of continued antibody formation after the interruption of RNA synthesis was relatively short. If antibody production had continued at an undiminished rate for one or more days, no more than about 50 percent reduction in antibody activity would have been expected within 2 days. The rapid decline in activity suggested that antibody formation was halted within 1 day after the inhibition of RNA synthesis and that the average lifetime of the residual RNA was consequently less than 1 day. Additional support for the idea that the lifetime of this RNA was shorter than 1 day was provided by the finding that a relatively brief inhibition of RNA synthesis (½ hour or 9 hours) caused very nearly complete cessation of antibody production. Further, the fact that interference with the synthesis of template RNA for only 5 or 15 minutes significantly reduced antibody formation suggests an average lifetime of less than ½ day.

If actinomycin D had no direct effect on the functional integrity of polyribosomes, the results would then suggest that in rabbit spleen cells the ribosome-associated RNA fraction that directs the synthesis of polypeptide chains of the H- or L-types, or both, which compose the 19*S* γ_1-globulin, has an average lifetime of ½ day or less. The confirmation of the messenger nature of this RNA must await studies of its sedimentation behavior and a comparison of its base composition with that of spleen cell DNA.

In exponentially growing *Bacillus subtilis* an average lifetime of about 2 minutes was estimated for mRNA (*4*), but in mammalian cells, particularly those which manufacture only a few types of proteins, mRNA has been reported to be fairly long lived. Protein synthesis in mouse fibroblasts was also reported to continue for several hours after inhibition of RNA synthesis to 99.9 percent (*10*), and the mean lifetime of mRNA in rat liver cells was estimated as 4 to 8 hours (*3*). In rabbit reticulocytes both actinomycin-sensitive and actinomycin-resistant protein synthesis was described, indicating the presence of short-lived as well as stable messenger (*11*).

The addition of 20 to 50 μg/ml of chloramphenicol results in complete inhibition of protein synthesis in bacteria (*12*), and even lower concentrations markedly suppress protein synthesis in cell-free systems (*13*). In contrast, protein synthesis in mammalian cells was only partially interfered with by concentrations of chloramphenicol 15 to 20 times higher (*14*). But such amounts of the drug are capable of causing reduced cellular respiration and even cell death (*15*). Only when amino acid incorporation directed by synthetic messenger (polyU) was measured in the reticulocyte ribosome system did chloramphenicol exert an inhibitory effect, which, however, was attributed to an effect on polyU rather than on the ribosomes *per se* (*16*). Thus, a puzzling feature of the chloramphenicol activity has been its apparent specificity for the bacterial ribosomes (*17*). However, in my study and in the recent extensive work by Ambrose and Coons (*9*), chloramphenicol concentrations of 40 to 50 μg/ml markedly suppressed antibody formation in vitro. A possible explanation for the discrepancy between the results in these two studies and in earlier work, with mammalian cell systems, is—as was pointed out by Ambrose and Coons—the longer exposure of the cells to the antibiotic in the studies of antibody formation. This proposed explanation is supported by our finding that treatment for 4 hours or less with chloramphenicol failed to suppress the continued formation in vitro of primary 19*S* (γ_1-globulin) antibody to PV.

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Induction of Several Adaptive Enzymes by Actinomycin D

Abstract. *Although actinomycin D has been used to prevent protein synthesis in experiments of several hours' duration, its effects on the synthesis of adaptive enzymes which are induced over a period of several days have received less attention. Treatment of young rats with doses of actinomycin D, which permitted survival for a period of 5 days, resulted in marked increases in the activities of four hepatic enzymes known to be induced by cortisol: alanine transaminase, tyrosine transaminase, serine dehydrase, and tryptophan pyrrolase. Actinomycin D also induced responses of two of these enzymes in adrenalectomized rats.*

Actinomycin D can block protein synthesis (*1*), inhibit the response of a number of adaptive enzymes (*2*), and prevent or delay antibody formation (*3*). The fact that actinomycin D can inhibit the DNA-mediated synthesis of RNA, presumably by reacting with guanine bases of DNA (*4*), has proved useful in studying the role of newly formed RNA in protein synthesis.

