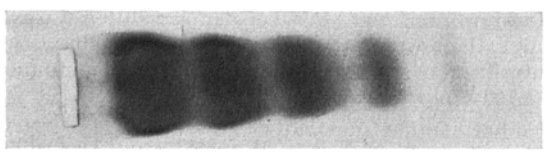
lesions consisted of diffuse myositis[11]. The isozymes in 10 or 20 λ aliquots of the extracts (200 mg mouse leg/ml 0.85% saline at 5 °C for 24 h) were separated by agar gel electrophoresis with barbital buffer, pH 8.6, and a current of 10 M.A./slide for 1.5 or 2.5 h in a Beckman Durrum-type electrophoresis cell. They were developed in a substrate containing lactate, DPN and nitrotetrazolium blue[12] cleared with an acid-alcohol wash, dried overnight at 37 °C on Mylar, and the relative enzyme activity measured in a Beckman Analytrol at 500 nm.

The isozymes separated into 5 anodal bands. The slowest moving band was predominant with the fastest band, LDH-1, the least prominent[4]. The same pattern was found in the extracts from infected and from non-infected mice; there was no difference with age (from 0–4 days), nor with type or length of virus infection (Figure). Total and relative activities of the enzyme fractions were similar in all the preparations.

Our failure to elicit a change in LDH pattern of mouse muscle by Group A Coxsackie virus infection and the normal pattern reported in dystrophic muscle disease of mice suggest that decreased LDH-5 is as much a species, as a disease specific expression.



LDH isozyme pattern in saline extract of leg muscles of 1-day-old normal mouse. Similar patterns were found in tissues of 2- and 4-day-old normal mice and in mice of the same ages after injection with Coxsackie virus Group A, types 20 and 21.

Résumé. Le zymogramme des LDH du muscle de souriceau nouveau-né souffrant de myosite diffuse causée par le virus de Coxsackie Groupe A est semblable à celui du souriceau sain de même âge, suggérant qu'un zymogramme anormal des LDH dans les affections musculaires n'est pathognomonique que chez certaines espèces.

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Meiotic Consequences of a Combined Treatment Fast Neurons-FUDR in *Vicia faba*

Some chemicals, like the bifunctional alkylating agent Myleran and the pyrimidine analogue 5-fluorouracil deoxyriboside (FUDR), have been reported to increase the effects of ionizing radiations in post treatment[1–4].

The synergistic effect obtained is higher after Co^{60} γ-rays than after fast neutrons[2–4]. Although the theory has been advanced that FUDR exerts its chromosome breaking effect by inhibition of the enzyme thymidilate synthetase, the mechanism by which the effects of radiations are modified is not yet clear but should be different from the previous one. At some Co^{60} γ-rays doses, it was found that all classes of chromosome aberrations are enhanced. Since it is well known that neutron effects are far more difficult to modify, see e.g. oxygen effect, it was in the scope of the present experiment to see if treatments by FUDR after fast neutron irradiations result in observable effects at meiosis.

Material and technique. Vicia faba dry seeds ssp. *minor* from Gembloux (Belgium) were irradiated by fast neutrons (fission) in the following experimental conditions: ITAL reactor (Wageningen, the Netherlands). Reactor power: neutron flux density was 2.10^7 $n/cm^2/sec$; neutron fluence applied was $2.2 \cdot 10^{10}$ n/cm^2 corresponding approximately to an absorbed neutron dose of 125 rad; γ contamination was about 140 rad/h. After irradiation, half of the seeds were treated by a solution of FUDR (concentration: 0.1 mg/100 ml). A control set (not irradiated) was also treated by FUDR and compared with a untreated set. All seeds were sown on perlite medium.

After 2 weeks, seedlings were transplanted in liquid medium (Hoagland) under bubbling conditions. They were grown in the following conditions: light intensity: 15.000 lux; photoperiod: 16 h light. Under these conditions they reached the flowering period in 2 months. At that time flower samples were collected for cytological investigation (5 samples in each series). They were fixed with Carnoy (2 h) then transferred into 70° alcohol. They were stained according to Feulgen technique and mounted in Depex.

