rats as compared with controls at the first MES; these changes persisted in those animals subjected to 6 hours of light alternated by 6 hours of dark and in animals exposed to an all light environment until the rats were sacrificed at 49 days of age (Fig. 1). Shortening of flexion and/or lengthening of extension of the tonic phase of a maximal seizure indicate increased convulsive activity(13).

Weights of thymus glands were: 370 ± 1.7 mg/100 g body weight, control; 299 ± 1.6, 300 ± 2.1, 340 ± 2.7 mg/100 g body weight for animals subjected to 6 hours of light alternated by 6 hours of dark, continuous light and continuous dark, respectively. Significance of difference between experimental and control animals is <0.01 for each of the light schedules. Adrenal and pituitary weights remained unchanged.

It is possible that light cycles, as produced in this experiment, altered hypothalamic-hypophyseal pathways. Obviously, the hypophyseal gonadotropins can be influenced as evidenced by rats subjected to constant illumination(6). Ovarian steroids administered during the first week of life hastened brain maturation as measured by electroshock seizure responses and were less effective when administered during the second week of life (14). On the other hand, administration of cortisol during the second week of life hastened brain maturation(8). Thus, the ACTH-adrenal cortical axis may be involved in the present study since a modest thymic atrophy was noted and is a tissue responsive to corticoids(15).

Summary. Alterations in light cycles hasten brain maturation of the young rat, produce greater brain excitability and involution of the thymus gland.

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Influence of Protein Synthesis Inhibitors on Circulatory Dynamics.* (31709)

S. CHARLES FREED
Harold Brunn Institute, Mount Zion Hospital and Medical Center, San Francisco, Calif.

It was recently demonstrated in this laboratory that actinomycin D, an inhibitor of DNA-dependent protein synthesis, lowered the blood pressure of normotensive and renal hypertensive rats(1). The degree of blood pressure depression was a function of the dosage, and was significant even in amounts well below that which induced general toxici-

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TABLE I. Systolic Blood Pressure of Normotensive Rats Treated with Acetoxycycloheximide.

Dosage per 100 g body wt (μg)	No. rats	Average blood pressure (mm/Hg)			
		Original	24 hr	48 hr	72 hr
10	12	112 ($\pm$ 8.7)*	96 ($\pm$ 7.3)	104 ($\pm$ 9.0)	109 ($\pm$ 8.1)
20	12	115 ($\pm$ 9.1)	85 ($\pm$ 7.7)	94 ($\pm$ 8.6)	105 ($\pm$ 8.9)
30	12	111 ($\pm$10.2)	77 ($\pm$ 8.4)	86 ($\pm$ 9.4)	101 ($\pm$10.4)

* Standard deviation.

ty. The mechanism of this response is obscure since this substance is able to alter protein metabolism of extra-vascular, vascular or intravascular tissues, any of which may influence blood pressure levels. In this regard it was demonstrated, however, that reduction of blood pressure apparently did not result from suppression of adrenal cortical steroid synthesis since administration of an anti-inflammatory steroid failed to prevent it. It was furthermore shown that the phasic contractile response to pressor drugs was not diminished when administered to rats whose blood pressure was depressed, suggesting that were lowering of peripheral resistance a factor it would probably involve resting state rather than contractile protein mechanisms.

To clarify the role of protein metabolism in the regulation of blood pressure, other inhibitors of protein synthesis were tested for possible effects. Three compounds were selected for this purpose, all of which are known to interfere with protein formation at the ribosomal level: (a) acetoxycycloheximide, which interferes with the transfer mechanism of amino acids(2); (b) chloramphenicol, which also disturbs such transfers but requires relatively large doses to be effective in mammalian tissues(3); and (c) puromycin, which releases nascent polypeptides prematurely from the ribosomes(4). These compounds were administered in various fractions of their lethal doses on the assumption that toxicity is due to protein synthesis inhibition.

In addition to obtaining blood pressure responses to these compounds, the effect of prednisolone on such responses was also studied. Furthermore, as was done in the previous study with actinomycin D, phasic contractile reaction to a pressor drug was investigated in rats treated with acetoxycycloheximide.