When cortisol is injected intraperitoneally into young rats, induction of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase occurs rapidly, the activity reaching a maximum within 6 hours (*5*). We have confirmed previous studies demonstrating that actinomycin D (150 μg/100 g) can block the rapid induction of tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase by cortisol (*2*). The induction of alanine-α-ketoglutarate transaminase and serine dehydrase after the administration of cortisol occurs slowly,

the activity reaching a maximum after several days (*6*). Experiments were undertaken to determine whether the induction of such enzymes could be prevented by treatment with doses of actinomycin D (15 and 50 μg/100 g of body weight) which allow survival of the rats for several days. Under these conditions it was apparent that actinomycin D did not inhibit the induction of these enzymes. Instead, this treatment actually produced marked increases in the activity of these enzymes as well as of tyrosine transaminase and tryptophan pyrrolase.

Fresh solutions of actinomycin D were prepared daily from a stock solution (2 mg/ml) in physiological saline which was stored frozen. A commercial preparation of cortisol (Cortef, Merck Sharp and Dohme) was diluted with saline and injected subcutaneously into male albino rats (Sprague-Dawley) (100 to 125 g). The control rats were injected with an equal volume of saline. Procedures for preparation of tissue homogenates, assay of alanine transaminase, tyrosine transaminase, tryptophan pyrrolase, and protein were the same as described previously (*6*). Serine dehydrase was assayed by a fluorometric procedure similar to that used for alanine transaminase.

After a single dose of actinomycin D each of these enzymes, with the exception of tryptophan pyrrolase, increased in activity within 24 hours, and each of the four enzymes showed a maximum response by 48 hours (Table 1). No significant depression or stimulation in the activity of any of these enzymes was observed after 12 hours. The values at 72 hours were lower than those obtained at 48 hours and were more variable, as is indicated by the larger standard deviations. Of the enzymes studied, serine dehydrase appears to be the most responsive to treatment with actinomycin D.

Although the possibility that actinomycin D may act as an inducer has not been excluded, the delayed responses indicate the likelihood of an indirect action. As a consequence of the initial temporary inhibition of protein synthesis after a single dose of the drug, a subsequent compensatory effect associated with an increased rate of formation or stabilization of template RNA might be related to the observed increases in enzyme activity. To sustain the direct effects of the drug, actinomycin D was injected daily for 5 days and was also given in combination with cortisol (Table 2). Each of the enzymes responded to cortisol. Alanine transaminase and serine dehydrase increased upon daily injection with actinomycin D alone to about the same extent as observed upon treatment with the corticoid, whereas tyrosine transaminase and tryptophan pyrrolase were more responsive to actinomycin D than to cortisol. When both actinomycin D and cortisol were administered at the same time, the severalfold increases in the activity of these enzymes were similar to those which occurred when these agents were used individually.

Because of the marked toxicity of this antibiotic, it was necessary to consider the possibility that its enzyme-inducing action might be related to an increase in plasma corticosterone that would be present in the stressed animal (*7*). In adrenalectomized rats, treatment with actinomycin D stimulated increases in tyrosine transaminase and serine dehydrase activity comparable to those seen in the intact animal (Table 3). Under the same conditions, the amounts of alanine transaminase in the livers were increased by about 50 percent and those of tryptophan pyrrolase by 75 percent after 72 hours. These data and the evidence reported elsewhere that tryptophan pyrrolase is more responsive than tyrosine transaminase to small doses of cortisol (*6*) suggest that increased amounts of circulating corticosterone cannot be the only factor responsible for the observed enzyme response induced by actinomycin D in intact animals.