Results. The criteria on which the present analysis is based have different meanings according to the stage investigated. The stages going from diplotene to metaphase I are generally suitable to identify translocations. Anaphase I allows us to observe the consequence of trans-

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Table I. Proportions of aberrations observed in different meiotic stages (5 different samples put together in each case)

Stages	Diplotene		Diakinesis		Metaphase I	
	No. of cells analysed	No. of abnormal cells	No. of cells analysed	No. of abnormal cells	No. of cells analysed	No. of abnormal cells
Control	150	0	200	0	700	0
FUDR	120	0	200	0	900	3
Neutrons	94	3	64	0	548	2
Neutrons + FUDR	93	3	116	2	387	13

Table II. Proportions of aberrations observed at different meiotic stages (5 different samples put together in each case)

Stages	Anaphase I		Anaphase II		Tetrads	
	No. of cells analysed	No. of abnormal cells	No. of cells analysed	No. of abnormal cells	No. of cells analysed	No. of abnormal cells
Control	400	1	1000	1	3000	3
FUDR	430	6	1200	9	5000	13
Neutrons	300	26	1260	13	2500	23
Neutrons + FUDR	344	90	862	31	1450	32

Table III. Proportions of different kinds of aberrations at anaphase

		Chromosome bridges	Chromatid bridges	Fragments	Attached fragments	Bridges + fragments	Micronuclei	Dissociation of translocations
Anaphase I	Neutrons	1	7	4	4	6	2	2
	Neutrons + FUDR	11	22	20	28	5	2	2
Anaphase II	Neutrons	–	4	6	–	2	1	–
	Neutrons + FUDR	–	12	15	–	2	2	–

The bar indicates that the category does not exist.

locations and also other structural changes. Mitosis II yields mostly information about the selection of the aberrations from which some inference can be made on the nature of these aberrations.

In Tables I and II, all types of aberrations have been put together.

No significant difference appears for diplotene and diakinesis at which some aberrations can remain unnoticed. On the other hand, there is a significant increase in the amount of aberrations scored at metaphase I. The aberrations observed are mostly rings of 4. No significant difference in the relative proportion of rings of 4 and figures of 8 exists for the 2 series, neutrons and neutrons + FUDR. The amount of aberrations induced by FUDR alone is low. The difference when FUDR is added is more striking for anaphases I and II as well as for resulting micronuclei observed in the tetrads (Table II).

Some aberrations at anaphase I are clearly identified as the consequence of translocations like the above-mentioned rings of 4 and figures of 8. The occurrence of attached fragments is somewhat more difficult to interpret. They are generally considered as subchromatid aberrations. When the possibility of an artefact is ruled out, the only explanation could be based on the existence of a delayed effect which is far higher when FUDR is added.

A comparison of the amount of aberrations is given for different types in Table III. Some are clearly of other origins than the ones mentioned above and were not detected at early stages. At anaphase I, some classes of aberrations are not increased. This is specially the case for bridges with 1 fragment, the probable origin of which being some kind of inversions.

For the series neutrons + FUDR, there is a selection of some aberrations from the first to the second mitosis although the figures remain still higher than for neutrons alone (Table II).

The distribution of the aberrations seems to be the same at anaphase II (Table III) since bridges and fragments are almost in equal numbers.

Conclusions. This research confirms the possibility of modifying the effects of fast neutrons by FUDR in post treatment. Moreover it shows that this effect results in a higher amount of aberrations at meiosis.

It should be pointed out that there is no difference in induced zygotic sterility between the two series, but this last statement should be extended to higher neutron fluences.

An increased sterility might possibly be entirely on the gametic side. Experiments designed to analyse *X2* generations for the mutation rate are being carried out[5].

Résumé. Des graines sèches de *Vicia faba* ont été irradiées par les neutrons rapides. Un lot a été traité par un analogue d'une base pyrimidique: FUDR. Ces graines sont semées et les plantes sont cultivées jusqu'à la floraison. Les bourgeons floraux sont alors fixés. On a pu mettre en évidence que le lot traité par FUDR après irradiation montre une fréquence plus élevée d'aberrations chromosomiques en méïose. La proportion des différents types d'aberrations chromosomiques a été étudiée. Cette recherche confirme la possibilité de modifier les effets des neutrons rapides par la FUDR en post traitement.

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