On the Radio-Sensitivity of Rat Liver

Although the effects of irradiation on various metabolic activities of liver have been extensively investigated[1], most of these studies were carried out after total body irradiation, making it difficult to distinguish between effects of irradiation on the liver per se and abscopal effects. Despite the general assumption of radioresistance of liver, data in this regard is sparse and even the few investigations involving irradiation of only the liver or liver area have yielded conflicting results[2–5]. In order to more directly study the sensitivity of G_0 hepatocytes to interphase radiation death, I have investigated the effect of irradiating exteriorized portions of rat liver, with the rest of the liver and body shielded, using incorporation of ^{3}H-leucine into Trichloro-acetic acid (TCA) precipitable material as a biochemical index of cell viability.

Materials and methods. Male Sprague-Dawley rats weighing 200–250 g were housed in holding cages for at least 1 week before experimental use and were given water and Purina chow ad libitum. Irradiation was carried out using a 250 kVp Maxitron with 1 mm Al added filtration in order to achieve high dose rates. The 2 anterior lobes were irradiated using a modification of the technique of WEINBREN et al.[6] with the animals under nembutal anesthesia. The rats were placed in a lead box (5 mm thick sides and bottom) with a 6 mm slit in a 3 mm thick lead top through which the liver lobes were exteriorized. That portion of the slit not filled with liver vessels was covered with lead. Less than 1% of the dose delivered to the anterior lobes was received by the posterior lobes and remainder of the body with the exception of a small mass of tissue directly under the slit. Dosimetry was carried out using a Model 570 Victoreen Condenser R-meter and Model 131 chamber. Dose rates to the exposed lobes on top of the lead box at 15 cm (> 9 kR) or 30 cm (0.94 kR) tube target-to-liver distances were extrapolated from a series of measurements made at 40 to 100 cm. After irradiation, the liver lobes were replaced in the peritoneal cavity and the incision closed with silk sutures. Sham-irradiated animals had their anterior liver lobes exteriorized from the lead box but were not irradiated. Following the time of irradiation, the animals received water but no food.

At various times after X-ray exposure, each animal was injected with 20 μCi of ^{3}H-L-leucine in saline solution (specific activity 36.6 Ci/m*M*. New England Nuclear Co, Boston) per 100 g body weight via the tail vein. 1 h following injection, the rat was killed by a blow on the head, the liver removed, washed with ice-cold 0.25 *M* sucrose, blotted dry and portions of anterior and posterior lobes weighed and homogenized in 9 volumes of cold 0.25 *M* sucrose in a Potter-Elvehjem homogenizer. Aliquots of the homogenates were precipitated with equal volumes of 10% TCA, the precipitate washed 4 to 5 times with 5 ml portions of 5% TCA and finally dissolved in 1 ml of NCS solubilizer (Amersham/Searle) and counted in a liquid scintillation counter. Corrections for quenching were made by addition of an internal standard. Portions of the homogenates were used for determination of DNA (as an index of cell number) by the double wave-length method of DISCHE[7]. The *t*-test was used to determine the significance of differences between means of experimental groups.

Results and discussion. The effect of different doses of radiation after varying periods of time on the incorporation of leucine into TCA insoluble material of the liver, expressed as dpm/mg DNA, is shown in the Table. Some animals were sham-irradiated to determine if manipulation of the exteriorized portion of the liver caused alteration in the pattern of incorporation of leucine. No consistent differences were observed in the extent of incorporation of leucine between the exteriorized and shielded portions of liver in these animals, and the incorporation is therefore reported as the average of the values for both portions. The shielded posterior lobes of the irradiated animals, which serve as a control for the irradiated anterior lobes in the same animal, did not differ significantly from the

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^{3}H-Leucine incorporation by irradiated liver

Experiment No.	Radiation dose [kR]	Time after irradiation [h]	Irradiated[a] [dpm/mg DNA] $\times 10^{-3}$			Sham-irradiated[a] [dpm/mg DNA] $\times 10^{-3}$	
			No. of animals	Exposed lobes	Shielded lobes	No. of animals	Whole liver
1	0.94	24	4	199 ± 24	197 ± 16	4	176 ± 6
2	9.1	24	3	162 ± 14	179 ± 13	3	150 ± 11
3	18	24	5	228 ± 6	204 ± 9	2	204 ± 3
4	9.1	48	4	204 ± 22	205 ± 16	3	182 ± 17
4	18	48	4	179 ± 11	185 ± 22		[d]
5	27	48	3[b]	134 ± 10	178 ± 6	3	180 ± 10
5	36	48	4[b]	61 ± 13	201 ± 9		[d]
6	45	48	3[b]	77 ± 12	252 ± 17	3	244 ± 9
6	91	48	3[b]	13 ± 2	272 ± 29		[d]
7	45	72	4[b]	38 ± 7	283 ± 21	3[c]	208 ± 4

[a] Values are means ± standard errors of means. [b] Indicates significant difference between mean of exposed and shielded lobes ($p < 0.05$). [c] Indicates significant difference between mean of whole livers and shielded lobes ($p < 0.05$). [d] Control animals are the same as those entered immediately above.

sham-irradiated whole liver values during the first 2 days after the time of irradiation.