Other observations of stimulatory, rather than inhibitory, effects of actinomycin D have been reported with quite different experimental systems. This antibiotic stimulated the induction of alkaline phosphatase in mammalian cells in culture (*8*) and increased the activity of penicillinase, but not β-galactosidase, in cultures of *Bacillus subtilis* (*9*). Also, Moog (*10*) recently observed that treatment of young mice

Table 1. Adaptive response of several hepatic enzymes to actinomycin D. A single intraperitoneal dose of actinomycin D (50 μg/100 g of body weight) was given to each rat. Control rats were injected with an equivalent volume of saline and killed at 12, 24, 48, and 72 hours; the values shown are the means (± S.D.) of individual determinations on the livers from five animals sacrificed at these different times. Activity is expressed as micromoles of product formed per milligram of protein per hour, except for tryptophan pyrrolase for which the activity is expressed as micromoles of product formed per gram of protein per hour.

Time after actinomycin D (hr)	Alanine transaminase	Serine dehydrase	Tyrosine transaminase	Tryptophan pyrrolase
Control	6.38 ± 1.3	1.68 ± 0.42	0.64 ± 0.14	12.3 ± 2.7
24	8.33 ± 1.4	3.26 ± 0.69	1.17 ± 0.27	13.3 ± 1.2
	$p < .05$	$p < .01$	$p < .05$	$p < 0.5$
48	20.8 ± 3.6	9.16 ± 1.7	3.71 ± 0.46	50.3 ± 17
72	16.9 ± 8.3	4.98 ± 2.2	3.16 ± 2.0	25.5 ± 10

Table 2. Effect of actinomycin D alone or in combination with cortisol on enzyme activity in liver. Cortisol was administered subcutaneously 30 minutes after the intraperitoneal injection of actinomycin D. The animals were killed 5 hours after the last injection of drug. Control rats received an equivalent volume of saline. The mean value (± S.D.) for five animals in each group is given. When compared to the control values, the p value for all groups is less than 0.01. Activity is expressed as micromoles of product formed per milligram of protein per hour, except for tryptophan pyrrolase for which the activity is expressed as micromoles of product formed per gram of protein per hour.

Five daily doses of:		Alanine transaminase	Serine dehydrase	Tyrosine transaminase	Tryptophan pyrrolase
Cortisol (mg/100 g)	Actinomycin D (μg/100 g)				
		7.72 ± 0.19	2.71 ± 0.89	0.45 ± 0.11	7.28 ± 1.5
3		24.2 ± 2.9	10.5 ± 2.0	1.40 ± 0.21	40.4 ± 14
	15	24.0 ± 4.5	12.1 ± 2.1	5.31 ± 2.5	80.1 ± 42
3	15	33.5 ± 5.2	13.0 ± 2.5	2.37 ± 1.0	79.1 ± 41

Table 3. Enzyme changes produced by actinomycin D in the livers of adrenalectomized rats. Bilateral adrenalectomy was performed by the technique of Grollman (*15*) 3 days prior to the start of treatment, and the animals were maintained on 1 percent saline in place of drinking water. Actinomycin D was injected intraperitoneally, and the control rats received an equivalent volume of saline. The mean value (± S.D.) for three to five animals in each group is given. Activity is expressed as micromoles of product formed per milligram of protein per hour.

Actinomycin D (μg/100 g body wt)	Tyrosine transaminase	Serine dehydrase
	0.45 ± 0.05	1.34 ± 0.20
5 (daily for	2.13 ± 1.1	5.20 ± 2.1
3 days)*	$p < .02$	$p < .01$
25 (single dose)†	1.81 ± 0.37	6.40 ± 2.8
	$p < .01$	$p < .01$

* Rats were killed 24 hours after the last injection. † Animals were killed 48 hours after this single dose.

with actinomycin D stimulated an increase in alkaline phosphatase in the duodenum to about twice the activity seen during the course of normal development.

The doses of actinomycin D in our experiments, although smaller than those in similar studies (*2*), were toxic to the animals as reflected by their loss in body weight, accumulation of fluid in the peritoneal cavity, and hemorrhagic areas in various tissues. The influence of such toxicity (*11*) on the altered enzymic activity produced by actinomycin D is difficult to evaluate. It is well known that fasting increases the activity of a number of the adaptive enzymes (*12*). The altered enzymic activity produced by the stress of fasting was compared with that found in actinomycin D-treated animals. The weight loss observed when rats were fasted for 2 days was twice that of the actinomycin D-intoxicated animals, but none of the enzymes in the livers of the fasted rats was more than doubled in activity.

