The Effect of Route of Administration and Radiation on ^{3}H-Actinomycin D Binding by Mouse Hepatocyte DNA[1] (34983)

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Several laboratory and clinical studies have suggested that actinomycin D may potentiate the effect of ionizing radiation (1–5). The mechanism of this potentiation in animals is as yet unclear and may be subject to several explanations. For example, radiation may be altering the rate at which the drug is absorbed from its site of injection. Another possibility is that radiation, which may alter the DNA–histone complex (6–8), makes the DNA more accessible to the action of actinomycin D. The present investigation was prompted by the observation that ionizing radiation enhances the action of small doses of actinomycin D in inhibiting enzyme induction by cortisol in rat liver (Yatvin, Lamar, and Pitot, unpublished).

The results reported herein, on the basis of both *in vivo* and *in vitro* studies, suggest that irradiation does not affect the binding of ^{3}H-labeled actinomycin D to mouse hepatocyte DNA. Shortly after whole-body irradiation, however, there is a marked though transitory effect on the distribution of intraperitoneally injected actinomycin D, but not when the drug is administered intravenously.

Materials and Methods. Experiments were performed using young adult male mice of either the ICR/HA or BALB/C strain. Prior to killing, all animals were fasted for 18 hr but allowed water *ad libitum* or, when adrenalectomized, a 1% NaCl drinking solution. Bilateral adrenalectomies were performed 3–5 days before use, employing a mid-line dorsal approach after either anesthetization. Animals received whole-body exposure from either gamma rays in a 4π ^{137}Cesium irradiator (1800 R/min) or unfiltered 300-kvp X-rays (20 mA) from a General Electric Maxitron 300 at a flux of 1000 R/min. At various times after radiation exposure ranging from ½ to 17½ hr, the mice were injected either ip or iv into the tail vein, with 2.5 μCi of ^{3}H-actinomycin D (2.69 μCi/μg). Thirty minutes later, the mice were killed by cervical dislocation. The ip-injected mice were given a peritoneal lavage with 2 ml of 0.9% NaCl which was then collected through the inguinal canal. Then the liver was removed. After homogenization, liver nuclei were collected (9), and their DNA content and associated radioactivity determined (10). An aliquot of the peritoneal lavage was counted for tritium-activity content.

In a second experiment, mice were treated in the same manner as above, except that ^{3}H-actinomycin was not administered prior to killing. The isolated liver nuclei were then incubated for 15 min in 0.9 ml of media (11) to which labeled actinomycin had been added. Then associated radioactivity of the washed nuclei was determined.

For the third experiment, nuclei from pooled livers of fasted mice were collected and suspended in 0.9 ml of the incubation media and exposed *in vitro* to doses of gamma rays ranging from 50–2500 R at a dose rate of 50 R/min from a 2000 Ci ^{137}Cesium source emitting 662 keV gamma rays. During

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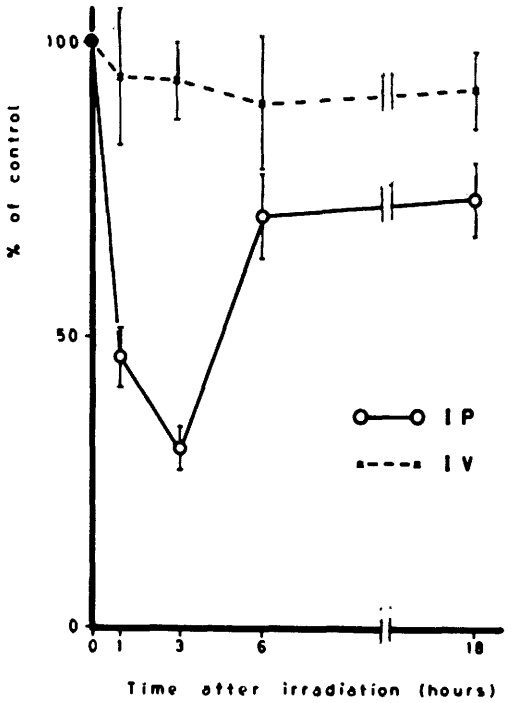

