Effects of Cycloheximide on DNA and Protein Synthesis in Rat Liver (33935)

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(Introduced by D. F. Flick)

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The effects of the antibiotic cycloheximide have been widely reported in the recent literature. In yeast, the drug inhibits protein and DNA synthesis and stimulates RNA synthesis (1–3), while in *Neurospora crassa*, it is reported to inhibit RNA synthesis and methylation (4). *In vivo* studies on rabbits (5) and mice (6) have shown that cycloheximide inhibits amino acid incorporation in the mammalian liver. Cycloheximide inhibits protein synthesis and the estrogen response in the rat uterus (7) and inhibits both DNA and protein synthesis in rat liver extract (8), mammalian cell cultures (9), and the protozoan tetrahymena (10). The present study was undertaken to determine the specific effects of cycloheximide on the components of the pathways of protein synthesis in rat liver and relate dosage of drug to its relative effect on protein synthesis, RNA synthesis, and DNA content of the cells.

Materials and Methods. Orotic acid hydrate-6-^{14}C (5.5 μCi/μmole) and L-leucine-4,-5-^{3}H (8250 μCi/μmole) were obtained from the New England Nuclear Coporation. Cycloheximide was purchased from Nutritional Biochemical Corporation.

Male Wistar albino rats weighing approximately 140 g were put into groups of four animals each. After the animals were fasted for 18 hr, they were given intraperitoneal injections of cycloheximide in mammalian Ringer's solution. The dosage levels used were 0.1, 0.2, and 0.4 mg of cycloheximide/100 g of fasted body weight; in a later experiment, levels of 0.05, 0.7, and 1.0 mg/100 g of body weight were used. Control rats received only the Ringer's solution. Two hr later, two rats in each group received

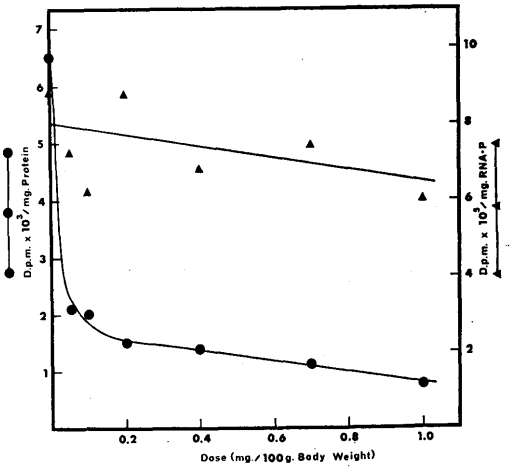

FIG. 1. Effect of cycloheximide dosage on orotic acid-^{14}C incorporation into liver RNA, and L-leucine-^{3}H incorporation into liver protein.

intraperitoneal injections of orotic acid-^{14}C (12.6 μCi each). After an additional 2 hr, during which time the animals had access to food, they were decapitated. The livers were rapidly excised, frozen in Freon and weighed, and sections were taken for protein analysis by the method of Lowry *et al.* (11), for RNA and DNA analysis by modifications of the Schneider (12) and Burton procedures (13), and for phosphate analysis by the method of Lowry *et al.* (14). The modifications of the RNA and DNA procedures are based on the recent report of Webb and Lindstrom (15), and are improved methods for quantitatively extracting the nucleic acids from tissues. The procedure used to prepare liver samples for counting the isotopes incorporated into protein and nucleic acid involved the addition of 1.3 vol of cold 0.6 *N* perchloric acid to aliquots of tissue homogenate to precipitate protein. The protein and nucleoprotein precipitates were washed several times by suspension and centrifuga-

