Hepatomas in Mice: Incidence Increased after Gamma Irradiation at Low Dose Rates

Abstract. *Irradiation of LAf_1 mice with gamma-rays (total doses of 618 to 1335 rad) at a rate of 1.45 rad per hour markedly increased the incidence of hepatomas and ovarian adenomas, compared with incidence of these tumors in mice irradiated with x-rays at 30 rad per minute. This was somewhat unexpected, since chromosome aberrations in the liver cells were less frequent after irradiation at the low dose rate. Other types of tumors were not increased by irradiation with gamma-rays at the low dose rate. It is suggested that indirect hormonal mechanisms may contribute to the production of the hepatomas as well as of the ovarian adenomas.*

The fact that ionizing radiations elicit chromosome aberrations and also induce neoplasms has stimulated interest in a possible causal relation between these two effects of radiation. Recently, we observed that liver cells with chromosome aberrations were less frequent in mice irradiated with γ-rays at low dose rates than in mice irradiated with x-rays at high dose rates (*1*). We have therefore investigated the subsequent incidence of hepatomas in such mice exposed to γ-radiation at low dose rates.

We observed the frequency of liver tumors and other neoplasms in a group of 50 female LAf_1 mice irradiated at 2 to 3 months of age with γ-rays from a Co^{60} source at a rate of 1.45 rad/hr, the total doses being 618, 964, 965, 1335, or 2408 rad (*1*). The mice were killed or examined at death between 20 and 28 months of age; complete autopsies were performed.

The incidence of hepatomas (32 percent), ovarian adenomas (80 percent), and lymphomas (24 percent) is given in Table 1. In addition, among the 50 irradiated mice, one renal adenoma and three hyperplastic lesions of the gastrointestinal tract were found. No carcinomas or sarcomas were observed, and no instances of nephrosclerosis or hepatic abscess were noted. Although several of the hepatomas and ovarian adenomas became very large, they were histologically similar to those previously reported in irradiated LAf_1 mice (*2*), and in no case was there definite evidence of malignant transformation. As in our previous studies with LAf_1 mice, the lymphomas were generally nonthymic in origin.

With respect to hepatomas and ovarian adenomas, the data in Table 1 stand in marked contrast with previous observations on comparable groups of LAf_1 mice receiving single doses of γ- or x-rays at high dose rates over the whole body. In previous studies (*2–5*), in which a total of 403 mice received x-ray doses ranging from 260 rad to 1100 rad delivered at rates of 28 to 30 rad/min (hematopoietic cells being replaced or the marrow protected by lead-shielding of the femur at the higher dose levels), overall incidence of hepatomas was only 2 percent; of ovarian adenomas, 35 percent. In unirradiated control mice, the comparable figures were: hepatomas, 6 percent; and ovarian adenomas, 6 percent (*3*). In the study of Upton *et al.* (*6*) on large groups of LAf_1 mice exposed in the field to ionizing radiations (chiefly γ-rays) from an atomic bomb explosion, the incidence of hepatomas ranged from 11 percent for the lowest total dose (223 rad) to 4 percent for the highest dose (687 rad); incidence in unirradiated controls was 4 percent. Ovarian adenomas were present in 35 percent of the irradiated mice and in only 2 percent of the controls. Thus, the present incidence of 32 percent for hepatomas in mice irradiated with γ-rays at low dose rates is markedly greater than any previously reported incidence of such tumors after comparable radiation delivered at high dose rates. This finding also extends that in another study (*7*) where, under certain circumstances, continuous exposure to γ-irradiation slightly increased the incidence of various tumors, including hepatomas, in mice.