The dose of actinomycin D used to inhibit the 5-hour response of tyrosine transaminase and tryptophan pyrrolase to cortisol (*2*) was more than twice the LD_{50} (50 percent lethal dose) of this antibiotic (40 μg/100 g) for the rat (*11*). We have observed that 25 μg of actinomycin D given as a single dose to rats weighing 100 g does not block the rapid induction of these enzymes by cortisol. On the other hand, the cortisone-induced increases in the activity of several glycolytic enzymes were impaired by treatment with as little as 8 μg of actinomycin D per 100 g of body weight given daily for 5 days (*13*). These different observations and the finding that enzyme induction by the antibiotic is achieved with doses in the range of the LD_{50} suggest the importance of comparing the amount of drug required to inhibit RNA synthesis with that necessary to inhibit enzyme induction.

Studies by Schwartz *et al.* (*14*) have indicated that inhibition of RNA synthesis in rat liver to the extent of 90 percent occurs within 30 minutes after the administration of actinomycin D (100 μg/100 g); thereafter this effect is slowly relieved and is no longer apparent after 16 hours. These results suggest that, after a single large dose of this antibiotic, protein synthesis should be markedly inhibited for a period of about 5 hours and not significantly impaired after 12 hours. Thus, different effects of actinomycin D may be obtained, depending on the dose and the intervals at which observations are made. However, the manner by which this antibiotic stimulates the induction of certain adaptive enzymes is not understood. It is conceivable that the negative nitrogen balance and cachexia which occurred after administration of toxic doses of the drug might enlarge the pool of free amino acids in liver and thus selectively promote the induction of hepatic enzymes responsive to increased protein intake or fasting (*12*). The evidence that treatment with actinomycin D induces the synthesis of certain adaptive enzymes in adrenalectomized rats indicates the need for comparative investigation of the mechanism underlying the induction of the same enzymes by either cortisol or actinomycin D.

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Transplantation of Rat Bone Marrow in Irradiated Mice: Effect of Exposure Rate

Abstract. *Mice were irradiated at several different exposure rates so that they received a total of 900 roentgens over the whole body. Within 2 hours they were injected with rat bone marrow. The success of the transplantation depended upon the rate at which the animals had been irradiated: the higher the rate, the greater the success of the grafts. Failure of grafts in mice subjected to low exposure-rates was associated with antibody formation. The data indicate existence of an immune mechanism which is dependent on the radiation exposure-rate.*

In a study of the survival of irradiated rats injected with allogeneic (homologous) bone marrow, Courtenay (*1*) found a relation between survival and the exposure rate. Her study suggested, by virtue of hematopoietic failure and death, that the lower rates of 0.28 r/min and 1.4 r/min (Cs^{137} γ-rays) were less effective in depressing the host's immune mechanism than the higher exposure rate of 29 r/min [250 kv (peak) x-rays]. In the study described here, heterologous (xenogeneic) transfer of rat bone marrow into irradiated mice was used to demonstrate the dependency of an immune mechanism on the exposure rate in the rejection of a foreign graft.

Mice of the $C3BF_1$ [$(C3H \times C57Bl)F_1$] strain were exposed to γ-radiation from two different sources. The first was a total-body irradiator designed for the treatment of patients; it consisted of eight 500-curie Cs^{137} sources (*2*). The positions of the sources ensured a uniform total-body exposure of mice in a compartmented lucite cage continuously rotating at 0.2 rev/min. The exposure rate, as measured in air with a Victoreen roentgen chamber, was 3.75 r/min. For greater exposure rates, a Co^{60} unit with a 260-curie source was used. By altering the distance between the lucite cage and the source, groups of mice could be irradiated at exposure rates of 19.8 r/min, 39.7 r/min, or 53.4 r/min. These values represent the mean of the center and end positions in a single