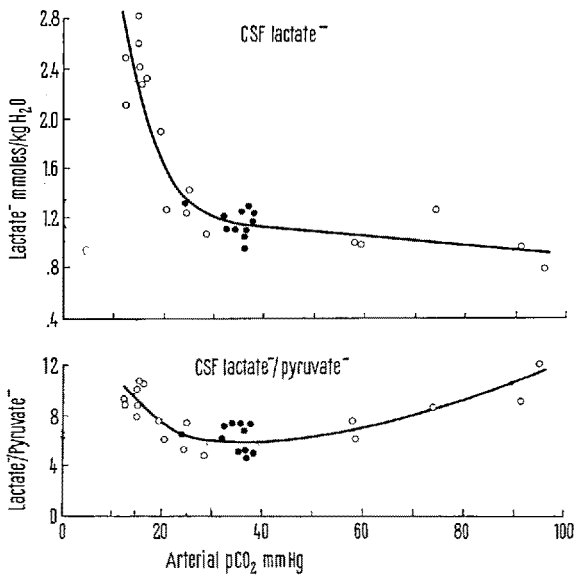
system, analyses of lactate/pyruvate ratios in cellular or extracellular systems might give important information on the redox state of the cells[3,4,7]. However, the steady state relation between the systems involves hydrogen ions

$$\frac{NADH}{NAD^+} = \frac{(Lactate)}{(Pyruvate)} \cdot \frac{K}{(H^+)}$$



The relation between the arterial CO_2 tension and the lactate concentration and the lactate/pyruvate ratio of cisternal CSF in anaesthetized and immobilized cats. The animals were either spontaneously breathing air or 7–9% CO_2, or they were mechanically hyperventilated for 60–90 min. Control samples are denoted by filled circles. Note increased lactate/pyruvate ratio at CO_2 tensions below 20–25 mm Hg.

where K is the equilibrium constant. The equation shows that at a constant redox state the lactate/pyruvate ratio will vary directly with the hydrogen ion concentration. Thus, we would expect the lactate/pyruvate ratio to increase in hypercapnia (see Figure) and decrease in hypocapnia. The fact that the lactate/pyruvate ratio increases during hyperventilation thus suggests an increased $NADH/NAD^+$ ratio, i.e. a state of hypoxia (cf. ref. [8] and [9])[10].

Zusammenfassung. Passive Hyperventilation anästhesierter und immobilisierter Katzen führt zu signifikanter Erhöhung des Laktat-Pyruvat-Quotienten in Zerebrospinalflüssigkeit und Gehirngewebe. Wegen der Verbindung zwischen Laktat/Pyruvat- und $NADH/NAD^+$-System ist somit anzunehmen, dass Hyperventilation (CO_2-Druck unter 20–25 mm Hg) zu zerebraler Hypoxie führt.

L. GRANHOLM and B. K. SIESJÖ

Department of Neurosurgery, University of Lund and Neurosurgical Service A, Lasarettet, Lund (Sweden), 6 November 1967.

[7] W. E. HUCKABEE, J. clin. Invest. *37*, 244 (1958).
[8] F. PLUM and J. B. POSNER, Am. J. Physiol. *212*, 864 (1967).
[9] B. K. SIESJÖ, Å. KJÄLLQUIST and N. ZWETNOW, Life Sci. *7* (2), 45 (1968).
[10] Acknowledgment. Supported by the Swedish Medical Research Council (Project No. 14X-263-03) and by contract No. F6 1052 67C 0052 between the ONR, Dept. of the U.S. Navy, and the University of Lund, Sweden.

Influence of a Mixture of Radioprotectors on the Mucosa of the Small Intestine of Mice Irradiated with 2000 R of X-Rays

Intestinal death occurs from the third to the fifth day after doses of 1–10 kR and is preceded by inhibition of cell division, destruction of the crypts, shortening of the villi and denudation of the intestinal epithelium (QUASTLER[1], MAISIN[2]). Mixtures of chemical protectors offer a better protection to the stem cells in the duodenum of mice than the most patent sulfhydryl radioprotectors given alone (MAISIN[2,3], MAISIN and MATTELIN[4]). The present communication reports data on the influence of mixtures of radioprotectors on the stem cells of the duodenum of mice during a period of 30 days after a dose of 2000 R of X-rays.