Since chromosome damage in the liver is greater in mice irradiated at high dose rates than in mice irradiated at low dose rates (*1, 8*), it is evident that the amount of chromosome damage induced by radiation is not a valid quantitative indicator of subsequent tumor development. This does not preclude the possibility that the hepatomas in this study resulted from the direct action of low-dose-rate radiation on the genetic material of the liver cells, but implies that if such an effect did occur it was apparently at a submicroscopic level. It may well be that for neoplasia induction the relation between the dose of radiation and the response depends on two distinct effects of radiation at the cellular level: (i) mutagenic damage or alteration of genetic materials at the subchromosomal level; and (ii) destruction of proliferative capacity, or cell sterilization, perhaps associated with visible chromosome aberrations (see *9*). Irradiation with γ-rays at the low dose rate may dissociate these two effects —that is, permit intracellular recovery from damage to the proliferative capacity of the cells (*10*) without permitting the corresponding recovery from the radiation-induced increased risk of mutation. Therefore, after irradiation with γ-rays at the low dose rate (with total doses of 618 to 1335 rad), there would exist a relatively large population of liver cells bearing radiation-induced genetic stigmata (necessary for tumor initiation), and these cells would still be able to proliferate in response to physiological stimuli (for example, growth hormone) or other stimuli which would promote the production of hepatomas (*2*).

That the incidence of lymphomas in mice irradiated with γ-rays at the low dose rates was not reduced below "background" (unirradiated) level is

Table 1. Incidence of tumors in mice irradiated with γ-rays at low dose rates. Eighty percent of the mice were killed; the others died.

No. of mice	Total dose (rad)	Age at death (months)	Tumors		
			Hepatomas	Ovarian adenomas	Lymphomas
5	618	21–28	3	4	1
15	964	24–28	4	11	3
15	965	21–27	4	13	4
13	1335	20–23	4	11	4
2	2408	20, 26	1	1	0
50		Totals	16 (32%)	40 (80%)	12 (24%)

also consistent with our hypothesis. X-rays or γ-rays, delivered at high dose rates in total doses above 600 rad, produce a "therapeutic" effect on the incidence of lymphomas in LAf_1 mice (*4*, 6), the incidence falling considerably below the 20- to 30-percent incidence in unirradiated controls. Therefore, with total doses of 618 to 1335 rad, lymphomas were more frequent after γ-irradiation at low dose rates than after x-irradiation at high dose rates, despite the fact that, as with the liver, chromosome aberrations in the hematopoietic tissues were more frequent after x-irradiation at the high dose rate (*1*, *11*).

Even if such an explanation of the carcinogenic effects of irradiating mice at low dose rates is accepted, there remains the question of why only hepatomas and ovarian adenomas were increased in frequency in this study; gastrointestinal tumors, renal tumors, and miscellaneous malignancies, though uncommon in both groups, appeared to be even less frequent than in earlier experiments in which high dose rates were used (*2–5*). The fact that induction of ovarian adenomas by radiation involves an indirect mechanism, hormonal imbalance (*12*), may be significant. Since the incidence of hepatomas in unirradiated mice is markedly influenced by hypophysectomy (*13*) and by castration in males (*14*), it is conceivable that hormonal imbalance was also a factor in development of the hepatomas in this study. Perhaps irradiation at low dose rates is more effective in producing such imbalance than are comparable total doses delivered at high dose rates; this possibility remains to be investigated.

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References and Notes

1. P. C. Nowell, D. E. Craig, F. A. Matthews, L. J. Cole, *Radiation Res.* **24**, 108 (1965).
2. L. J. Cole and P. C. Nowell, in *Biological Effects of Neutron and Proton Irradiations* (International Atomic Energy Agency, Vienna, 1964), vol. 2, pp. 129–141.
3. P. C. Nowell and L. J. Cole, *Radiation Res.* **11**, 545 (1959).
4. L. J. Cole, P. C. Nowell, J. S. Arnold, *ibid.* **12**, 173 (1960).
5. L. J. Cole and P. C. Nowell, *ibid.* **18**, 487 (1963).
6. A. C. Upton, A. W. Kimball, J. Furth, K. W. Christenberry, W. H. Benedict, *Cancer Res.* **20** (pt. 2), 1 (1960).
7. J. Hollcroft, in *Symp. Fundamental Cancer Res. 12th, Houston, 1958* (1958), p. 409.
8. H. J. Curtis, J. Tilley, C. Crowley (*2*), pp. 143–155.
9. L. H. Gray, in *Symp. Fundamental Cancer Res. 18th, Houston, 1964*, in press.
10. L. F. Lamerton and B. I. Lord, *Natl. Cancer Inst. Monogr.* **14** (1964), p. 185.
11. P. C. Nowell and L. J. Cole, *Science* **141**, 524 (1963).
12. M. K. Deringer, E. Lorenz, D. E. Uphoff, *J. Natl. Cancer Inst.* **15**, 931 (1955).
13. W. E. Heston, *ibid.* **31**, 467 (1963).
14. H. B. Andervont, *ibid.* **11**, 581 (1950).
15. Work supported in part by PHS career award K6-GM-15004 (to P.C.N.) and by the Bureau of Medicine and Surgery, U.S. Navy.

