Decrease in Pethidine Demethylating Activity of Isolated Rat Liver Microsomes Following Whole-Body X-Irradiation

Enzymic oxygenase system[1] involved in the metabolism of drugs and other foreign compounds is attached to the endoplasmic reticulum of mammalian liver[2,3]. The activities of these hepatic microsomal O_2- and NADPH-dependent enzymes[3] are influenced by a variety of factors[4,5] including X-irradiation, after which degradation of hexobarbital by liver homogenate is markedly suppressed[6]. Individual components of the mixed function oxidase system were not tested. There are several reports on the impairment by radiation of the development of drug-metabolizing enzymes in young male rats[6,7] and the inhibition of microsomal enzyme development of prenatal X-irradiated rats[6,8,9].

In an earlier electron-microscopic study of thin rat liver sections, we observed gradual disappearance of the endoplasmic reticulum and large apparently structureless cytoplasmic areas after X-irradiation[10]. These findings stimulated us to look for changes in the drug metabolizing capacity of the endoplasmic reticulum (microsomal fraction) and in the level of the cytochrome P-450 component. This topic might have practical implications for pharmacotherapy in post-irradiation states.

Materials and methods. The present study deals with the influence of in vivo whole-body X-irradiation (600 R, 800 R, 1000 R using the X-ray equipment Supersanap, 180 kV, 15 mA, 0.5 mm Cu + 1 mm Al filter, exposure rate 27 R/min) on the enzymic pethidine demethylating system of isolated rat liver microsomes at various time intervals. Groups of male rats weighing 220–240 g were irradiated and killed on various days after irradiation. The activity of the enzymic system was measured in isolated microsomes[11] by the estimation of formaldehyde released[12] from pethidine after one-hour incubation at 37 °C of the following mixture: suspension of microsomes (2 mg of microsomal protein), NADPH (3 nmoles), pethidine (11 nmoles), semicarbazide (100 nmoles), sodium phosphate buffer (0.3 *M*, pH 7.4) made to a total volume of 2 ml. The optical density of yellow colour was measured using the spectrophotometer Spectromom 360 (MOM, Budapest, Hungary) at 420 nm, and was expressed in μmoles of formaldehyde per 1 g of microsomal protein. Protein was estimated with the phenol reagent[13]. The level of cytochrome P-450[14], probably acting as terminal oxygenase[15] of the system, was also measured[16].

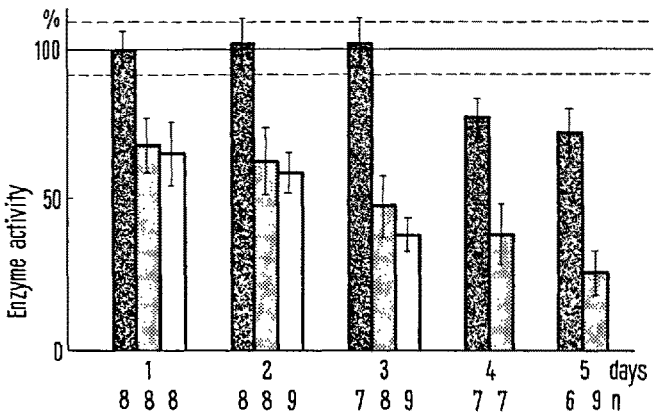
Results and discussion. Figure 1 shows the activity of the demethylating system expressed as percentage of the controls. Statistically significant decreases of the activity of the system demethylating pethidine from the 1st to the 5th day after 800 R and from the 1st to the 3rd day after 1000 R X-irradiation were observed. The dose of 600 R did not influence the activity till the 3rd day, but led to a statistically significant decrease on the 4th and 5th day after irradiation. In the present study, the decrease of the pethidine demethylating activity was not accompanied by the decrease of cytochrome P-450, whose levels were without significant changes (Table). The amount of microsomal protein per g liver wet weight changed only slightly, therefore the results are practically not altered if they are expressed on tissue wet weight basis. Our observations are another case of lack of parallelism between the mixed-function oxidase activity and P-450 levels (cf. references [15,17]).

Changes in the activity of the pethidine demethylating system followed in isolated microsomes in vitro need not be directly correlated with the activity of the pethidine demethylating enzymic system of the liver in vivo. In our experiments, NADPH is added in excess whereas in vivo it might become the rate-limiting factor. HILGERTOVÁ et al.[18] described a drop of liver NADPH level after whole-body irradiation. This would point in the

The levels of cytochrome P-450 at various days after X-irradiation expressed as percentage of controls

Doses	Days after X-irradiation				
	1	2	3	4	5
600 R	102 ± 7.2	99 ± 6.5	100 ± 7.0	98 ± 7.9	102 ± 8.0
800 R	100 ± 9.0	97 ± 4.8	103 ± 7.4	100 ± 8.5	102 ± 7.5
1000 R	103 ± 9.4	102 ± 8.7	102 ± 6.7	–	–

Numbers of the animals are the same as indicated in the Figure.



The effects of different doses of X-irradiation on pethidine demethylating activity of the microsomal fraction. The results are expressed as percent of enzyme activity in the controls. Standard deviation is expressed as dashed lines for the controls and as vertical lines for the irradiated groups. n, numbers of animals in the irradiated groups; 20 control animals. Black columns – 600 R, dashed columns – 800 R, open columns – 1000 R.