Materials and methods. Twelve-week-old male mice of the BALB/c strain weighing 25–30 g were used. The treated mice were given 16 mg of reduced glutathione by stomach tube 25 min before irradiation with 2000 R of X-rays (300 kV, 20 mA, 1mm Al, 2 mm Cu; dose rate 100 R/min). Fifteen min later, the mice were injected i.p. with 15 mg of cysteine and 10 mg of AET (both neutralized to pH 7.2 with NaOH) and, 20 min after administration of glutathione, with 1 mg of serotonin creatinine sulphate. The number of nuclei, mitoses, karyorrhexis and pycnosis were determined in at least 75 crypts/time point. The mice were killed by cervical dislocation at various time intervals after X-ray exposure. The duodenum was fixed in Bouin or neutral formaline, or in Carnoy. Slices were stained with hematoxylin eosine or with Feulgen.

Results and discussion. On the first day after exposure to 2000 R of X-rays, some cellular debris and fewer, often abnormal, mitoses are visible, but otherwise the mucosa of the duodenum appears normal. On the third day the crypts are atrophic and irregular, most of the villi have a normal aspect. Seven days after irradiation, some crypts are still atrophic, others are irregular and deeper than normal; the villi appear normal. From the ninth to the thirtieth day, the mucosa of the duodenum regains its normal aspect.

The number of the nuclei in the crypts column is presented in Figure 1. In the irradiated protected mice, the number of the nuclei decreases until day 2 (38% of the

[1] H. QUASTLER, Radiat. Res. *4*, 303 (1956).
[2] J. R. MAISIN, *Radiations ionisantes, radioprotecteurs et syndrome gastro-intestinal* (Ed. Masson, Paris 1966).
[3] J. R. MAISIN, Nature *204*, 196 (1964).
[4] J. R. MAISIN and G. MATTELIN, Nature *214*, 207 (1967).

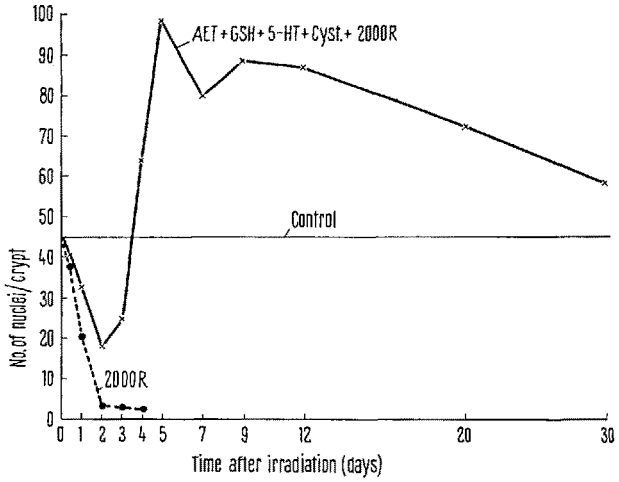


Fig. 1. The effect of a mixture of 2-β-aminoethylisothiouronium Br-HBr (AET) + glutathion (GSH) + creatinine serotonine sulphate (5-HT) + cysteine (Cyst.) on the number of nuclei in the duodenal crypts of BALB/c mice at different times after 2000 R.

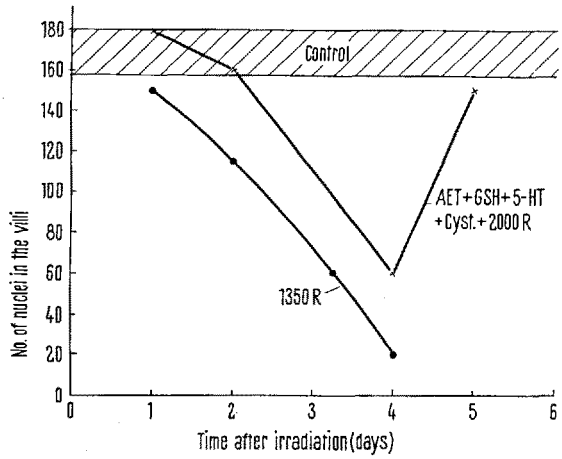


Fig. 2. The effect of a mixture of radioprotectors on the number of nuclei in the duodenal villi of BALB/c mice at different times after 2000 R.

normal value), then increases rapidly to reach more than twice the normal value by day 5 and declines gradually until the thirtieth day. At this time, the average number of the nuclei is still slightly above the normal value ($\pm$ 16%). In the villi, the number of nuclei reaches a minimum ($^1/_3$ of the normal value) 4 days after irradiation and returns almost to normal on day 5 (Figure 2).