FIG. 1. Effect of radiation (10,000 R) on binding of ^{3}H-actinomycin D to mouse hepatocyte nuclear DNA. Mice were injected with labeled drug either ip or iv, 30 min before killing and collecting nuclei at the times indicated (6 min per point). The uptake of controls after iv injection is three times that of ip controls. Vertical bars are standard deviations.

radiation, the suspended nuclei were maintained in an ice bath at 0°. After irradiation, labeled actinomycin D was added, and the nuclei were incubated for 15 min at 37°. The nuclei were then washed and their bound radioactivity determined.

Results. The binding of ip- and iv-injected ^{3}H-actinomycin D to hepatocyte DNA at various times after irradiation is depicted in Fig. 1. When the labeled drug was administered ip, there was a marked decrease in the amount bound to DNA at 1 and 3 hr after radiation. By 6 hr binding was 70% of control and continued at this level through 18 hr. In similarly treated mice, ^{3}H-actinomycin disappeared less rapidly from the peritoneal cavity 1 and 3 hr after exposure. After 6 hr, the movement of the drug from the peritoneal cavity was back to the control value (Fig. 2). In contrast, when the actinomycin was injected iv, radiation did not affect binding to liver nuclei at any of the times studied and three times as much ^{3}H-actinomycin was bound per milligram of DNA.

No effect of radiation on binding of actinomycin D by liver nuclei was observed either in *in vitro* binding studies with nuclei isolated from both intact and adrenalectomized animals killed at various times after irradiation (Table I), or in experiments with nuclei irradiated *in vitro* (Table II). There is, in fact no effect of either *in vivo* or *in vitro* irradiation on binding of ^{3}H-actinomycin by liver nuclear DNA *in vitro*. Furthermore, adrenalectomy did not significantly affect the ability of the nuclei to bind the labeled drug (Table I).

Discussion. The data reported in this paper indicate that although radiation inhibits

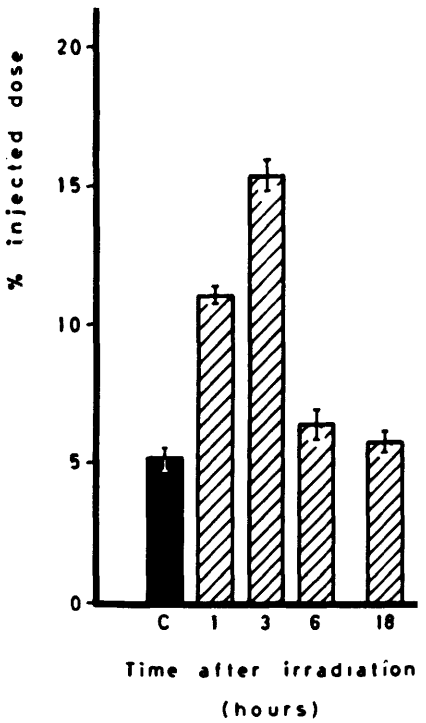

FIG. 2. Effect of radiation (10,000 R) on movement of ip-injected ^{3}H-actinomycin D from the peritoneal cavity of mice, 30 min after isotope injection. Residual peritoneal activity determined by counting peritoneal lavage. Solid column is the unirradiated control (seven mice per group). Vertical bars are standard deviations.

TABLE I. *In Vitro* Uptake of ^{3}H-Actinomycin D by Nuclei Irradiated *in Vivo*.

	Mice (hr)[a]					
	Control	0	0.5	1.5	6	18
	dpm ($\times 10^{-4}$)[b]					
Intact	3.44	3.54	3.23	3.38	3.35	3.50
Adrenalectomized	3.37	3.22	3.55	3.42	3.56	3.54

[a] Hours after the end of irradiation (10,000 R) at which mice were killed.

[b] Average disintegrations per minute of nuclear preparation (nuclei from 0.137 g of liver incubated with 0.5 μCi ^{3}H-actinomycin D) from three mice.

