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Impairment of rat hepatic microsomal demethylating activity and structural protein after X-irradiation of the head

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RECENTLY, we observed a decrease of enzymic pethidine-demethylating system of isolated rat liver microsomes after *in vivo* whole body X-irradiation without concomitant decrease of cytochrome P-450 levels.[1] After whole body X-irradiation (1000 R), two components became undetectable on polyacrylamide gel electrophoreograms of a rat liver microsomal structural protein preparation.[2]

The present paper deals with the influence of X-irradiation (1000 R), when the body of rats was shielded and only the head was irradiated: electrophoretic patterns of rat liver microsomal protein together with the pethidine-demethylating activity, total microsomal protein and cytochrome P-450 levels were studied to elucidate whether the changes described earlier[1,2] were due to the direct effect of ionizing radiation or whether radiation exerted an indirect effect on liver endoplasmic reticulum.

Enzymic demethylating activity of isolated rat liver microsomes was assayed as formaldehyde (acetylacetone method[3]) released from pethidine on 60 min incubation in a NADPH-containing medium.[1] In a supplementary series of experiments the incubation lasted 2 min only. Cytochrome P-450 levels were also determined.[4] Proteins were estimated by the Folin reagent.[5] Polyacrylamide gel electrophoresis was carried out according to Takayama *et al.*[6] (7·5% acrylamide, 35% acetic acid, 5 M urea in the gels, 10% acetic acid as buffer, constant current of 5 mA per tube for 75 min at 4°) with aliquots of 0·25 mg of microsomal structural proteins. Staining of the gels was described previously.[2] Microsomal structural proteins were prepared by the method involving 6·5 per cent saturation with $(NH_4)_2SO_4$ of the material solubilized with deoxycholate.[7] The heads were exposed to a single irradiation with 1000 R,[1] the radiation received by the shielded rest of the body was less than 100 R.[8]

It can be seen in Fig. 1 that the pethidine-demethylating activity of isolated rat liver microsomes was significantly decreased on the second and third day after the irradiation of the head by 1000 R. Similar results were obtained in the supplementary experiments involving 2 min incubation periods. The concentrations of cytochrome P-450 and total microsomal protein (Table 1) were not changed at all intervals under study. Whole-body irradiation with 100 R showed no difference against non-irradiated controls.

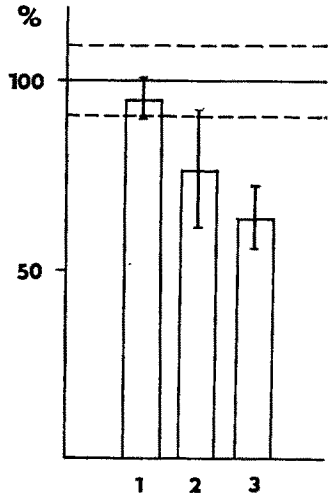

FIG. 1. The effect of head irradiation on pethidine demethylating activity of the microsomal fraction. 1–3. Days after 1000 R head X-irradiation. The results represent enzyme activity percentage referred to the controls (average control value 0·819 μmole CH_2O released by microsomes from g tissue during 60 min). Standard deviation is expressed as dashed horizontal lines for the control (19 animals) and as vertical bars for the post-irradiation groups (6–7 animals per group).

TABLE 1. CYTOCHROME P-450 AND TOTAL PROTEIN OF RAT LIVER MICROSOMES AFTER X-IRRADIATION OF THE HEAD

	No. of rats	P-450* (% of control)	Protein† (% of control)
Control	19	100 ± 6·1	100 ± 14·7
1 day after 1000 R	6	100 ± 7·0	98 ± 20·6
2 days after 1000 R	6	104 ± 9·9	97 ± 18·7
3 days after 1000 R	7	101 ± 9·0	99 ± 15·9

* $E_{450-490}$ per milligram microsomal protein recovered, average of the control group 0·066.
† Microsomal protein (mg) (Lowry method) recovered from g liver, average of the control group 10·1.
(Averages ± standard error).

Of the electrophoretic patterns obtained on the second and third day after head irradiation by 1000 R, two protein components were missing (see arrows on Fig. 2) compared with the microsomal structural protein of intact animals or of rats first day after head irradiation by 1000 R.

The present results suggest that the decrease of pethidine-demethylating activity in isolated rat liver microsomes[1] and the disappearance of two components* from the isolated microsomal structural protein[2] after whole body X-irradiation probably is not due to direct impairment of liver endoplasmic reticulum by radiation. Nothing is known on the actual (neural and/or humoral) links mediating this "abscopal" effect of ionizing radiation on the mixed function oxidase system and structural protein of the rat liver microsomes. A humoral pathway may be considered in view of the reports[10–12] describing the role of the pituitary (its adrenocorticotrophic and gonadotrophic function) in the effect of radiation on the oxidation of barbiturates in unresolved liver homogenates.

* In view of the aggregation phenomena taking place in deoxycholate treatment and preparation of the structural protein[9] and sensitivity of gel electrophoresis against such effects, the absence of an electrophoretic zone does not necessarily reveal the absence of a protein entity *in vivo*; e.g, changes in the lipid moiety of lipoprotein membranes may be thus shown.

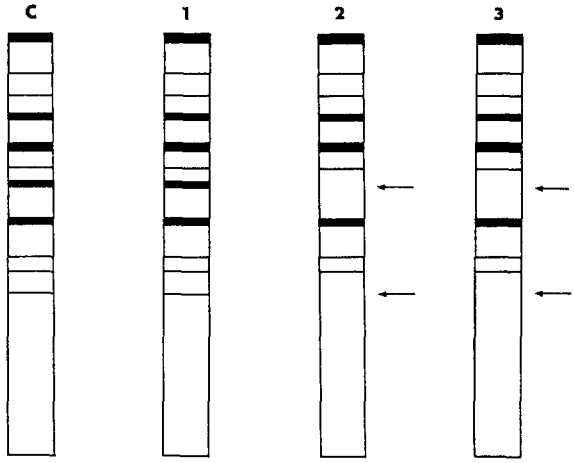

FIG. 2. Diagrams of polyacrylamide gel electrophoretic patterns of rat liver microsomal structural proteins obtained with pooled samples containing the material from two animals of each group. Cathode at the bottom; area of application at the top. C: Control. 1–3: Days after 1000 R X-irradiation of the heads.

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Enhanced antitumor effect of cytosine arabinoside given in a schedule dictated by kinetic studies *in vivo*

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THE SCHEDULING of cancer chemotherapy has been largely empirical because of the lack of precise information about the site, mode and duration of action of many of the presently useful antitumor