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same direction as our findings. A possible explanation of our results would be that the irradiation directly damages enzymic protein(s) or the structural integrity of the system (for example on the basis of the impairment of microsomal lipids[19,20]); alternatively enzyme activator(s) may be reduced. Finally, the decrease in the proteosynthesis of this enzymic system could be considered.

A possible effect on the pituitary, with the resulting changes in the functions of the adrenal cortex or the sexual glands, has also to be kept in mind[9,21].

Résumé. L'irradiation (800 R) entraîne une diminution de la déméthylation in vitro de la péthidine par la fraction microsomale du foie de Rat, du premier au cinquième jour après l'irradiation. Une réduction marquée a été aussi observée après 1000 R du premier au troisième jour et après 600 R –, le quatrième et le cinquième jour. Le taux du cytochrome P-450 ne change pas après ces doses dans les intervalles mentionnés. C'est un cas de manque de parallélisme entre le taux du P-450 et les changements de la fonction de la monooxygénase microsomale déjà connue.

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The Effects of Heat on the Interactions Between Leucyl-tRNA Synthetases and tRNA's

Several studies have revealed the importance of tRNA secondary structure for ensuring its aminoacylation by activation enzymes[1]. The thermostable leucyl-tRNA synthetase from *Bacillus stearothermophilus*[2,3] seemed to be particularly suitable for the investigation of the effects of heat on the biological properties of tRNA's in aminoacylation systems in such conditions that a direct effect of heat on enzyme stability can be, if not neglected, at least largely avoided. As cross-reactions between *B. stearothermophilus* and *Escherichia coli* tRNA's and leucyl-tRNA synthetases are complete[2], we were also able to study the effects of heat on heterologous systems.

Materials and methods. The enzymes used in this study were *E. coli* 112-12 AS3 fractions[4] and *B. stearothermophilus* highly purified enzyme fractions[3]. The preparation, purification and properties of tRNA's, labelled products, chemicals and standard reaction mixtures for the loading of tRNA and for the leucine-dependent ATP-PP_i exchange have been fully described elsewhere[3,5]. Melting of tRNA's were performed in standard reaction mixtures (in the presence of $6\times10^{-3}M$ $MgCl_2$) without addition of enzyme preparation.

Results and discussion. We reported earlier that *B. stearothermophilus* leucyl-tRNA synthetase is very resistant against denaturation by heat[2,3]: Figure 1 shows that its kinetics of denaturation are very slow at 68 °C, whereas the corresponding enzyme from *E. coli* is rapidly destroyed at 53 °C. The same figure also shows that both functions of these enzymes – the loading of leucine on tRNA and the ATP-PP_i exchange – possess the same heat stability. Figure 2 represents the effect of heat on the maximum amount of leucine that can be loaded on a limiting amount of tRNA: it can be seen that those loadings are affected differently by heat when *E. coli* or *B. stearothermophilus* tRNA's are allowed to react with the thermostable enzyme from the thermophilic bacteria. In the first case, there is a drop in amino acid accepting activity at 60 °C while this phenomenon only occurs at 65 °C in the second case. Furthermore, at 66.5 °C, the *B. stearothermophilus* tRNA's accept 95% of the amount of leucine loaded at 37 °C, whereas this value only reaches 52% for the *E. coli* tRNA's. The loss of activity occurs when the hyperchromicities of the unfractionated tRNA's are only 2 or 3%; at the T_m of the latter, the residual aminoacylation is almost negligible. The difference between the 2 tRNA species can still be increased when this time the effects of heat on the rates of the loading reactions are investigated. Figure 3 shows that in systems containing tRNA's from *E. coli* and activation enzymes either from *E. coli* or from *B. stearothermophilus*, there is a sharp drop of the initial velocity of the aminoacylation reaction at 30 °C. As the enzyme from *E. coli*

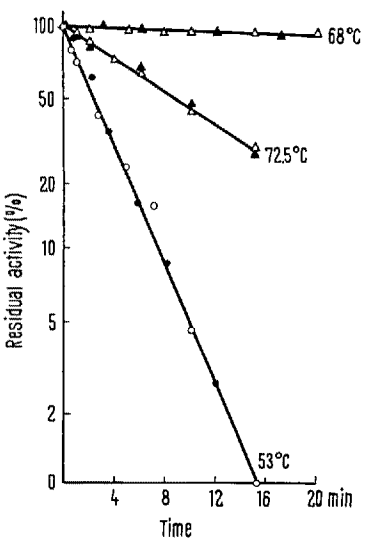

Fig. 1. Thermal stability of the leucyl-tRNA synthetases from *E. coli*: ○—○, loading of leucine on tRNA; ●—●, ATP-PP_i exchange; and from *B. stearothermophilus*: △—△, loading of leucine on tRNA; ▲—▲, ATP-PP_i exchange. The enzymes were incubated in 0.01 *M Tris*-HCl buffer pH 7.4 containing 10^{-3} M reduced glutathione. Aliquots were taken at various time intervals, cooled at 0 °C and assayed for enzyme activity at 37 °C in the standard reaction mixtures.

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