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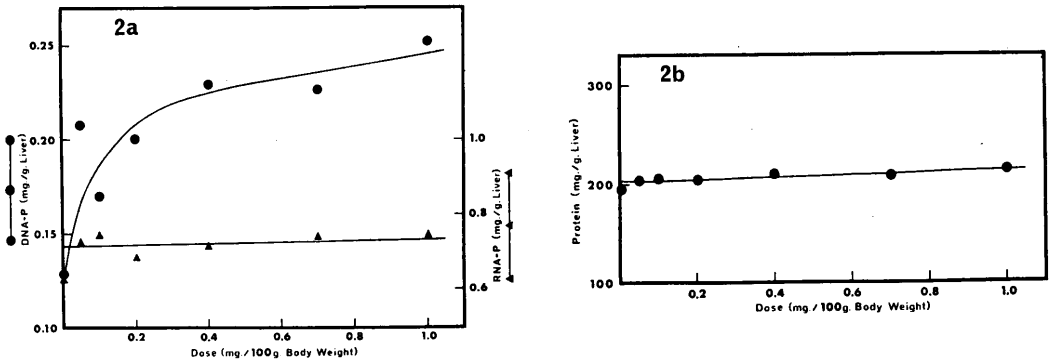


FIG. 2. Effect of cycloheximide dosage on liver DNA-P and RNA-P (a); and on liver protein (b).

tion in 0.33 *N* perchloric acid to eliminate unincorporated radioactivity. The final precipitate was dissolved in NCS (Nuclear Chicago Corporation) reagent and aliquots were removed for counting in 15 ml of a toluene–scintillation system or Bray's solution (16). Radioactive counting was carried out in a Nuclear Chicago mark 1 liquid scintillation counter and measurements were corrected for quenching using appropriate values derived from known quenched samples and external standard channels-ratio techniques (17). Sections of the fresh livers were taken for fixing in 10% formalin, stained with H. and E. imbedded in paraffin, and sections of 6–8 μ in thickness were taken for counting of nuclei.

Results. Figure 1 shows that cycloheximide markedly affected the incorporation of L-leu-

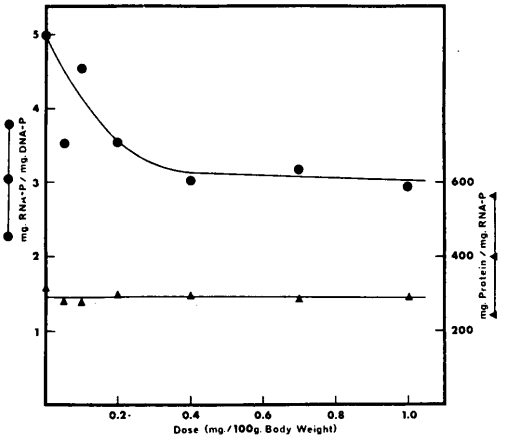


FIG. 3. Effect of cycloheximide dosage on the RNA-P: DNA-P and protein: RNA-P ratios of rat liver.

TABLE I. The Effects[a] of Various Dosages of Cycloheximide on Liver Weight, RNA-P, DNA-P, and Protein of Rats.

Cycloheximide (mg/100 g of body wt)	(mg/g of liver)			(g)	
	DNA-P	RNA-P	Protein	Total liver protein	Liver wt
Control	0.117 ± 0.006	0.624 ± 0.023	196 ± 4	0.984 ± 0.033	5.02 ± 0.21
0.1	0.170 ± 0.018	0.747 ± 0.010	205 ± 5	0.903 ± 0.031	4.40 ± 0.07
0.2	0.201 ± 0.020	0.686 ± 0.009	205 ± 5	0.865 ± 0.017	4.24 ± 0.11
0.4	0.239 ± 0.011	0.719 ± 0.010	210 ± 6	0.927 ± 0.014	4.42 ± 0.14
Control	0.140 ± 0.009	0.631 ± 0.034	197 ± 5	0.988 ± 0.039	5.01 ± 0.10
0.05	0.208 ± 0.009	0.727 ± 0.012	203 ± 3	0.961 ± 0.004	4.73 ± 0.08
0.7	0.237 ± 0.016	0.736 ± 0.038	208 ± 8	0.909 ± 0.036	4.37 ± 0.13
1.0	0.252 ± 0.006	0.742 ± 0.007	214 ± 4	0.996 ± 0.021	4.66 ± 0.06
Control (av)	0.128 ± 0.007	0.628 ± 0.019	196 ± 3	0.986 ± 0.024	5.02 ± 0.11

[a] Each value is the mean of four animals and is given with the SE.