16 December 1964

Coxsackie A9 Virus: Mutation from Drug Dependence to Drug Independence

Abstract. *During the multiplication of Coxsackie A9 virus dependent on 2 - (α - hydroxybenzyl) - benzimidazole there arise some drug-independent virus particles, which are either drug-resistant or drug-sensitive. We studied the properties of such revertant particles under conditions which did not favor the selection of resistant over sensitive virus particles. These particles exhibited a wide range of responses to 2-(α-hydroxybenzyl)-benzimidazole, from high sensitivity to high resistance. Subclones showed the same degree of sensitivity or resistance as the respective parent clones.*

Mutants of Coxsackie A9 virus which require 2-(α-hydroxybenzyl)-benzimidazole (HBB) for multiplication give rise among their progeny to some HBB-resistant or HBB-sensitive virus particles (*1*). We have now determined the proportions of the drug-resistant and drug-sensitive revertant particles and their degree of resistance or sensitivity. Previous work provided only an estimate of the relative proportions of resistant and sensitive revertant particles, and HBB may have selectively favored the multiplication of drug-resistant particles by inhibiting the multiplication of drug-sensitive revertants. This difficulty can be overcome since unsubstituted benzimidazole permits optimal multiplication of HBB-dependent Coxsackie A9 virus at concentrations which do not inhibit the reproduction of sensitive particles (*1*).

The HBB-dependent Coxsackie A9 virus was plated in the presence of 1 m*M* unsubstituted benzimidazole in the overlay medium (Fig. 1) on monolayer cultures of monkey kidney cells (*2*). The infectivity titer was 2.6×10^8 plaque-forming units (PFU) per milliliter as compared to a titer of 1.7×10^8 PFU per milliliter with 0.1 m*M* HBB in the overlay. Three plaques that developed in the presence of unsubstituted benzimidazole and at a high dilution of virus were picked and suspended individually in 1 ml of phosphate-buffered saline (*p*H 7.2). The plaques contained 0.9×10^6, 1.3×10^6, and 1.1×10^6 PFU of HBB-dependent virus and 2.8×10^3, 2.9×10^3, and 5.7×10^3 PFU of HBB-independent virus, respectively, as determined by titration in the presence or absence of 0.1 m*M* HBB. These results indicate a high probability that each plaque had been initiated by a single HBB-dependent virus particle. To isolate HBB-independent revertants, virus from plaques 1, 2, and 3 was plated in the absence of the compound. The developing plaques, now initiated by drug-independent virus particles, were picked at random and subjected to one passage in monkey kindey tube cultures in the absence of the compound to eliminate residual HBB-dependent virus which might have contaminated the drug-independent clones. A total of 79 clones of drug-independent virus, derived from the HBB-dependent virus contained in plaques 1, 2, and 3, were thus prepared.

The HBB sensitivity of the drug-independent clones was determined by plaque titrations without and with 0.1 m*M* HBB in the agar overlay. Sensitivity to HBB was expressed as the ratio of the number of plaques formed in the absence of the compound to the number formed in its presence. Under the experimental conditions, clones of HBB-sensitive Coxsackie A9 stock virus gave ratios in the range from 1×10^3 to 8×10^3. The virus resistant to 0.1 m*M* HBB gives a ratio of 1. The procedure, though adequate, has limitations in that only plaque number, and not plaque size, is taken into account, and testing is confined to one concentration of HBB.

Tests of the 79 drug-independent clones revealed many degrees of sensitivity to HBB. At one extreme, the most sensitive clone showed a ratio as high as 1.7×10^5, whereas at the other extreme, some clones were completely resistant to 0.1 m*M* HBB.