No significant effect of irradiation was detected 1 or 2 days after exposure to doses up to 18 kR. This absence of detectable effect at doses of 18 kR or less is not the result of in vivo elimination from the liver of dead or injured cells because no significant liver weight changes due to these radiation exposures occur during the first several days after-irradiation. In contrast, mean incorporation was significantly reduced by 2 days after irradiation, following doses equal to or greater than 27 kR. Greater inhibition was observed 3 days following 45 kR than 2 days after the same exposure, showing that hepatocyte radiation injury requires several days for expression even at these large doses. There was a significant increase in incorporation of leucine by the shielded lobe 3 days after 45 kR as compared to the corresponding sham-irradiated control value. This probably reflects the onset of compensation for cell killing in the irradiated lobes. The highest exposure (91 kR) resulted in almost complete cessation of incorporation at 2 days. Exposure to a dose of 36 kR or higher caused the liver to become necrotic and yellowish in appearance and difficult to homogenize.

There have been very few reports on radio-sensitivity of the liver to early damage utilizing local liver irradiation and these results are not consistent. GERSHBEIN[4] irradiated the exteriorized lobes remaining after partial hepatectomy in rats and observed no liver pathology below a dose of 20 kR at 11 days after exposure. In dogs receiving 2.8 to 5.9 kR to the exteriorized liver, necrosis of hepatic parenchyma was evident in certain animals from 1 day to 8 months after exposure[8]. ARIEL[2] carried out localized irradiation of rabbit livers and observed necrotic damage after 30 kR and higher doses but not after 3 kR or less.

The results of the present study correlate with the earlier morphological work of ARIEL[2] and GERSHBEIN[4], showing the extreme radioresistance of hepatic cell to early interphase death. This is in marked contrast to the very great radio-sensitivity of rat thymocytes which die in interphase within 2 days in vivo[9] or in vitro[10,11] following exposures of 1 kR or less. The mechanism of these large differences in radio-sensitivity among various cell types remains unkown[12].

Résumé. Les lobes antérieurs du foie de rats ont été prélevés et exposés aux rayons-X jusqu'à des doses de 91 kR et en employant comme indicateur biochimique de survivance l'incorporation de ^{3}H-leucine. Une dose de 45 kR fit diminuer l'incorporation au cours des 3 premiers jours suivant l'exposition; ce type de blessure de radiation nécessite donc plusieurs jours pour se manifester. Ces rêsultats montrent qu'au commencement de l'interphase l'hépatocyte est extrêmement résistant à la mort par radiation.

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Therapeutic and Immunologic Effects of Iodoacetate in Mature AKR Mice[1]

It has been suggested that certain sulfhydryl inhibitors (e.g., iodoacetate, iodoacetamide, oxophenarsine) may act preferentially on neoplastic cells[2–5]. Iodoacetate has been utilized as a chemotherapeutic adjunct to surgery for the management of selected cancer patients[3–5], and also has been employed to modify tumor cells for immunoprophylaxis in certain experimental murine model tumor systems[6–8]. The effect of this agent, however, on immunologic responsiveness in patients or in these model systems has not been examined.

The present report summarizes the effects of iodoacetate on survival and on splenic plaque formation in mature AKR mice, a model system in which there is a uniform incidence of spontaneous lymphoid leukemia (100% in our colony), and a relative constancy of the death rate.

Materials and methods. 6-to-7-month old female AKR mice, purchased from Jackson Laboratories, Bar Harbor, Maine, were randomized into treated and control groups (90 mice/group), and included only those animals with palpable thymomas and splenomegaly.

Mice in the treated group received 5 i.p. injections each of 0.5 ml quantities of $10^{-3}M$ sodium iodoacetate at 10-day intervals. Placebo control mice received the same regimen of the diluent, Hank's balanced salt solution. As the mice died, their thymuses and spleens were weighed. 6 days after each injection, sampling of mice from the 2 groups (4–6 mice/sample) were administered 0.5 ml of 25% suspension of sheep erythrocytes (SRBC). 4 days later, spleens from these animals were removed and assayed for total numbers of plaque forming cells (PFC), using a modification of the hemolysis-in-gel plaque procedure described by JERNE and NORDIN[9]. Rabbit anti-mouse IgG was added to each test plate which permitted the development of both IgM and IgG plaques.

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