A. Acetoxycycloheximide.† *Methods and results.* This compound was administered to normotensive male Long-Evans rats weighing 250-300 g and to those made hypertensive by figure-of-eight renal ligation. Groups of 12 rats were injected intraperitoneally twice at a 6-hour interval at 3 dosage levels totalling 30 μg, 15 μg, and 10 μg per 100 g body weight. Under these conditions one LD/50 was approximately 60 μg per 100 g body weight. In about 50% of the rats treated with 30 μg per 100 g body weight there were signs of moderate toxicity for a day or two as shown by lessened activity, ruffled fur, and diarrhea, and one hypertensive animal died on the fourth day. The lesser doses elicited no apparent signs of illness, and there were no deaths. Systolic blood pressures were obtained by microphonic manometer prior to the first and second injections and daily thereafter for 6 days following the day of treatment.

Blood pressures of normotensive rats were significantly depressed at the 3 dosage levels of acetoxycycloheximide (Table I). The depression of blood pressure in hypertensive rats was even more pronounced, as depicted in Fig. 1. For purposes of comparison, the average daily blood pressure of a group of 12 hypertensive rats treated similarly with 30 μg per 100 g body weight of actinomycin D are included in Fig. 1.

It can be seen that a marked depression of blood pressure followed the administration of acetoxycycloheximide at all dosage levels, and that the amount of reduction was a function of the dosage. The blood pressure decline was quite rapid, averaging approximately 40 mm Hg 6 hours after the administration of the first injection of 15 μg per 100 g body weight. Blood pressures reached their lowest level

† Furnished through the courtesy of T. J. McBride, M.D., Medical Research Laboratories, Chas. Pfizer & Co.

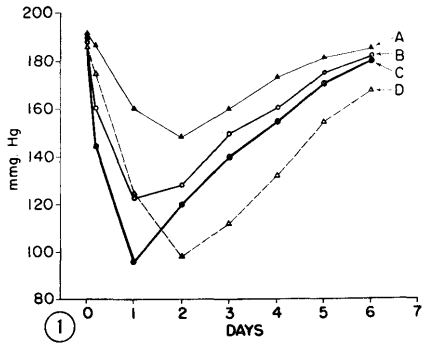
about 18 hours after the second injection, and thereafter gradually climbed to their original levels by the sixth day. The blood pressures of animals treated with actinomycin D were at their lowest point a day later.

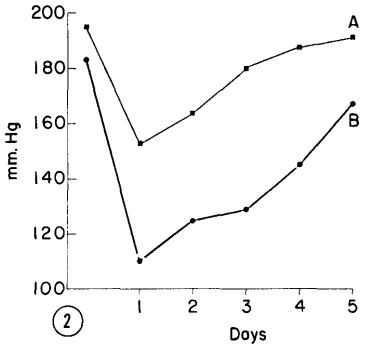
B. Chloramphenicol. Methods and results. Two groups of 12 hypertensive rats were injected in the same manner with amounts of chloramphenicol totalling 150 mg and 75 mg per 100 g body weight. Under these conditions LD/50 is approximately 300 mg per 100 g. The rats receiving the larger dosage were not as active as the controls, but did not otherwise appear under toxic influences. The smaller dosage apparently did not affect the vitality of the rats. The average daily systolic blood pressures of the hypertensive rats following this treatment are shown in Fig. 2.

It can be noted that the blood pressure response to chloramphenicol followed the same general pattern as that observed after acetoxycycloheximide administration, except that at one-fourth LD/50 dosage there was somewhat lesser depression than after an equivalent dose of the latter.

C. Puromycin. Methods and results. Three groups of 4 renal hypertensive rats were injected in a similar manner with puromycin in dosage totalling approximately 20 mg, 10 mg and 5 mg per 100 g body weight, or 50 mg, 25 mg and 12 mg, respectively, per rat. The highest dosage was approximately one LD/50(5). There was no significant change in blood pressure in rats treated at any of the above dosage levels.