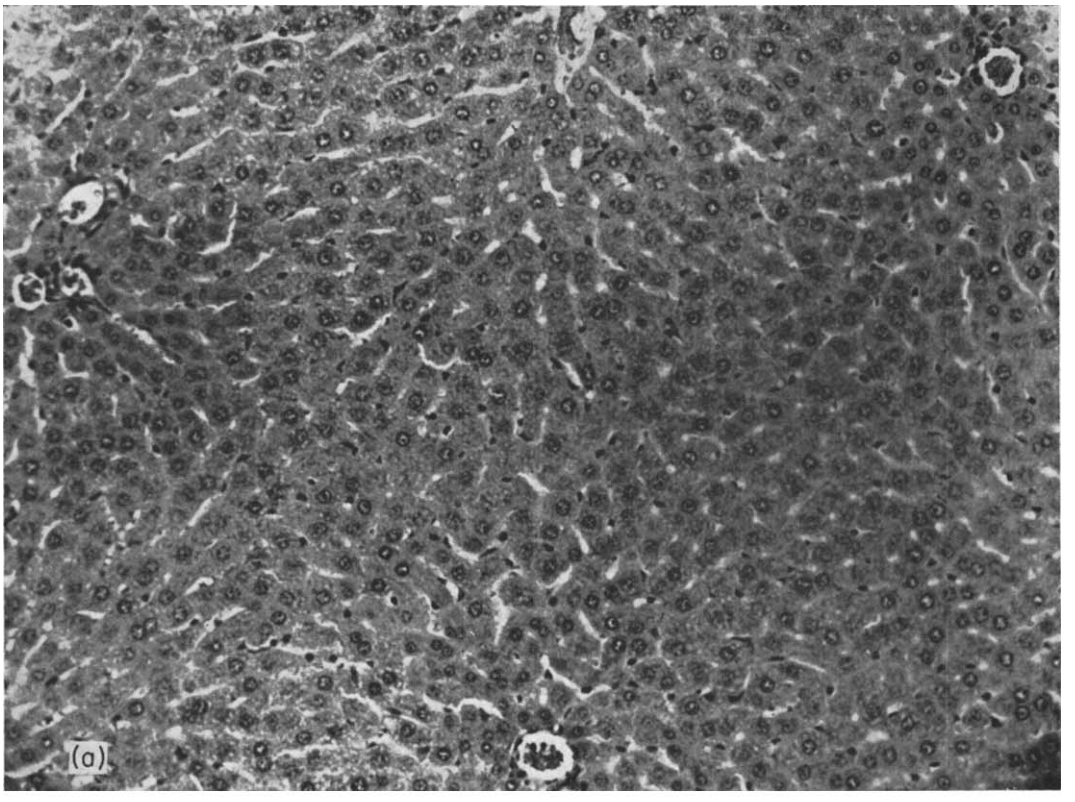
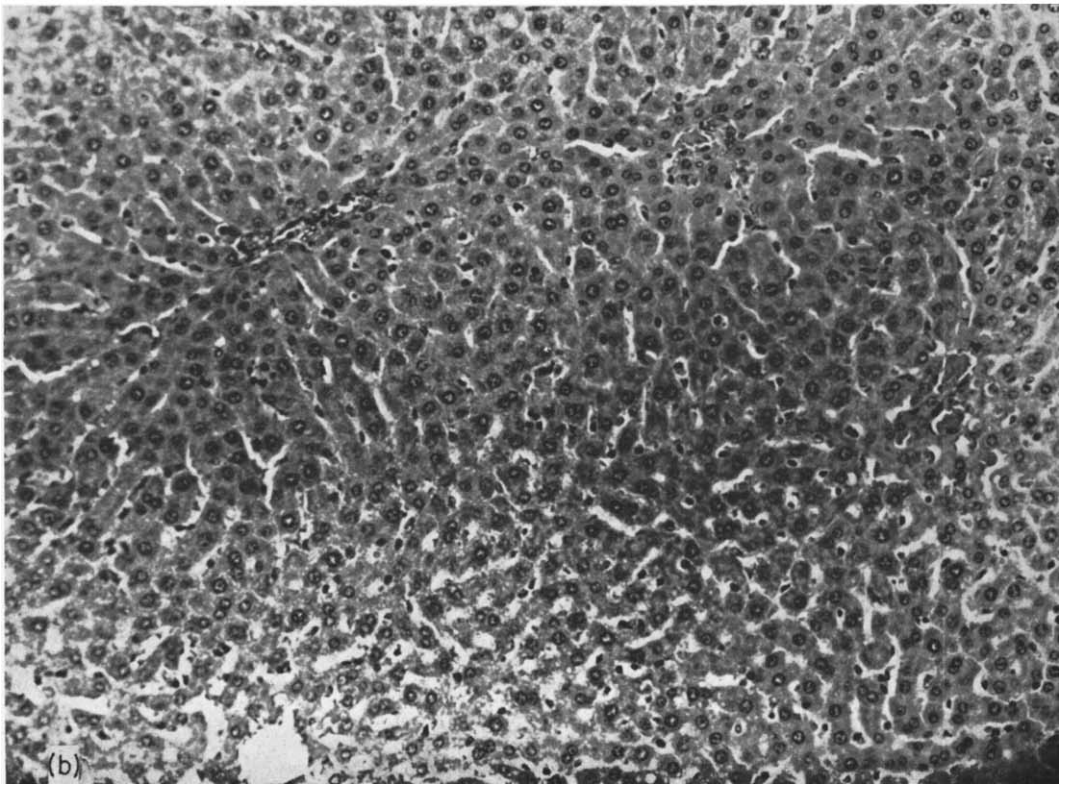


