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.

M. HAIDER[13]

Division of Radiation Biology, Department of Radiology, University of Washington School of Medicine, Seattle (Washington 98105, USA),
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[13] Present address: Solano Laboratories, Berkeley, California 94705, USA.

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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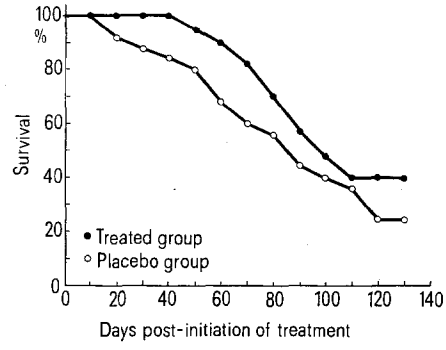
Values reported represent the mean number of PFC/10^6 nucleated cells from 4–6 plates.

Results. 100% survival was noted in iodoacetate-treated AKR mice through 40 days post-initiation of treatment, as compared with 84% survival in the placebo group (Figure). After termination of treatment (40 days), the survival rate of the treated mice declined gradually through 70 days, followed by a more rapid decline between the 70th and 90th day. 40% survival was noted for this group at the termination of the experiment (129 days post-initiation of treatment). The survival rate of the control group declined at a relatively steady rate, with 24% of the mice surviving the test period. The mean survival time of the treated mice was 99 ± 5.7 days, as compared with 84 ± 7.7 days in the placebo control group. The survival curve of untreated AKR mice is not depicted, since it simulated that recorded for the placebo control animals.

The mean thymus weight of treated mice was markedly less than was noted for the control groups (248.7 mg ± 54.3 mg vs. 399.6 mg ± 84.4 mg), while the mean spleen weight of iodoacetate-treated mice was greater (338.2 mg ± 61.2 mg as opposed to 286.3 mg ± 59.8 mg).

Although the total number of splenic PFC was markedly increase in the treated mice groups at all test intervals, the greatest increase occurred following the 3rd injection of iodoacetate. The magnitude of the splenic plaque response in treated AKR mice following the 4th and 5th injections of iodoacetate, although significantly elevated, was somewhat less than the 4-fold increase noted after the 3rd injection of the agent. The splenic plaque formation of the placebo control mice was relatively constant through the course of the experiment (Table).



Effect of 5 injections of iodoacetate (10-day-intervals) on the mean survival rate of leukemic AKR mice.

Splenic plaque formation in mature AKR mice during treatment with iodoacetate

Test intervals (days)	No. of injections	Total number PFC[a] Treated group	Placebo group	p-value[b]
10	1	535 ± 64.9	199 ± 8.4	< 0.025
20	2	510 ± 19.6	119 ± 30.3	< 0.001
30	3	759 ± 42.5	179 ± 31.5	< 0.001
40	4	387 ± 31.2	158 ± 18.7	< 0.01
50	5	453 ± 14.4	177 ± 6.4	< 0.001

[a] Total number of plaque forming cells/10^6 nucleated spleen cells.
[b] p-values determined by Student's t-test.

Discussion. The results of these experiments demonstrate that the sulfhydryl inhibitor, iodoacetate, can effectively reduce the mean thymus weight of AKR mice and increase their mean survival. Moreover, leukemic AKR mice given this agent demonstrated an enhanced immune response to SRBC, as reflected by statistically significant increases in numbers of splenic PFC throughout the treatment period.

While it has been suggested that certain sulfhydryl inhibitors may preferentially attack neoplastic cells[2–5], the specific site(s) of attack by these agents in tumor cells is not presently known[10,11].

Pertinent to the present study, it has been shown that tumor cells treated with iodoacetate in vitro protect mice against subsequent challenge with viable tumor cells[6–8]. Additionally, BLACK et al.[2], and more recently KNOCK et al.[3–5] have intimated that certain sulfhydryl inhibitors may exert their effects via stimulation of the host's immune mechanism, but these suggestions require further clarification.

While we are unable to relate the palliative effects noted in iodoacetate-treated AKR mice to their apparent increase in immunocompetence, similar adjuvant-like effects have been reported following administration of 6-mercaptopurine[12], uracil mustard[13] and cyclophosphamide[14].

Thus, the present data may indeed reflect iodoacetate-mediated immunostimulation, or may be a consequence of testing a selected population of lymphoid cells due to preferential lysis of tumor cells.

These experiences suggest that certain agents currently employed in the chemotherapeutic sense to treat malignant disease may have an adjuvant-like effect on the immune mechanism, and warrant further investigative attempts to obtain both a maximum antineoplastic activity, and an increased level of immunocompetence in the cancer patient.

Résumé. A des souris AKR leucémiques on administre un total de 5 injections de sulfhydryle inhibiteur, iodoacétate, à 10 jours d'intervalle. Ce régime produit une augmentation de survivance moyenne de 15 jours par rapport au groupe témoin. 40% des souris traitées vivent delà de la période d'expérimentation. Chez certaines souris traitées, on constate l'augemtation statistiquement assez importante des nombres totaux de cellules spléniques formatrices de plaquettes à tous les stades d'expérimentation.

M. S. RHEINS and ANNA D. BARKER[15]

Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus (Ohio 42210, USA),
28 September 1972.

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[15] Present address: Battelle Memorial Institute, 505 King Avenue, Columbus (Ohio 43201, USA).