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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neutron flux $8.8 \times 10^{10} n/cm^2$, which corresponds to approximately 500 rad with neutron flux density about $10^7 n/cm^2$ sec; γ-contamination 140 rad/h) and treated with FUDR in the same way. In the third experiment barley seeds were treated with FUDR for 2 h followed by treatment with EMS for 3 h at 0.3 g/100 m/concentration. The treatments were done on dry seeds, and after pre-soaking for 18 and 30 h.

Table I. Effect of FUDR with γ-rays and neutrons at different pre-soaking times on *Nigella damascena* (FUDR: 0.5 mg/100 ml/5 h). 300 cells analysed in all treatments except treatments Nos. 4, 8 and 12 in which 100 cells were analysed

Treatments	% abnormal cells	% of aberrations		
		Bridges	Fragments	Total
1. Control	1	0	1	1
2. FUDR, 0 h	1	1	1	2
3. FUDR, 16 h	3	2	3	5
4. FUDR, 30 h	5	3	6	9
5. γ-rays	21	16	18	34
6. γ + FUDR, 0 h	24	19	21	40
7. γ + FUDR, 16 h	27	17	29	46
8. γ + FUDR, 30 h	31	15	41	56
9. Neutrons	15	14	20	34
10. N + FUDR, 0 h	15	15	20	35
11. N + FUDR, 16 h	19	15	28	43
12. N + FUDR, 30 h	25	21	28	49

Table II. Effect of FUDR with γ-rays at different pre-soaking times on barley (FUDR: 0.5 mg/100 ml/5 h). 300 cells analysed except treatment No. 8 in which 100 cells were analysed

Treatments	% abnormal cells	% of aberrations		
		Bridges	Fragments	Total
1. Control	1	0	1	1
2. FUDR, 0 h	1	0	2	2
3. FUDR, 16 h	2	1	3	4
4. FUDR, 30 h	6	3	5	8
5. γ-rays	18	14	16	30
6. γ + FUDR, 0 h	21	16	21	37
7. γ + FUDR, 16 h	26	19	26	45
8. γ + FUDR, 30 h	31	15	35	50

Table III. Effect of FUDR with EMS at different pre-soaking times on barley. FUDR: 0.5 mg/100 ml/2 h. EMS: 0.3 g/100 ml/3 h

Treatments	No. of cells analysed	% of abnormal cells	% of aberrations		
			Bridges	Fragments	Total
1. Control	600	0.3	0.0	0.3	0.3
2. EMS	600	0.8	0.0	1.0	1.0
3. FUDR, 0 h	600	0.7	0.2	0.5	0.7
4. FUDR, 18 h	300	1	1	1	2
5. FUDR, 30 h	300	5	0	9	9
6. FUDR-EMS, 0 h	600	4.3	0.7	5.2	5.9
7. FUDR-EMS, 18 h	300	5	1	5	6
8. FUDR-EMS, 30 h	300	8	1	8	9

Chromosome aberrations were investigated at anaphase of the first mitotic cycle after germination. In all the treatments of dry seeds the ultimate germination was not affected. The results show that FUDR exerts a strong mitotic inhibition, and induces chromosome aberrations at a low rate in dry seeds but at a higher rate in pre-soaked seeds. The effect is pronounced with 30 h pre-soaking. When FUDR is combined with γ-rays, there is usually a greater total effect than the individual effect. The results are presented in Tables I and II. It may be seen that this synergic effect is greater when total aberrations are considered than total aberrant cells. This effect is almost absent with neutron in nigella which is different to that observed in vicia under different experimental conditions[7]. The total number of bridges is less than the fragments but not so low as expected from previous findings, suggesting that a considerable amount of reunion has been possible in the presence of FUDR. The reason for synergism may be different to that due to an added effect in absence of reunion. Additional evidence in favour of other interpretation comes from observation on *Vicia faba*[8].

From the different pre-soaking times it may be seen that a greater effect is noted at the later time, i.e. after 30 h pre-soaking, of the treatment with FUDR. The synergic effect is also higher at that time. (It should be mentioned that strong mitotic inhibition after this treatment makes it difficult to score the aberrations.) There are two possible reasons. One being the effect of FUDR at early prophase time. The second factor is the different physiological conditions of seeds after pre-soaking. From this preliminary experiment no conclusions on this effect can be made.

From Table III it can be seen that when barley seeds were pretreated with FUDR before EMS a synergic effect was observed (which is less evident with 30 h pre-soaking). Since EMS does not induce chromosome breakage under normal conditions this interaction, giving a greater number of aberrations, particularly fragments, is of interest. The results of further experiments will be reported in a later communication[9].

Résumé. Des graines de *Nigella damascena* et des graines d'orge ont été irradiés par les rayons γ du Co^{60} ainsi que par des neutrons rapides. Ils ont ensuite été traités par la déoxyfluorouridine (FUDR) après différents temps de prégermination. On a observé que FUDR accroissait de manière nette les effets totaux des rayons γ sur les chromosomes (taux d'aberrations en anaphase). Par contre les effets des neutrons rapides n'ont été que faiblement modifés. Des effets synergiques ont également été obtenus chez l'orge après des traitements consécutifs FUDR-EMS.

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