FIG. 4. Liver sections from control (a); and cycloheximide-treated (b) rats; ×160.



cine-^{3}H into rat liver protein, and that even the smallest dose level, 0.05 mg/100 g of body weight, resulted in 68% inhibition. The inhibition was slowly increased by the larger dose levels, and reached 88% at 1.0 mg/100 g of body weight. However, at the same time that L-leucine-^{3}H incorporation into liver protein fell rapidly, the incorporation of orotic acid-^{14}C into nucleic acids decreased only slightly.

The data in Table I show some of the effects of cycloheximide when administered to rats. The DNA content of liver rose sharply with increasing dosage of drug; the animals treated with the high levels of cycloheximide had as much as twice the DNA-P content of control livers (Fig. 2a). The RNA-P and protein concentrations in these livers remained remarkably constant at all levels of the drug tested (Fig. 2b). The decrease in the RNA-P/DNA-P ratio, from 5 to 3, as the dosage of cycloheximide is increased, further reflects the increase in DNA-P at constant RNA-P levels (Fig. 3). At the same drug levels the protein/RNA-P ratio remains constant. The significance of liver weight changes that take place in a 2-hr period is doubtful and it would appear that controls gained because they ingested food during this period.

A typical liver section from a control and cycloheximide treated rat is shown in Fig. 4. It is immediately apparent that there is an increase in the number of nuclei of parenchymal cells in the livers of drug-treated rats. Numerous nuclei counts made on a variety of such liver sections show the increase to be approximately 1.5-fold for rats receiving the highest dosage (1 mg/100 g of body wt) of drug.