D. Effect of prednisolone on blood pressure depression. Methods and results. A group of 12 renal hypertensive rats received 30 μg acetoxycycloheximide per 100 g body weight, divided into 2 intraperitoneal injections 6 hours apart. A second similar group received, in addition to this, 2 mg prednisolone acetate subcutaneously at each injection and 2 mg daily thereafter. A third group of 10 hypertensive rats received 150 mg chloramphenicol per 100 g body weight in 2 intraperitoneal injections 6 hours apart, and a fourth group of 10 such rats was injected in the same way, but with the addition of 2 mg prednisolone acetate at each injection and daily thereafter. Systolic blood pressures were obtained in these four groups prior to treatment and daily thereafter.





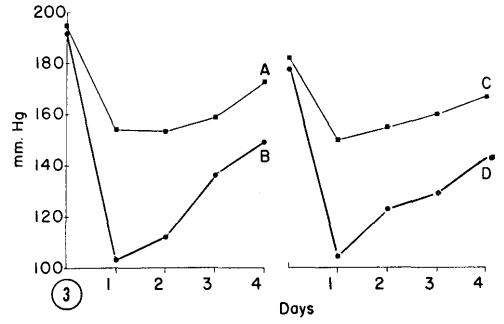


FIG. 1. Average systolic blood pressures of hypertensive rats following acetoxycycloheximide administration on day zero. A = 10 μg, B = 15 μg and C = 30 μg per 100 g body weight. D = after 30 μg per 100 g body weight actinomycin D.

FIG. 2. Average systolic blood pressures of hypertensive rats after administration of chloramphenicol. A = 75 mg and B =150 mg per 100 g body weight.

FIG. 3. Average systolic blood pressures of hypertensive rats receiving A—acetoxycycloheximide and prednisolone; B—acetoxycycloheximide alone; C—chloramphenicol and prednisolone; D—chloramphenicol alone.

The results of this experiment are shown in Fig. 2. It is quite apparent that prednisolone effectively protected the rats against

the blood pressure depressor effects of acetoxycycloheximide and chloramphenicol.

E. Pressor response to l-norepinephrine. Methods and results. A group of 12 normal rats was injected with 30 μg acetoxycycloheximide per 100 g body weight divided into 2 injections 6 hours apart. Twenty-four hours after the first injection they were studied for pressor response as follows. The abdominal aorta was cannulated at the femoral bifurcation and connected to a mercury manometer. After a 5-minute interval for blood pressure stabilization they were injected intravenously with 1 μg l-norepinephrine. The blood pressure response was recorded at its peak, and then at one-minute intervals for 5 minutes. This procedure was repeated an additional 3 times at 5-minute intervals. A similar study was made in 10 untreated control rats.

The average mean blood pressure of acetoxycycloheximide-treated rats before administration of the pressor drug was 68 mm Hg as compared to that of the controls, which averaged 94 mm Hg. There appears to be no significant difference in responses to the pressor agent between the treated and control groups of rats.

Discussion. The data presented above demonstrate that acetoxycycloheximide and chloramphenicol are effective in reducing blood pressures of renal hypertensive rats. The degree of reduction is dose-dependent and is appreciable following doses which are below those which cause general toxicity. These results suggest that the lowering of blood pressure is independent of "non-specific" toxic factors. In addition, such factors as interference with nutrition or reduction in potassium intake, which have been demonstrated to lower blood pressure of hypertensive rats, may be ruled out of the present experiment. It requires several days or even weeks for such a response, in contrast to the marked lowering of blood pressure within several hours after administration of the antibiotics.

The blood pressures fall quite rapidly, and can be readily observed within 6 hours after administration of these substances. The lowest level is reached in about 24 hours, after which there is a gradual recovery to pre-injection pressures by the sixth day. In comparison, blood pressures following administration of an amount of actinomycin D with an equivalent depressor effect reached the lowest levels a day later. This result is not surprising on the assumption that inhibition of protein synthesis is responsible for blood pressure reduction. Thus both acetoxycycloheximide and chloramphenicol are reported to exert their influences by acting directly on the ribosomes to interfere with formation of polypeptides essential for protein synthesis. Actinomycin D, on the other hand, has been shown to inhibit protein synthesis at an earlier cellular level by suppressing the formation of messenger RNA from DNA. It may therefore require an additional 24 hours for ribosomes to utilize the already available m RNA before maximum inhibition of arterial protein synthesis is reflected in fall of blood pressure.

