Action of Actinomycin D on the GI Tract: Implications for Enzyme Induction Studies (35417)

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Recently I reported (1) that actinomycin D, which has a number of actions besides the inhibition of DNA-directed RNA synthesis, markedly alters gastrointestinal amino acid metabolism in adrenalectomized rats. It had previously been observed (2) for anesthetized rats that absorption of amino acids injected into jejunal loops was inhibited at various times after prior actinomycin D treatment. With the exception of the above report (1), however, the action of actinomycin D on gastrointestinal physiology has not been considered in any of the studies employing this drug in dietary induction of hepatic enzymes with orally intubated substrates. Because of the great influence that any action of actinomycin D on gastrointestinal physiology and inducer availability would have on the interpretation of previous dietary induction studies (3-6), an investigation of the problem seemed particularly worthwhile. With the above rationale, experiments to ascertain the effect of actinomycin D on stomach emptying and gastric physiology in intact rats were performed. The results indicate that actinomycin, in addition to its other actions, affected both.

Materials and Methods. Male albino Wistar rats (150–175 g), bred in the Radiobiology Department of the Centre d'Etude de l'Energie Nucléaire, MOL, Belgium and maintained on a standard laboratory rat diet, were used in the present studies. To determine the effect of actinomycin D on stomach emptying and its relation to subsequent availability of inducer, rats were fasted for 24 hr. They were then orally intubated, under light ether anesthesia, with 3 ml of a 33% casein hydrolysate, containing 5 μCi of a reconstituted protein hydrolysate (Schwarz Bioresearch Inc.) labeled with ^{14}C. Then they were immediately injected intraperitoneally with actinomycin D (100 μg/100 g) (Cosmegen, Merck, Sharp, Dohme) dissolved in 0.2 ml of saline or the same volume of saline alone. After 2 and 4 hr, the rats were killed by exsanguination, and blood was collected and centrifuged to obtain serum. The stomachs and their contents, the small intestines and their contents, and the livers were then collected and weighed. They were homogenized in 8 vol of cold water, using an Ultra-Turrax homogenizer (Janke and Kunkel KG), and perchloric acid (PCA) was added for a final concentration of 7%. The stomach and intestine homogenates were heated for 1 hr at 80°, and after centrifugation, a 1-ml aliquot of the supernatant was mixed with Insta-gel (Packard Inst. Co.) and counted for radioactivity. Aliquots of the liver homogenates and serums to which PCA was added were centrifuged and the supernatants were counted as above. The precipitates were dissolved in soluene (Packard Inst. Co.) and radioactivity was determined. All carbon counting was done in Packard Tri-Carb liquid scintillation spectrometer and corrected for quenching by addition of internal ^{14}C standard.

Results and Discussion. The rate of stomach emptying was significantly inhibited in rats given actinomycin D (Table I). During the first 2 hr after orally intubating 3 ml of a

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TABLE I. Distribution of Radioactivity in Rats After Stomach Intubation of Casein Hydrolysate Containing ^{14}C Amino Acids.

Time after casein administration (hr)	Treatment	Animal no.	Stomach content (g)	Percentage of total administered radioactivity					
						Liver PCA		Serum[a] PCA	
				Stomach	Intestine	Precipitate	Supernate	Precipitate	Supernate
2	Saline control	5	1.6 ± 0.3	32.6 ± 6.2[b]	9.6 ± 0.7	4.3 ± 0.3	2.8 ± 0.2	1.1 ± 0.1	1.2 ± 0.2
	Actinomycin D	6	2.7 ± 0.1	51.1 ± 3.3	5.4 ± 0.4	3.5 ± 0.2	1.6 ± 0.1	1.1 ± 0.0	0.5 ± 0.1
4	Saline control	5	0.6 ± 0.1	3.4 ± 0.8	5.1 ± 0.6	6.8 ± 0.5	2.9 ± 0.3	2.0 ± 0.1	0.4 ± 0.1
	Actinomycin D	6	3.3 ± 0.3	45.3 ± 8.5	3.4 ± 0.3	4.0 ± 0.4	1.4 ± 0.1	1.2 ± 0.2	0.4 ± 0.1
	Actinomycin D[c]	4	5.5 ± 0.6	82.0 ± 5.1					

[a] Total serum radioactivity was estimated by assuming 7% of body weight was blood with 55% being serum.

[b] Total activity per organ ± standard deviation from the mean after correcting for quenching by addition of ^{14}C standard.

[c] In this group, the drug was administered 1 hr prior to casein; in the other groups, actinomycin D was given immediately following casein intubation.

33% casein hydrolysate containing ^{14}C amino acids, stomach radioactivity of saline control rats declined 70% and the content weight was 50% of the amount initially administered. In contrast, in actinomycin D-treated animals, the administered radioactivity dropped 50% but weight of stomach contents only 10%, suggesting that nonradioactive material was somehow entering the stomachs of these drug-injected animals. Within the next 2 hr there was no significant decrease in radioactivity for the treated animals while there was an additional 90% drop for the controls. Apparently, sometime between 0 and 2 hr a block had been established in the treated stomachs, preventing further emptying. In fact, when actinomycin was injected 1 hr prior to casein hydrolysate administration, more than 80% of the dose was found in the stomach 4 hr after intubation. In addition, an examination of the stomach 4 hr after casein hydrolysate intubation revealed an actual increase in contents over that given the animal. Either enhanced gastric secretion, retention of secretion, antiperistalsis, or a combination of factors could have been the cause.

Because of the greater amount of material leaving the stomachs of control animals, it is not surprising that levels of radioactivity in the small intestines at both 2 and 4 hr were more than 50% higher than those of treated animals.