The number of mitoses falls rapidly in the irradiated protected mice after X-irradiation, so that 12 h after irradiation hardly any mitoses can be found (Figure 3). Later, the number of mitoses increases steeply, reaching more than 3 times the normal value on day 7 and stays at this level until day 12. On day 30 the number of mitoses is still above the normal value.

The number of the pycnotic and karyorrhectic nuclei shows 2 peaks 12 h and 7 days after irradiation (Figure 4). The first peak corresponds to the early damage of irradiation. The second peak coincides with the second mitotic peak and may result from abnormal cells dividing during or just after division.

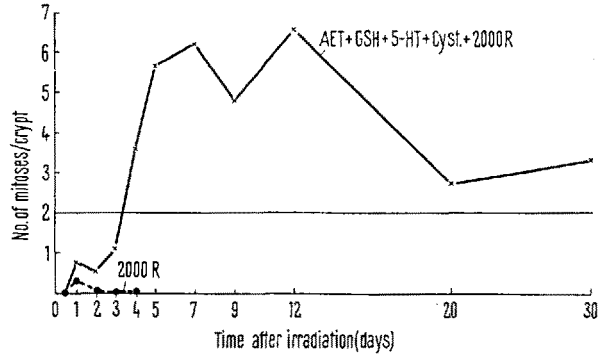


Fig. 3. The effect of a mixture of radioprotectors on the number of mitoses in the duodenal crypts of BALB/c mice at different times after 2000 R.

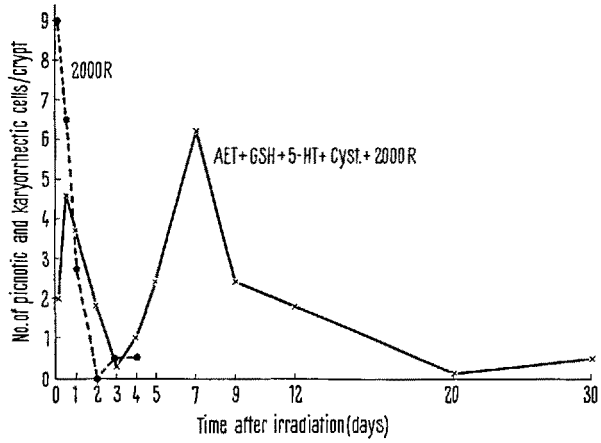


Fig. 4. The effect of a mixture of radioprotectors on the number of pycnosis and karyorrhexis in the duodenal crypts of BALB/c mice at different times after 2000 R.

Conclusions. Our results on the number of nuclei, pycnoses, karyorrhexis and mitoses in protected irradiated mice demonstrate that mixtures of chemical protectors largely diminish the lesions produced in the stem cells of the duodenum of mice irradiated with 2000 R of X-rays, but a delay of about 30 days is nevertheless needed to allow complete recovery of the duodenal mucosa[5].

Résumé. Nos résultats sur le nombre de noyaux, de pycnoses, de caryorrexis et de mitoses, chez les souris protégées et irradiées, montrent que des associations de substances radioprotectrices diminuent fortement les lésions produites dans les cellules souches du duodénum de souris irradiées avec une dose de 2000 R de rayons X; un délai d'au moins 30 jours est néanmoins nécessaire avant que la muqueuse duodénale ait retrouvé un aspect entièrement normal.

J. R. Maisin and M. Lambiet-Collier

Département de Radiobiologie, Centre d'Etude de l'Energie nucléaire, Mol (Belgique), 5 September 1967.

[5] This work was supported in part by research contract A.I.E.A. No. 347/RB and by grants from the 'Fonds de la Recherche scientifique fondamentale collective'.