Actually the mechanisms involved in the relationship between inhibition of protein synthesis and hypodynamic response are quite obscure. In the report on the depressor effect of actinomycin D, it was suggested that blood pressure reduction may be mediated by alterations in protein metabolism in extravascular tissues such as myocardium, nerves and kidney, or on arterial muscle proteins(1). There is limited evidence to indicate that these inhibitors may interfere with the proteins of the peripheral vasculature. This evidence consists of preliminary data in this laboratory showing that actinomycin D suppressed the uptake of tritiated uridine by the rat aorta. Physiological evidence indicates that acetoxycycloheximide may lower blood pressure without interfering with the phasic contractile mechanisms since a normal response to a vasoconstrictor drug was obtained during the hypodynamic state. This would suggest that if lowering of peripheral resistance were significantly responsible for the fall in blood pressure, such an effect would involve tonal rather than contractile protein functions. Obviously, additional data are required before a firmer conclusion can be made.

No explanation can be offered to account for the failure of puromycin to lower blood

pressure. The first question to be resolved is whether puromycin in the dosages used was capable of effectively inhibiting the synthesis of proteins. It would seem that adequate doses were administered; thus puromycin was administered in about 6 times the effective dosages, based on toxicity, of acetoxycycloheximide and actinomycin D. Likewise the dose of 50 mg puromycin per rat, which failed to reduce blood pressure, is 3 to 5 times the amount which other investigators used to demonstrate *in vivo* inhibitory effects on various tissue proteins(6). This failure to lower blood pressure may be related to the mechanism by which puromycin inhibits protein synthesis in inducing release of nascent polypeptides from the ribosomes, rather than suppression of polypeptide synthesis obtained with the other compounds. Whether this feature is coincident with, or has a physiologic significance in blood pressure regulation remains to be demonstrated.

Another illustration of how the specific mechanisms involved in inhibition of protein synthesis may influence circulation may be noted in the difference in blood pressure response to prednisolone between rats treated with actinomycin D and acetoxycycloheximide or chloramphenicol. The reduction of blood pressure by the first compound, which suppresses DNA-dependent RNA synthesis, including m RNA, is not corrected by this steroid. Chloramphenicol and acetoxycycloheximide, on the other hand, which have no effect on m RNA but with the orderly transfer of amino acids to form polypeptides, induce a depressor effect which is opposed by prednisolone. While on the surface it may appear that the presence of m RNA is essential for this hemodynamic response of prednisolone, it is obviously premature to attempt to explain the above results on the basis of available information.

Summary. Acetoxycycloheximide, an inhibitor of protein synthesis at the ribosomes, reduces the blood pressure of normotensive and hypertensive rats. Chloramphenicol, which has a similar but not identical action, also reduces blood pressure of hypertensive rats. The degree of blood pressure depression to both substances is dose-dependent and is appreciable in non-toxic amounts. Puromycin, which inhibits ribosomal synthesis of protein, but through release of rather than suppression of polypeytides, fails to reduce blood pressures at much higher dosage levels relative to toxicity. Blood pressure depression following acetoxycycloheximide or chloramphenicol administration is corrected by prednisolone. Rats treated with acetoxycycloheximide respond to l-norepinephrine with normally vigorous blood pressure elevation suggesting that phasic contractile proteins remain intact.

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Rheumatoid Factor and Immuno-Conglutinin Responses Following Various Vaccinations. (31710)

K. AHO, T. SOMER,† AND O. P. SALO (Introduced by C. L. Christian)
State Serum Institute, Helsinki, Finland, and the Finnish Defence Forces

Rheumatoid factors exhibit a considerable degree of specificity for rheumatoid arthritis. They can be detected in the great majority of sera from active cases of rheumatoid arth-

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† Research fellow of Finnish Medical Research Council.