Discussion. Cycloheximide has been recognized as a potent inhibitor of protein synthesis. Our results emphasize two notable changes that occur when rats are treated with this drug. First, protein synthesis is rapidly inhibited, as evidenced by decreased incorporation of L-leucine-^{3}H into liver protein. This primary effect is probably due to an interference with the formation of the peptide chain. Secondly, the DNA-P content is markedly increased in the livers of drug-treated rats. This increase in DNA-P at times twofold compared to controls, is much greater than the small variations in the protein and RNA-P values and in the liver weights. This was further reflected in a 1.5-fold increase in liver nuclei. In comparison with the abrupt inhibition of protein synthesis, the increase of DNA-P is gradual in its response to increasing amounts of the drug and suggests that the two effects are separate. These studies indicate that cycloheximide may be valuable in the investigation of DNA synthesis and cell replication.

Summary. Cycloheximide markedly decreases protein synthesis (68%) at a time when RNA synthesis is only slightly inhibited. The DNA content of the liver increases with increasing dosage of the drug; animals treated with cycloheximide and having 1.5 times as many nuclei had twice as much DNA-P in their liver as control animals. The abrupt inhibition of protein synthesis contrasts with a gradual, dose-dependent rise in DNA-P and suggests a separation of these two effects of cycloheximide.

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The Cardiovascular Response to Intrapulmonary Pressure Inflation in Dogs Treated with Reserpine, Propranolol, and Phenoxybenzamine (33936)

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Abnormal responses to the Valsalva maneuver have been described in derangement of the autonomic nervous system (1, 2), in individuals treated with ganglionic blocking agents or guanethidine (3, 4), and in conditions in which the adrenergic neurotransmitter norepinephrine is depleted from myocardium, such as congestive heart failure (5) or after reserpine treatment (5, 6). Since the introduction of newer classes of pharmacological agents, it is now possible to study the specific alpha- or beta-adrenergic functions (7, 8).

In the present study an attempt was made (a) to demonstrate the role of specific alpha- or beta-adrenergic function, and (b) to describe the effect of altered myocardial norepinephrine metabolism after reserpine administration, as reflected by arterial blood pressure and heart rate responses to intrapulmonary pressure inflation (IPPI) in anesthetized dogs.

Materials and Methods. Nineteen mongrel dogs weighing 9–20 kg were studied. Pentobarbital sodium (Nembutal) anesthesia was employed initially in a dose of 25–40 mg/kg. Additional small doses were given, when necessary, to maintain the desired light-to-moderate anesthetic depth. The technique for IPPI and recording femoral arterial and endotracheal pressures, and ECG were described previously (9).

Eight dogs were studied before and after alpha- and/or beta-adrenergic blockade. In 2 dogs, propranolol was administered intravenously in a dose of 0.25 mg/kg. In 4 dogs, intravenous phenoxybenzamine (PBZ) was given in a dose of 2.5 mg/kg. In one dog, PBZ (1.25 mg/kg) was given first, followed by administration of propranolol. In another dog, intravenous injection of propranolol was followed by PBZ (1.25 mg/kg). Observations were made every 5–30 min following drug administration for about 2 hr in this group of dogs.

Eleven dogs were given reserpine. These dogs were put into two groups: Group A, acute treatment group; 4 dogs received reserpine 0.5 mg/kg intravenously on the day of experiment and responses to IPPI were observed over 4–5 hr. Two dogs were given 0.1 mg/kg and were similarly studied. Group S, subacute treatment group; 2 dogs received 0.5 mg/kg intravenously 18 hr prior to the experiment. One dog received 0.5 mg/kg subcutaneously 18 hrs prior to the experiment. Two dogs received 0.5 mg/kg subcutaneously each at 24 and 48 hr prior to the experiment. Group A dogs before reserpine administration served as controls for the cardiovascular response to IPPI.

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