The Effect of Gamma Radiation and Actinomycin on Cortisone Induction of Liver Enzymes[1] (36333)

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Previous studies from this laboratory (1) have described the effects of exposure to high energy gamma radiation on the synthesis of the enzymes serine dehydratase, tyrosine transaminase, and tryptophan pyrrolase in the liver of adrenalectomized rats. In those experiments administration of either actinomycin-D or lethal doses of radiation at zero time to rats force-fed dietary protein resulted in an inhibition of serine dehydratase synthesis. Both agents were ineffective when administered at later time periods. Thus actinomycin-D in this system mimicked the action of radiation on enzyme synthesis. In contrast where cortisone induction of both tryptophan pyrrolase and tyrosine transaminase was inhibited by actinomycin-D administration, radiation had little or no effect on induction at the times studied. This latter phenomenon was difficult to explain, especially in view of the fact that both ionizing radiation and actinomycin-D have been implicated in the inhibition of genetic transcription (2, 3). The studies reported in this paper were initially designed to gain an understanding of the mechanism of this discrepancy. Although the total mechanism is as yet unclear, the results reported herein suggest that irradiation and actinomycin-D when combined act synergistically in dramatically reducing induction of tyrosine transaminase and tryptophan pyrrolase by cortisone.

Materials and Methods. In these experiments Rolfsmeyer rats obtained from the Rolfsmeyer Rat Company, Madison, Wisconsin were anesthesized with ether and bilaterally adrenalectomized by a midline dorsal incision. After the operation the animals were individually caged and allowed *ad libitum* access to a drinking solution of 1% sodium chloride, but were restricted to a Purina chow diet during the hours from 9:00 AM until 5:00 PM. The lighting in the room was adjusted so that the lights were on from 9:00 PM until 9:00 AM and off during the day between these hours. Animals were cycled in this manner for one week prior to the operation as described by Watanabe *et al.* (4). The same regimen was maintained for 5 days after the operation; then the experiments reported herein were performed.

Rats were irradiated in a plastic cage at a 95 cm distance from a 9000 Ci 3 cm diameter source of a Picker rotational ^{60}Cobalt unit for 37.5 min at 160 R/min for a total dose of 6000 R. After the radiation was completed the animals were removed from the case and actinomycin in the form of Lyovac Cosmegen (Merck, Sharp, and Dohme) or N_2-dimethyl-actinomycin-C_3 (a generous gift from Dr. W. Müller of Göttingen, West Germany) was immediately administered ip at the doses indicated and then again in 2 hr. Twenty minutes after irradiation cortisone in the form of cortef acetate (Upjohn Co.) was administered ip (100 mg/kg body wt) and in all experiments animals were sacrificed 4 hr after the administration of cortisone. The animals were killed by decapitation and the liver rapidly removed. After removal, the liver was homogenized in 4 vol of a buffer containing 0.05 *M* Tris–HCl, pH 8.2, containing 0.14 *M* KCl, 10^{-4} *M* dithiothreitol. Homogenates

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TABLE I. The Effect of Gamma Radiation and Actinomycin-D (200 μg/kg) on the Cortisone Induction of Tyrosine Transaminase and Tryptophan Pyrrolase.

Nonirradiated	Tyrosine transaminase		Tryptophan pyrrolase	
	Units ±	SE	Units ±	SE
Control	82.6 ± 4.7	(21)[a]	2.8 ± 0.4	(11)
Cortef	487.2 ± 16.4	(19)	15.2 ± 1.6	(16)
Act-D	77.2 ± 7.8	(9)	3.2 ± 0.3	(11)
Cortef + Act-D	382.8 ± 31.3	(7)	10.7 ± 1.4	(11)
Irradiated				
Control	61.6 ± 6.2	(12)	2.7 ± 0.4	(9)
Cortef	434.5 ± 26.1	(11)	12.9 ± 1.2	(11)
Act-D	45.7 ± 4.0	(12)	3.3 ± 0.5	(11)
Cortef + Act-D	206.2 ± 19.4	(10)	6.0 ± 0.8	(12)

[a] The number in brackets refers to the total number of animals studied. Enzyme units are expressed as micromoles product/hr/g liver ± SEM. See text for dosages of actinomycin-D (Act-D) and hydrocortisone (Cortef).

were prepared using a Polytron homogenizer (Brinkman Instruments, Inc.). Aliquots of the homogenate were then spun at 100,000*g* for 90 min in a Spinco Model L or Model L-2 in a 30.2 head. At the end of this time aliquots of the clear supernatant were removed and frozen at —70° for later enzyme assay. The assay for tryptophan pyrrolase and tyrosine transaminase was carried out by the automated methods previously described (5, 6).

Results. The effect of gamma radiation and actinomycin on the cortisone induction of tyrosine transaminase and tryptophan pyrrolase is seen in Table I. Cortisone administration alone results in a marked increase in the level of both enzymes 4 hr following the initial administration of the hormone. Administration of actinomycin-D does very little to the base level, however at a 200 μg/kg dose it does inhibit the cortisone induction of tryptophan pyrrolase and of tyrosine transaminase by approximately 25%. In animals that have been irradiated as indicated by both previous experiments and the current studies, the administration of cortisone results in a virtually normal induction of both enzymes at 4 hr. However, when actinomycin-D at these low doses is administered together with the cortisone, there is a rather dramatic inhibition (induction of both enzymes was less than 40% that obtained in livers from rats treated with cortisone only). This combined effect of irradiation together with cortisone does not appear to be additive, but rather seems to be a potentiation by irradiation of the effects of actinomycin-D on enzyme synthesis.