TABLE II. *In Vitro* Uptake of ^{3}H-Actinomycin D by Nuclei Irradiated *in Vitro*.

	Radiation dose (R)							
	0	50	100	600	900	1400	1800	2500
dpm ($\times 10^{-4}$)[a]	4.99	5.45	5.35	5.52	4.54	5.04	5.15	4.89

[a] Average disintegrations per minute from two tubes each containing 0.137 g equivalents of mouse nuclei and 0.75 μCi ^{3}H-actinomycin D. Nuclei were prepared from pooled homogenates of mouse liver.

the binding of ip-injected ^{3}H-labeled actinomycin by liver nuclei, the radiation is apparently not acting directly on the liver. It seems likely that the effect of irradiation on binding of ^{3}H-actinomycin D to hepatocyte DNA is mediated by decreased availability of the antimetabolite. This is of interest in the light of the studies suggesting that radiation *in vivo* and *in vitro* alters the DNA–histone complex (6–8), particularly since it appears that the amount of ^{3}H-actinomycin D bound by DNA is contingent on the state of chromatin repression, with histone removal increasing binding (12). It should be noted, however, that radiation had little effect on histone/DNA content in liver of irradiated rats (13). Perhaps with irradiated hepatocyte nuclei, no dissociation of the nucleoprotein complex occurs, or else it is either rapidly repaired or not detectable by ^{3}H-actinomycin D binding techniques. High doses of radiation (20,000 R) resulted in a slight (17%) decrease in binding of ^{3}H-actinomycin D to euchromatin but not to heterochromatin of ascites cells in culture (14). Since no statistics are offered, it is difficult to evaluate the significance of the ascites cell data.

The observation that after ip administration less actinomycin D is bound by the liver of irradiated animals seems incompatible with the observation of a synergistic effect that low levels of actinomycin and radiation have on inhibition of enzyme induction by cortisol in rat liver (Yatvin, Lamar, and Pitot, unpublished results). Perhaps the drug is functioning by inhibiting repair of radiation-damaged DNA. Such an action is not unlikely, as indicated by the studies of Merz and Pempre (15) in which they demonstrated that actinomycin D inhibited rapid repair of chromatid strand breaks induced by X-ray treatment of human lymphocytes in culture. Studies are presently underway to test this possibility with mammalian liver.

The finding that the movement of ^{3}H-actinomycin from the peritoneal cavity decreased was somewhat surprising since we have found that radiation markedly increased the movement of ip-injected ^{125}I glucagon in rats (16). However, the two compounds differ markedly in their physical chemical properties.

Finally, the smaller amount of actinomycin D bound to control liver when administered ip rather than iv could explain the report (4) that epilation occurred more frequently on

irradiated legs of mice treated with actinomycin when the drug was administered ip rather then iv. More drug could be available in the periphery as a result of the decreased liver uptake via the ip route.

The results of the current studies and those cited above (16) serve to reemphasize the importance of molecular composition and route of administration of agents used in comparing both intact and irradiated animals.

Summary. The ability of hepatocyte nuclei from irradiated mice to bind ^{3}H-actinomycin D was investigated at various times after exposure. Shortly after irradiation (1–3 hr), there was a marked drop in the amount of actinomycin bound by liver nuclei of ip-injected mice. The decreased binding after ip injection was probably an indirect action of radiation since it had no effect on binding iv-administered material. The decrease in actinomycin movement from the peritoneal cavity of similarly treated animals supports the hypothesis of an indirect action of radiation on binding by liver nuclei of ip-injected ^{3}H-actinomycin D. Further evidence for the hypothesis derives from the *in vitro* experiments using nuclei irradiated either *in vivo* or *in vitro*, in which there was no influence of exposure on binding. When iv administration is used, the amount of actinomycin bound to nuclei is three times that when the drug is given ip. These data call attention to the importance of the route used to administer compounds when intrepreting either the action of the compound or those of radiation in animals.

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