	9.94	11.158	11.158
	S.D.	2.31	2.871	2.189	2.77	1.51	1.8	1.88
	n	30	12	12	12	12	12	12
Free tryptophan	µg/ml	2.507	2.6	3.67[c]	3.6[c]	3.57[c]	3.317[a]	3.23[a]
	S.D.	0.78	0.83	0.93	0.93	0.49	1.23	0.75
	n	30	12	12	12	12	12	12

n, number of observation; [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$.

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.

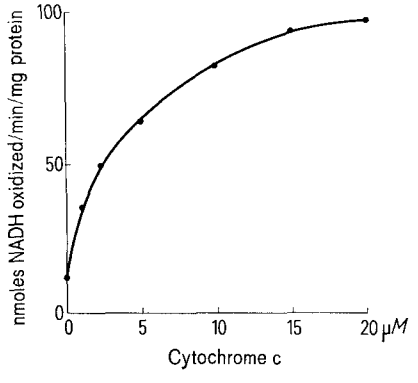

Fig. 1. The dependence of NADH oxidation by human placental mitochondria on cytochrome c concentration. NADH oxidation was measured by following the decrease of absorbancy at 340 nm using Unicam SP-800 recording spectrophotometer in the medium described under materials and methods. The medium contained 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).

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