Reflecting the blocked state of the stomachs in the actinomycin D animals was their decreased rate of amino acid incorporation into liver protein. At both 2 and 4 hr, control levels were higher than treated levels, but, more significantly, protein-bound radioactivity in the liver of treated animals showed no increase (between 2 and 4 hr), in contrast to the control increase of 50%, while at both times, liver free amino acid content in control rats was twice that of treated animals. Likewise, at 2 hr, there was no difference between groups in serum protein-bound radioactivity. In the next two hr, there was no significant change in the actinomycin D group while there was a doubling of radioactivity in the controls.

Such close reciprocal relationships among stomach, intestines, and liver radioactivity

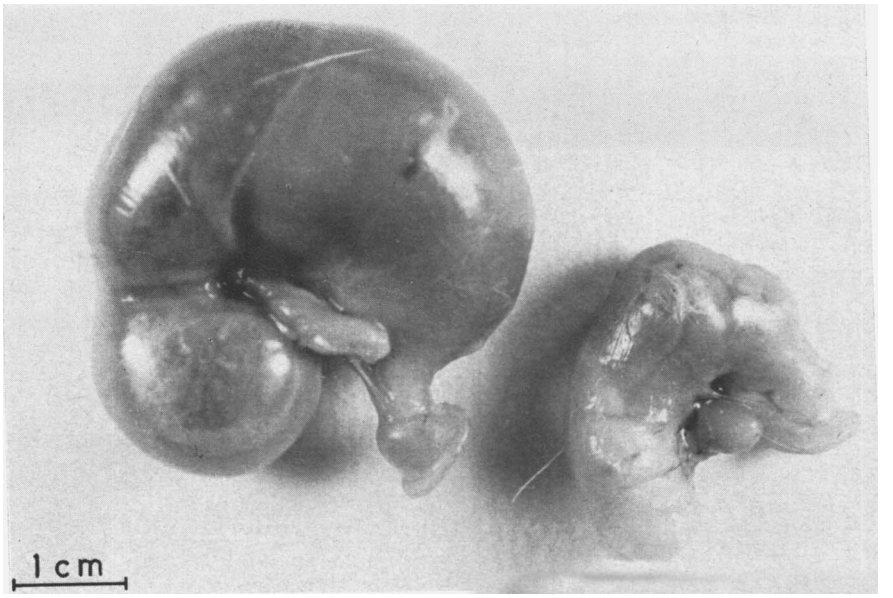

FIG. 1. Stomach of rat fasted 20 hr, injected with actinomycin D, and killed 4 hr later (left); and stomach of rat fasted 24 hr. The rats had access to their own fecal matter during the 4 hr from actinomycin D administration until they were killed (×1.5).

levels in actinomycin D-treated rats strongly suggest that failures to get induction in hepatic enzyme induction studies could have been the result of insufficient availability of inducers, rather than the more commonly credited inhibition of RNA synthesis.

To investigate the nature and source of the material accumulating in the stomach, rats were fasted 20 hr, then administered actinomycin D (100 μg/100 g). The rats were killed at 4-hr intervals after drug treatment, and stomach content was examined.

Actinomycin results in the appearance of fluid in the stomach (about 1.5 ml). It is not known whether this fluid is the result of retention and/or enhanced gastric secretion. Besides increasing fluid, the drug stimulates a bizarre appetite. If allowed, the rats will indulge in a marked degree of coprophagy. In addition, they may eat wood shavings, as well as the neoprene covering from coated cages. The fecal consumption combined with the fluid produces the striking enlargement in stomach size depicted in Fig. 1.

Mice also respond to actinomycin D with an increase in stomach content in fasted animals, but to a lesser extent than rats. (Yatvin, M. B., unpublished results). Perhaps vomiting observed in man after actinomycin treatment (7) is the result of similar actions of the drug: gastric retention and/or enhanced fluid secretion, whereas rats which do not have a vomiting reflex accumulate the material in their stomachs.

These experiments demonstrate that studies utilizing actinomycin to inhibit enzyme induction by orally administered substrates should consider the effect of this drug on gastrointestinal physiology and the resultant reduced availability of precursor.

Summary. Stomach emptying was significantly inhibited by actinomycin D administration (100 μg/100 g), after a 24-hr fast, to rats intubated with a ^{14}C-labeled casein hydrolysate. Four hr after intubation 45% of the radioactivity remained in the stomachs of actinomycin D-treated rats; whereas, only 3% was present in those of saline-treated controls. The decreased rate of amino acid incorporation into liver and serum protein reflected the blocked state of the stomachs of drug-treated rats. In addition to the retention of radioactivity there was an actual increase in total stomach content of the animals receiving actinomycin D. In drug-treated rats an increase in stomach content (1.5 ml of fluid)

was also observed 4 hr after administration of actinomycin D in fasted rats that were not intubated with casein. In addition, the drug stimulated a bizarre appetite. Treated rats consumed large quantities of wood shavings and neoprene if accessible, besides indulging in a marked degree of coprophagy. The reciprocal relationship between stomach and liver radioactivity levels suggests that failure to observe induction of hepatic enzymes by diet in studies using actinomycin D-treated rats could result from insufficient availability of inducers, rather than the more commonly credited inhibition of RNA synthesis.

This work was supported by funds from the "Fonds de la Recherche scientifique fondamentale collective" and U.S. Public Health Service Grant CA 03818 from the National Cancer Institute. I thank Dr. Pol Charles and Georg Gerber for their interest and advice.

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Received Sept. 1, 1970. P.S.E.B.M., 1971, Vol. 136.