It has been argued previously (7–9) that actinomycin-D may have effects other than inhibiting RNA synthesis in mammalian systems. Therefore, in order to determine whether or not the remainder of the structure of the molecule might have secondary effects, an analogue of actinomycin, (N_2-dimethylene-actinomycin-C_3) reported not to bind to the DNA molecular strand (10), was administered in doses equimolar to levels of actinomycin D which almost completely inhibit the hormonal induction of tyrosine transaminase and tryptophan pyrrolase. As can be seen from the data of Table II, whereas actinomycin at doses of 700 μg/kg, given together with cortisone inhibited enzyme induction almost 90%, the analogue of actinomycin had very little effect on enzyme induction. The minor effect noted at these levels of the analogue may be accounted for by small contaminating concentrations of actinomycin-D which is one of the breakdown products of the analogue.

Discussion. The data reported in this paper indicates that although radiation is only slightly inhibitory to the steroid induction of the enzymes tyrosine transaminase and tryptophan pyrrolase, at the doses and times

TABLE II. The Effect of Actinomycin D (700 μg/kg) and N_2-dimethylene Actinomycin C_3 (700 μg/kg) Administration on the Cortisone Induction of Tryptophan Pyrrolase and Tyrosine Transaminase in Rat Liver.[a]

	Tyrosine transaminase	Tryptophan pyrrolase
	Units ± SE	Units ± SE
Control	92.78 ± 6.09	2.12 ± 0.67
+ Cortef	443.52 ± 37.76	11.22 ± 1.86
+ Act-D	76.02 ± 4.90	3.52 ± 1.16
+ Cortef + Act-D	158.52 ± 13.00	2.83 ± 1.41
+ N_2-dimethylene Act-C_3	125.57 ± 7.98	3.25 ± 0.90
+ N_2-dimethylene Act-C_3 + Cortef	396.34 ± 42.93	9.64 ± 2.39

[a] See legend of Table I and text for details. Act-C_3 = Actinomycin C_3.

studied, it potentiated the action of low doses of actinomycin-D in inhibiting the induction of these enzymes. After these studies were begun similar results were reported in studies with mice subjected to irradiation and actinomycin-D treatment (11). The previously noted absence of the effect of lethal doses of gamma radiation on the induction of these enzymes (1, 12) suggested that synthesis of these enzymes may have occurred on a stable mRNA template and that the steroid regulated enzyme synthesis at the translational level. The data reported in this paper are not sufficient to either support or refute this concept. It would appear from the studies with N_2-dimethylene-Actinomycin-C_3 that the potentiation effect of Actinomycin-D is not related to the possible secondary effects of the antibiotic due to other structures in the molecule such as the polypeptide side chains or the heterocyclic 3-membered ring.

A number of studies from other laboratories, and clinical experience as well, indicates that irradiation may potentiate the effect of actinomycin-D or *vice versa* (13–18). In studies by Elkind *et al.* (16), the administration of actinomycin to cultured cells after sublethal doses of irradiation markedly enhanced the lethality of the x-rays. The potentiation effect of actinomycin and irradiation in the treatment of certain neoplasms, particularly certain sarcomas found in children (17, 18), may also be a reflection of this same general mechanism. The possibility that the administration of the small doses of actinomycin is inhibiting repair of DNA has been considered and partially tested in this laboratory. As yet no evidence for this type of mechanism has been forthcoming in rat liver. The observation of Pinkel (19) that the skin of patients who had been irradiated months previously, responds with erythema to a subsequent dose of actinomycin-D is of interest. Since according to accepted time requirements for the repair of radiation damaged DNA, this late effect would seem to argue against assigning the potentiating effect only to its inhibition of repair of radiation-damaged DNA. Such an action cannot, however, be excluded as indicated by the studies of Merz and Prempre (20) in which they demonstrated that actinomycin inhibited the rapid repair (90 min) of chromatid strand breaks induced by x-ray treatment of human lymphocytes in culture. The mechanism of potentiation of irradiation and actinomycin is as yet not fully understood and may be subject to several explanations. For example radiation may be enhancing the rate at which actinomycin-D is adsorbed from the peritoneal cavity and bound to hepatocyte DNA. However, studies in this laboratory indicate the opposite (21). Another possibility is that radiation which is known to alter the DNA-histone complex (22, 23) makes the DNA more accessible to the action of the actinomycin D. A third possible explanation, which is compatible with the known action of actinomycin-D on liver polyribosomal organization and ribosome attachment to membranes (7) and of radiation on membranes and enzyme release (24, 25) is that actinomycin

and radiation enhance the rate of enzyme degradation. Studies are presently under way in this laboratory to investigate this latter possibility.

Summary. Ionizing radiation and actinomycin-D were employed in an attempt to gain further insight into the mechanisms by which cortisone induces hepatic tyrosine transaminase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) and tryptophan pyrrolase (L-tryptophan: H_2O_2 oxidoreductase, EC 1.11.1.4). Administration of the antibiotic at a dose of 200 μg/kg resulted in slight inhibition of the induction of tyrosine transaminase and tryptophan pyrrolase. Concomitant gamma radiation (6000 R) markedly enhanced this inhibition, while irradiation of the animals wthout actinomycin administration had little or no effect on the basal or induced levels of the enzymes studied. N_2-dimethylene-actinomycin-C_3, an analogue of actinomycin-D which does not bind to DNA, had no effect on enzyme induction.

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