Effects of X-rays on polyamines, nucleic acids and amino oxidase and catalase activity in rat liver 48 h after irradiation

	Control	Irradiated with 800 r	Δ%	Irradiated with 1000 r	Δ%
Spermine (γ/g fresh tissue)	97 ± 4.2[a]	163 ± 11.2	+ 68	206 ± 19.6	+ 112
Spermidine (γ/g fresh tissue)	84 ± 3.7	120 ± 10.4	+ 43	189 ± 16.9	+ 125
RNA (mg/g fresh tissue)	12.16 ± 0.11	14.18 ± 0.18	+ 17	14.70 ± 0.15	+ 21
DNA (mg/g fresh tissue)	2.64 ± 0.07	2.97 ± 0.08	+ 12	2.86 ± 0.08	+ 9
Amino oxidase (μM NH_3/6 h/mg N)	0.48 ± 0.03	0.47 ± 0.04	–	0.47 ± 0.03	–
Catalase (μM/min/mg protein)	12.00 ± 1.10	9.26 ± 0.98	– 23	9.00 ± 0.91	– 25

[a] Values represent the means ± S.E.M. of 8 animals.

The nucleic acids were evaluated by the spectrophotometer at 260 nm after extraction and separation according to the SCHNEIDER technique[6]. The amino oxidase activity was measured by the ammonia content in CONWAY capsules[7].

The reaction system contained 0.1 N phosphate buffer pH 7.4 and spermine 75 μM; 10% liver homogenate in the buffer solutions centrifuged at 1000 g for 15 min corresponding to 3.4 mg of protein. The final volume was 3 ml.

The ammonia was collected in 0.01 N H_2SO_4 and determined spectrophotometrically by means of the Nessler reaction.

Catalase activity was evaluated spectrophotometrically[8]. The system was composed of 0.05 M phosphate buffer pH 7 and liver homogenate to 0.025% containing 0.2–0.3 mg of proteins. The proteins were determined according to FOLIN and CIOCALTEU[9].

From the results reported in the Table, one can see that the hepatic polyamine content of spermine and spermidine increases considerably in the first 48 h after irradiation, either in the groups that received 800 r or 1000 r; such an increment is higher for those receiving the higher dosage. A smaller increase was observed in RNA, while DNA shows less evident changes.

The values of the 2 enzymatic activities correlated to the polyamine catabolism, i.e. the amino oxidase and catalase, show no modification of the first enzymatic activity (which shows a different behaviour, however, every time there is an increase of the amines), while the second enzymatic activity decreases for both radiation doses given.

The results obtained, and in particular the decrease of catalase activity, lead us to suppose that at least after 48 h the X-rays determine a stimulatory effect of biosynthesis of some constituents rather than a slowing down of metabolic processes.

Riassunto. Un considerevole aumento di spermina e spermidina è stato osservato nel fegato di ratti dopo 48 h dalla pan-irradiazione con dosi totali di raggi X di 800 e di 1000 r. Gli acidi ribonucleici si modificano nello stesso modo, quantunque in misura minore.

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Stability of Erythrocytic Reduced Glutathione and Nicotinadenine Dinucleotide Phosphate in HbE-Thalassaemia Disease

Earlier reports by the present workers have shown a high incidence (30%) of instability of erythrocytic reduced glutathione (GSH) in HbE-thalassaemia disease[1]. This instability could not be explained on the basis of any deficiency in the related enzymes, glucose-6-phosphate dehydrogenase (G-6-PD) and glutathione reductase (GR)[2,3]. It was, therefore, thought worthwhile to investigate the status of nicotinadenine dinucleotide phosphate (NADP) in these subjects[4]. Preliminary results are reported in the present communication.

The series included the following subjects: HbE-thalassaemia disease 11, thalassaemia trait 2, and normal 7. GSH stability test was done according to the technique employed by BEUTLER[5]. Activity of G-6-PD

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G-6-PD activity and NADP levels of red cells

	G-6-PD U/l cells		NADP μM/l cells	
	Mean[a]	Range	Mean	Range
HbE-thalassaemia				
GSH unstable (5)	6,246 ± 2,781	4,750–11,400	7.1 ± 6.76	3.4–19[b]
GSH stable (6)	6,171 ± 984.5	4,500– 7,300	26.2 ± 5.30	19.4–33.4
Thalassaemia trait (2)		4,100–4,500		10.7–12.0
Normal (7)	3,081 ± 374	2,700– 3,600	23.5 ± 4.16	17.7–29

[a] Mean ± Standard deviation. [b] Value of 19 μM was obtained in only 1 subject; all other values were below 6 μM/l of cells.

was quantitated by the method of KORNBERG and HOREKAR[6] as modified by MARKS[7], and that of GR according to the technique of RACKER[8] as adapted for red cells[9]. Levels of NADP were estimated by the technique of SLATER and SAWYER using phenazine methosulphate and 2,6-dichlorophenol indophenol as the intermediary electron acceptors[10].

On the results of the GSH stability test, HbE-thalassaemia patients could be divided into 2 broad groups: (a) Those showing instability of erythrocytic GSH[5], and (b) those with a normal pattern of GSH stability[6]. G-6-PD and GR activities were normal or elevated in both the groups. The levels of NADP and G-6-PD activity in different subjects are shown in the Table. These data indicate that erythrocytes of HbE-thalassaemia patients showing an instability of GSH (group a) are deficient in NADP. Slight deficiency may also be present in thalassaemia trait.

Résumé. La cause de l'instabilité du GSH érythrocytaire dans la thalassémie HbE est recherchée. Les activités des enzymes G-6-PD et GR sont normales ou élevées; le taux de NADP est diminué, d'où probablement vient l'instabilité du GSH.

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On Combined Effects of Deoxyfluorouridine (FUDR) with Radiations and Ethyl Methane Sulphonate (EMS) on Chromosomes

The effect of fluorodeoxyuridine (5-fluorouracil deoxyribose, FUDR) alone and in combination with X-rays has been studied by several workers on the roots of *Vicia faba*[1–5]. In general, the results showed that FUDR, in effect, serves as an inhibitor of DNA synthesis[1]. It induces chromosome breakage and enhances the effect of X-rays on chromosomes. The exact mechanism of the synergy is not yet known. There is conflicting opinion regarding the time of action of the agent and evidence has been obtained to support the contention that FUDR produces cytological damage not only during the synthetic period but also after it[4] and during early stages of mitosis. All these studies are confined to the roots and it should be seen whether FUDR is equally incorporated in the dry seeds.

Since some combinations of alkylating agents are known to be more efficient than their individual effect, as well as combinations of alkylating agents and ionizing radiations[6], it may be worth testing whether FUDR can also modify the effect of other chemicals besides radiation. Such investigations are interesting since they might ultimately yield to a different mutation spectrum and also help to specify the effect of one agent if the action of the other agent is well known, at least in some respects.

In the present experiment dry seeds of *Nigella damascena* var. Miss Jekyll and barley var. Piroline were treated with FUDR (0.5 mg/100 ml) after γ-irradiation from a cobalt-60 source with a dose rate of 43,000 rad/h. The doses for nigella and barley were 5,500 and 10,100 rad respectively. The seeds were treated with FUDR for 5 h immediately after irradiation and after 16 and 30 h of pre-soaking.

In another experiment nigella seeds were irradiated with fast-neutrons from ITAL (reactor power 10 kW;

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